# King George Whiting (*Sillaginodes punctatus*) spawning dynamics in South Australia's southern gulfs

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

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# Abbreviations

| Acronym | Meaning |
|---|---|
| CPUE | Catch Per Unit Effort |
| DEPM | Daily Egg Production Method |
| DNA | Deoxyribonucleic Acid |
| DVM | Diel Vertical Migration |
| FAACC | Formalin Acetic Acid Calcium Chloride |
| FRDC | Fisheries Research and Development Corporation |
| GSV | Gulf St. Vincent |
| HRP | Horseradish Perioxidase |
| ISH | In Situ Hybridisation |
| IS | Investigator Strait |
| KI | Kangaroo Island |
| LTRANS | Larval Transport Model |
| MFA | Marine Fishers Association |
| MSF | Marine Scalefish Fishery |
| NGSV | Northern Gulf St. Vincent |
| NSG | Northern Spencer Gulf |
| RNA | Ribonucleic acid |
| ROMS | Regional Ocean Modelling System |
| RDVM | Reverse Diel Vertical Migration |
| SAFCOL | South Australian Fishermens Co-Operative Limited |
| SESG | South-east Spencer Gulf |
| SWSG | South-west Spencer Gulf |
| TGM | Two Gulf Model |
| WC | West Coast |

# Executive Summary


### *Overview*

The overarching aim of this study was to investigate the spawning dynamics of King George Whiting (*Sillaginodes punctatus*: Perciformes) in South Australia's southern gulfs and Investigator Strait. This study was the first to apply a modified daily egg production method (DEPM) to King George Whiting. This was achieved by the development of a molecular *in-situ* hybridisation (ISH) technique which facilitated the accurate identification of King George Whiting eggs and larvae from mixed plankton samples. This species-specific molecular probe targets King George Whiting ribosomal RNA sequences and when conjugated with a reactive molecule creates a colour change for positive reactions. The application of this ISH technique has reduced the potential for bias in egg counts through misidentification. This allowed for daily egg production to be estimated for King George Whiting, overcoming the issues of accurate egg identification that have prevented previous applications of the DEPM. However, issues with adult sampling during the egg survey's prevented spawning fraction (a key DEPM parameter) from being accurately estimated. Consequently, an accurate estimate of spawning biomass could not be produced for these two surveys. Despite this, this study determined that estimates of spawning biomass can be produced for King George Whiting; acknowledging that issues with adult sampling present in this study must be overcome. When available, these can be further included in integrated stock assessment models by providing a fishery independent estimate of spawning biomass. Egg density information from the DEPM was incorporated into a bio-physical model that predicted important larval pathways and key areas of spawning with increased settlement success. The culmination of this early life history information has been synthesised to inform future management decisions, including the evaluation of spatial closures to protect future spawning stocks. Therefore, key results of this study have been integrated with the on-going assessment and management of the resource.


### *Background*

King George Whiting is one of the most valuable and iconic coastal finfish species of southern Australia. In South Australia's Marine Scalefish Fishery, it is considered a 'primary' species, attracts the highest finfish price per unit weight for commercial fishers, and is highly sought after by the recreational and charter boat sectors. Recent trends in South Australian commercial catch and effort statistics and modelled estimates of fishable biomass indicate that the status of the gulf stocks, including the southern gulfs spawning grounds, were in decline. In the 2014 stock assessment, both Gulf St. Vincent / Kangaroo Island stocks and Spencer Gulf stocks were classified as 'transitional-depleting'. This classification initiated a review of the fishery during 2016, which resulted in changes to management regulations from 1 December 2016 that included reductions in bag and boat limits for the recreational fishing sector and for all sectors an increase to the minimum size limit to 32 cm west of 136°E, and a spatial closure that aimed to protect the spawning population in May. Furthermore, stock status assessments are based on fishery-dependent catch and effort data which does not include improvements in 'effective' effort and changes in fleet dynamics and so are likely to have underestimated the actual rate of stock decline. As such, there was a need to develop a fishery-

independent method to estimate King George Whiting biomass to supplement and underpin the future fishery-dependent estimates of stock.

Previous attempts to apply a DEPM to King George Whiting and obtain a fishery-independent measure of biomass was unsuccessful due to the difficulties in egg identification and the relative rarity of eggs encountered during the sampling program. The development of molecular validation for egg and larvae identification has been successful for Snapper (*Chrysophrys auratus*) and shown to be essential for DEPM programs with challenges in morphological egg identification. Additionally, advancements in bio-physical modelling have enabled the prediction of larval pathways and the identification of critical spawning areas for larval settlement success. Given the advancements in these areas, it was suggested that further investigation into the spawning dynamics of King George Whiting and the development of a fishery-independent estimate of biomass was now possible.

### *Objectives*

1. To determine key King George Whiting spawning areas throughout the southern gulf systems of South Australia.
2. To quantify links between larval source and sink populations.
3. To develop a fishery-independent technique that provides the most accurate estimate of spawning biomass (i.e. daily egg production method) for King George Whiting and integrates with the on-going assessment and management of the resource.
4. To evaluate the potential benefits of strategic management options to protect the spawning stock and ensure the sustainable harvest of King George Whiting.

### *Methodology*

Two DEPM surveys were undertaken from *RV Ngerin* in April 2017 and 2018. Plankton samples were collected from 126 stations spaced in a 2 x 4 nm grid pattern using oblique bongo net tows to within 5m of the seabed. All plankton samples were stored in 95% ethanol and refrigerated prior to sorting and processing. All eggs and larvae were sorted under a dissecting microscope into ‘possible’ and ‘other’ eggs and larvae. The ‘possible’ eggs were then validated through the application of the *in-situ* hybridisation molecular technique. Once validated, the King George Whiting eggs were assigned an egg development stage.

During the plankton sampling period of the DEPM surveys, adult King George Whiting were collected from a combination of commercial fisheries (Marine Scalefish Fishery, Gulf St. Vincent Prawn Fishery and Spencer Gulf and West Coast Prawn Fishery), recreational fishers and scientific sampling. Adults samples collected were assessed for size and biological information for estimating key reproductive parameters (i.e. sex ratio, spawning fraction, batch fecundity). Total daily egg production, spawning area and spawning biomass estimates were then calculated for both DEPM surveys.

A bio-physical model was created based on larval behaviour and known settlement locations, observed and forecasted regional oceanography and a particle tracking model to assess key spawning areas for successful larval settlement and larval pathways.

### *Results*

Through the successful application of the *in-situ* hybridisation technique to validate the identification of King George Whiting eggs, a total of 541 confirmed eggs were collected in 2017 and 944 in 2018. Overall, King George Whiting eggs were distributed across ~70% of the plankton survey area in relatively low densities (<5 eggs.m$^{-2}$). Peaks in spawning activity, with egg densities ~18 eggs.m$^{-2}$ were identified at four differing stations in both 2017 and 2018. A spatial shift in spawning activity was identified between years from southern Spencer Gulf in 2017 to central Investigator Strait in 2018. The bio-physical model predicted low levels of settlement success (1 – 2%) for all particles released in the simulation, with the majority (~75%) of seeded particles predicted to move out of the model boundaries. The bio-physical model also predicted successful settlement from two key spawning areas in the south-east Spencer Gulf in 2017 and western to central Investigator Strait in 2018. The culmination of this observed and predicted spawning information highlights the importance of the southern Spencer Gulf and Investigator Strait for the replenishment of the gulfs King George Whiting stocks.

Issues with adult sampling (namely an inability to estimate spawning fraction [*S*] from available samples), prevented reliable estimates of spawning biomass from being determined. However, methods were still developed within this study that allow DEPM to be applied for King George Whiting, and if sufficient adult samples could have been attained, unbiased estimates of spawning biomass (*B*) would have been produced in this study.

Two main issues that previously prevented precise estimates of spawning biomass from being determined for King George Whiting were overcome in this study: 1) the use of *in situ* hybridisation to identify King George Whiting eggs, which provided accurate estimates of egg density and determined primary spawning grounds, and, 2) the application of a size-based DEPM (DEPMWt) that reduced the variance of spawning biomass by accounting for female population structure in its estimation. These provide strong evidence that future applications of the DEPM can be applied for King George Whiting and other demersal fish stocks in South Australia and other jurisdictions.

### *Implications*

The culmination of the spawning dynamics information derived from this study has identified that the southern Spencer Gulf and Investigator Strait are key areas of spawning for King George Whiting. This information will support the future development and refinement of spatial closures to ensure the continuation of successful recruitment and replenishment of King George Whiting stocks.

This study has demonstrated that the application of the DEPM is viable for King George Whiting. As King George Whiting are a demersal species, the application of DEPM was not routine and required sophisticated molecular techniques to overcome issues with egg identification. The successful adaptation of these

techniques could be widely applied for other King George Whiting stocks or developed for any multiple spawning species that produces pelagic eggs. However, issues with adult sampling prevented definitive estimates of spawning biomass from being achieved. A further refinement would be to separate the second egg development stage into multiple shorter stages. As this second stage is substantially longer than the other stages, separating it further would produce more precise estimates of age. This study therefore represents a proof of concept where better addressing adult parameters in the DEPM will provide estimates of spawning biomass. The integration of an unbiased estimate of biomass derived from the DEPM (when available) into the traditional fishery-dependant catch and effort based stock assessments will lead to greater confidence in future modelled outputs and will be beneficial for future management measures (i.e. harvest strategies, allocations, and reformation of the Marine Scalefish Fishery).

***Keywords***

**King George Whiting, *Sillaginodes punctatus*, Daily Egg Production Method, *In-situ* Hybridisation, Bio-physical Modelling.**

# Introduction

## Background

King George Whiting (*Sillaginodes punctatus*: Perciformes) is one of the most valuable and iconic coastal finfish species of southern Australia. It occurs in coastal and shelf waters along the southern Australian coastline from Perth in Western Australia to Sydney in New South Wales (Kailola, 1993). King George Whiting has a complex life history, exhibiting ontogenetic shifts in habitat at different life history stages (Fowler and Jones, 2008). Spawning occurs during late autumn and early winter at offshore reefs, shoals, and mounds in relatively deep water (30–100 m) in exposed locations (Fowler, 2000, Fowler et al., 1999, Steer et al., 2018). The larvae of King George Whiting can be advected across hundreds of kilometres over a prolonged pre-settlement period (3–5 months), dispersing larvae from the offshore spawning grounds to the shallow water nursery areas in the northern gulfs, West Coast and Kangaroo Island. Known nursery areas are characterised by semi-enclosed, protected shallow bays, with intertidal seagrass beds of *Zostera* and *Heterozostera* spp. (Fowler et al., 2000b, Rogers, 2019). Juvenile fish develop and grow in the vicinity of these nursery areas until approximately three-years of age (Fowler et al., 2000a). After this juvenile stage, these fish move to their offshore spawning grounds, which ultimately replenish the spawning stock (Fowler et al., 2000a, Fowler et al., 1999). As a result of the ontogenetic shift in habitats, the King George Whiting population size and age structure varies geographically (Fowler et al., 2000a). The northern gulfs and inshore bay area support populations with younger, truncated age structures, ranging predominately from one to four-years of age. In comparison, the offshore spawning areas have a broader age structure with ages up to 20 years (Steer et al., 2018). The population structure of King George Whiting of South Australia has genetic homogeneity, indicating that there is at least a small degree of mixing across all the states coastal and shelf waters (Kent et al., 2018). However, due to previously identified relationships between spawning grounds and settlement locations and suggested localised self-seeding, the commercial fishery is managed as three separate stocks of, west of Eyre Peninsular (West Coast), Spencer Gulf, and Gulf St. Vincent / Kangaroo Island.

In South Australia's commercial Marine Scalefish Fishery, King George Whiting is considered a 'primary' species which attracts the highest price per unit weight for commercial fishers and is highly sought after by the recreational and charter boat sectors. Commercial and recreational fishers have typically targeted the small, immature fish within inshore waters as they transition from the shallow protected nursery grounds to the deeper offshore spawning areas. The offshore movement of maturing juveniles and young adults must pass through a 'gauntlet' of fishing lines and nets to reach the spawning areas. Historically, the exploitation of the spawning aggregations was low, as they were largely

inconspicuous and difficult to access. As a result, the recruitment of King George Whiting into the fishery was believed to be relatively stable.

Recent trends in commercial catch and effort statistics and modelled estimates of fishable biomass indicate that the status of the gulf stocks, which include the southern gulfs spawning grounds were in decline (Fowler et al., 2014). In the 2014 stock assessment, both Gulf St. Vincent / Kangaroo Island stocks and Spencer Gulf stocks were identified as 'transitional-depleting'. This assessment initiated a review of the fishery in December 2016 and subsequently, management regulations were enhanced. Alternatively, the West Coast stock has maintained its period of stability and has continued to be assessed as 'sustainable' (Fowler et al., 2014, Steer et al., 2018)

In the most recent assessment, the status of both gulf stocks improved and were classified as 'sustainable'. This was driven by modelled increases in recruitment and fishable biomass (Steer et al., 2018). The previous declines in biomass and recruitment appear to be caused, at least in part, by increased fishing pressure on offshore spawning aggregations associated with advances in fishing technology (e.g. Global Positioning Systems, echosounders, increased horsepower) and rapid communication throughout the angling community (e.g. chat forums, social networks). Contemporary fishers are now able to confidently target large 'trophy' King George Whiting with increased efficiency. Current assessments of stock status are entirely based on fishery-dependent data and do not account for improvements in 'effective' effort and changes in fleet dynamics, which likely underestimates the actual rate of stock decline. Given these changes, there is a fundamental need to establish management measures to protect spawning aggregations and integrate fishery-independent population data into future assessments of stock status.

King George Whiting stocks in South Australia are heavily targeted by commercial, recreational and charter boat sectors. The commercial sector, which is a combination of three fisheries, Marine Scalefish, Northern Zone Rock Lobster and Southern Zone Rock Lobster, has seen a long term decline in total catches of King George Whiting. There has been a 68.4% decline since its peak of catch of 776 t in 1992, to the lowest recorded catch of 245 t in 2017 (Steer et al., 2018). Total catches have remained between 245 and 281 t for the commercial sector in the past five years (Steer et al., 2018). The Marine Scalefish Fishery targets King George Whiting with handlines, gill nets, and haul nets and contributes to ~99% of the commercial catch. The remaining 1% of catch is captured between both of the Rock Lobster fisheries. Estimates of recreational King George Whiting catch have been relatively consistent at ~ 350 t annually, based on three telephone surveys conducted in 2000/01, 2007/08 and 2013/14 (Giri and Hall, 2015, Steer, 2018). The most recent estimate of recreational King George Whiting catch was 367 t in 2013/14, which represents 58.1% of the total state-wide catch (Giri and Hall, 2015, Steer, 2018).

## Management Arrangements

Management regulations for the commercial King George Whiting fishery are complex and have evolved through time in response to the dynamics of this fish stock. The principal means of controlling effort has been the reduction in the number of licence holders operating in the fishery over time. There have been a complexity of regulations that apply to gear used to take King George Whiting. These restrictions include the number of handlines and hooks that can be used and gear specifications and spatial and temporal closures for fishing with hauling nets and gill nets. The commercial fishery is also regulated by legal minimum size limits and spatial closures.

Management regulations for both commercial and recreational fishers were recently amended following the 'transitional-depleting' status that was assigned to the two gulfs in 2014 and after the ensuing review of the fishery which took place through 2016. The changes that were implemented in December 2016 were: (1) an increase in legal minimum total length (LML) from 310 mm to 320 mm in all waters east of longitude 136°E, the LML to remain at 300 mm for the West Coast; for both commercial and recreational fishers (2) a State-wide reduction in bag limit for recreational fishers from 12 to 10 legal fish per person and a boat limit reduction of 36 to 30 fish per boat; (3) A possession limit of either 72 fish or 10kg of fillets, or 36 fish and up to 5 kg of fillets for recreational fishers; (4) A spatial closure for sections of the southern Spencer Gulf and Investigator Strait for 1 – 31 May for all fishing sectors was first implemented in May 2017 and continued through to 2019.

## Source to Sink

Resolving the link between key spawning areas, paths of larval dispersal and settlement is essential for understanding a species habitat use to inform spatial management (Jenkins et al., 2000). Despite extensive research into the life history of King George Whiting across southern Australian, there is considerable uncertainty about specific spawning locations and the connectivity to nursery areas. King George Whiting, similar to many demersal marine fish species depend on the transport of larvae from offshore spawning grounds to inshore nursery areas (Hamer and Jenkins, 1997, Teodosio et al., 2016). This species possesses a prolonged larval duration (80 – 130 days) which has the potential for extended large-scale advection influenced by physical oceanographic processes. In early stages of larval development for most marine fishes, larval transport is considered to be passive through the pelagic environment. During late-stage larval development, larvae have been identified to have increased swimming abilities and to use visual, auditory and olfactory cues to establish orientation, which may assist in actively moving towards potential nursery areas pre-settlement (Teodosio et al., 2016, Leis, 2010).

Recent developments in biological oceanography have led to the application of bio-physical models becoming a leading approach to predict patterns of larval dispersal and investigate population

connectivity in marine ecosystems. A bio-physical model is a three dimensional particle tracking model, and when used to simulate transport throughout the larval stage it couples a hydrodynamic model, incorporating oceanographic, topographic and atmospheric data, with a biological model, which includes larval development and behaviour. These models simulate larval dispersal patterns, enabling the prediction of dispersal pathways, prediction of spawning origins and estimation of successful larval settlement (North et al., 2008, Mcleay et al., 2016).

## Spawning Biomass Estimate

Estimates of biomass derived from fishery-independent surveys have been increasingly incorporated into stock assessments and used as a biological indicator to underpin harvest strategies. One routinely used method of estimating spawning biomass is the Daily Egg Production Method (DEPM), which was developed in the late 1970s to assess Northern Anchovy (*Engraulis mordax*) (Lasker, 1985) and has now been successfully applied to a range of small pelagic (Wolf and Smith, 1986, Ward et al., 2011) and demersal fish species (Murua et al., 2010, Jackson et al., 2012). This method estimates the spawning biomass of a stock by combining measurements of species specific egg density with a suite of adult biological parameters obtained during simultaneous sampling. The underlying premise of applying a DEPM is that the biomass of spawning adults can be calculated by dividing the mean number of eggs produced per unit mass of adult fish (Steer et al., 2017, Lasker, 1985).

$$SB = \frac{P_0 \times A}{(R \times F \times S/W)}$$

This technique was initially developed for small pelagic fisheries, such as anchovy and sardines, where obtaining adequate adult sample size and egg identification is not an issue. The adaption of DEPMs to demersal species has identified issues with egg identification, due to similarities in co-occurring species egg morphology, creating a lack of confidence in visual identification. To resolve these challenges in egg identification, species validation through molecular techniques have been applied to a sub-set of samples (Ward, 2016) and to create a 'correction factor' for egg counts (Neira et al., 2015). However, these first techniques have relied on destructive sampling where, after visual identification and staging, the DNA or RNA is chemically extracted for analysis, destroying the specimen (Steer et al., 2017; Oxley et al., 2017). A recent study of Snapper (*Chrysophrys auratus*) in South Australia used *in-situ* hybridisation (ISH) molecular validation for all possible Snapper eggs and larvae that were sorted from mixed ichthyoplankton samples. The ISH technique allowed for a more accurate, streamlined and relatively non-destructive method of validating the Snapper DNA (Oxley et al., 2017). This ISH technique developed a Snapper-specific oligonucleotide probe, which when bound with its targeted ribosomal RNA sequence creates a coloured reaction. The coloured eggs and larvae were able to be identified and extracted from the sample under a stereo microscope and then staged and archived. The success of this molecular validation for Snapper has enabled the ISH technique to be applied for other

similar demersal species, potentially resolving the challenges with egg identification in species such as King George Whiting.

A preliminary DEPM for King George Whiting was conducted in South Australia in the late 1990s, which identified the potential for incorporating the estimates of biomass into future stock assessments (Fowler, 2000). However, this study highlighted several limitations for the application of a DEPM for King George Whiting. One key finding was that King George Whiting eggs, especially early-stage eggs, were rare in comparison to other pelagic eggs. In particular, identification of early-stage eggs was further exacerbated by the difficulties in visual identification. King George Whiting eggs are considered a 'generic' fish egg, which is common in size (0.8 – 1 mm) and lacks any easily distinguishable features, which likely led to conservative egg counts and therefore, underestimated egg densities (Fowler, 2000). The development of a King George Whiting oligonucleotide probe, as proposed in this study, will resolve the ambiguity and improve accuracy in egg and larvae identification. Another issue identified by Fowler (2000), was the insufficient adult sample size and bias as a result of relying solely on fishery-dependant sampling. Reliance on sourcing adult fish samples from the commercial sector was cost inhibitive and also led to sampling a restricted size class due to processors grading the fish prior to sale (Fowler, 2000). Consequently, a future focus of obtaining a larger quantity of adult samples from both the commercial and recreational sector was proposed.

Citizen science initiatives such as recreational fishing tag and recapture and fish frame donation programs have been running for decades in many of Australia's state jurisdictions. In Western Australia, the 'Send Us Your Skeletons' initiative was implemented in the late 2000s and annually receives > 4,000 fish frames of key demersal and coastal finfish species. The New South Wales game fish tagging program extends beyond the states boundaries and has deployed ~460,000 tags since 1973. Recreational fishing programs have shown to encourage a sense of collaboration and investment in the fishery, which extends through to future management arrangements (Fairclough et al., 2014). Recreational fishing programs can also sample a species over greater spatial and temporal scales due to increased and more broadly dispersed effort than can be achieved by fishery-dependant and scientific fishery-independent sampling (Fairclough et al., 2014).

To resolve the previous DEPM adult sampling issues, this study aimed to sample King George Whiting from commercial and recreational fishers by implementing a targeted fish frame donation program during key spawning times. The intent was to encourage the estimated ~270,000 recreational fishers in South Australia to deposit their post-filleted frames and fishing location data at designated drop-off locations around the state.

## Need

Additional scientific based evidence around spawning is needed to support management actions that will ensure 1) that the King George Whiting stocks of Spencer Gulf and Gulf St. Vincent / Kangaroo Island

regions maintain current sustainable status and 2) management of recreational catches within the allocated catch shares in the Management Plan for the South Australian Marine Scalefish Fishery. Although spawning spatial closures have been identified as a management option for the fishery, the relative benefit of their proposed locations (south-east Spencer Gulf and Investigator Strait) in protecting spawning productivity is unknown. Similarly, it is not understood how key spawning grounds south of the gulfs support the regional stocks. Therefore, there is a fundamental need to characterise these spawning aggregations from an ecological, economic and social perspective to ensure that spawning closures are designed and implemented appropriately.

The previous 'transitional-depleting' status assigned to the gulf stocks in 2014 was predominantly based on commercial catch and effort data integrated with limited information from the recreational and charter boat sectors. Given the recent changes in the fishing fleet dynamics, which includes an increase in the estimated share of the state-wide catch by the recreational sector (by ~ 10%) (Giri and Hall, 2015) and an increased pressure on spawning fish, there is a need to develop a fishery-independent means of assessing biomass to enhance future assessments of stock status.

# Objectives

1. To determine key King George Whiting spawning areas throughout the southern gulf systems of South Australia.
2. To quantify links between larval source and sink populations.
3. To develop a fishery-independent technique that provides the most accurate estimate of spawning biomass (i.e. daily egg production method) for King George Whiting and integrates with the on-going assessment and management of the resource.
4. To evaluate the potential benefits of strategic management options to protect the spawning stock and ensure the sustainable harvest of King George Whiting.

# Method

## Key spawning areas and spawning biomass

### Adult sampling

Adult King George Whiting were collected in April 2017 and 2018 from a combination of commercial fisheries (Marine Scalefish Fishery, Gulf St. Vincent and Spencer Gulf King Prawn Fisheries), recreational fishers and fishery-independent sampling. All adult samples were grouped by their region of fishing, which was Kangaroo Island (KI), Investigator Strait (IS), northern Spencer Gulf (NSG), southern Spencer Gulf (SSG), northern Gulf St. Vincent (NGSV), southern Gulf St. Vincent (SGSV), and the West Coast (WC). The assigned regions of origin in this study are at a finer scale than the stocks are managed within the commercial fishery.

Adult samples were sourced from the commercial fishing sector via the centralised SAFCOL market. This sampling program occurred over two stages: the first involved targeting commercial catches from regions of interest (i.e. SSG, IS, SGSV) and measuring the size of each King George Whiting within the catch. The second stage involved processing a sub-sample of the catch to obtain further biological information according to the procedure outlined below. The landing details of capture date and location of these market-sourced fish were cross-referenced from the fisher's compulsory catch return logs. Additional adult samples were collected during fishery-dependent observer surveys in both Gulf St. Vincent and Spencer Gulf King Prawn fisheries. All King George Whiting caught as by-catch during each survey shot were collected and snap frozen for future processing, and the capture time and location was recorded for each survey shot.

Recreational and charter boat fishers contribute to ~60% of the total King George Whiting annual catches in South Australia. As a result, a targeted recreational fish frame donation program was established, encouraging recreational fishers to participate in research by donating their filleted King George Whiting frames. This was implemented by a callout to fishers through RecFish SA, South Australia's peak body in recreational fishing and an extensive media engagement through posters (Figure 1), television, social media, newspapers, magazine articles and radio interviews. The participating fishers were encouraged to provide catch details on the waterproof flyer provided (Figure 1) and deposit either fresh or frozen frames at the designated drop-off locations.

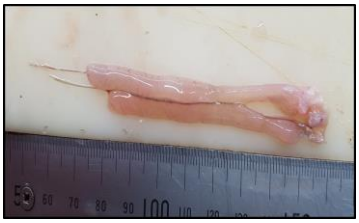
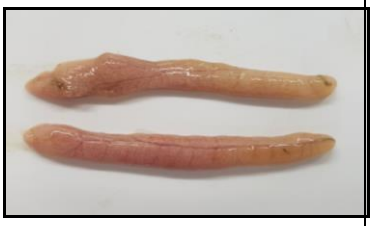
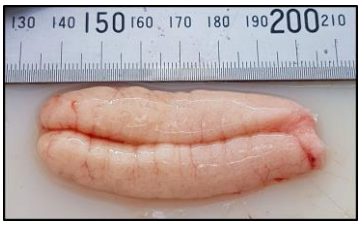
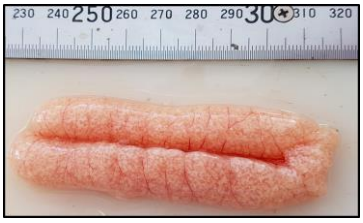
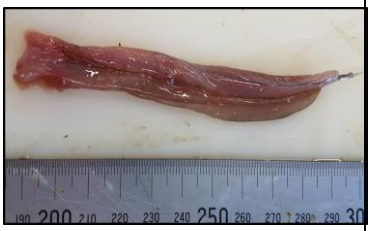
Fishery-independent sampling took place during the two DEPM surveys. The *RV Ngerin* was anchored in well-known fishing grounds in the southern Gulfs and Investigator Strait to target adult King George Whiting using baited hand-lines.


**Figure 1.** Recreational fishing King George Whiting frame donation poster for 2018, (see Appendix 1 Project Materials developed for A5 flyers and posters for 2017).

## Processing of adult samples

Adult fish were measured (total length (TL) and standard length (SL) to the nearest mm) and weighed (not applicable for donated fish frames) using a marine balance (0.01 kg). Total weight (Wt) was estimated for the donated frames by a linear regression ($Wt = 3.3172 \times TL - 731.87$, $R^2=0.92$) formulated from 9,200 length-weight records from SAFCOL market sampling. Fish, including the fish frames where possible were eviscerated, sexed and macroscopically assigned a maturity stage according to Fowler et al. (1999) (Table 1). The gonads were dissected and weighed (0.01 g). For fish captured during fishery-independent sampling, a subsample of advanced staged ovaries (Stage 3 and 4) were subjected to detailed batch fecundity analyses. For hydrated ovaries (Stage 4), they were weighed (0.01 g) and one ovarian lobe cut longitudinally and the oocytes were 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 batch fecundity analysis.

**Table 1.** Macroscopic stages of development for female King George Whiting as derived and modified from Fowler et al. 1999

| Stage | Macroscopic appearance | Microscopic characteristics | |
|---|---|---|---|
| **1** Immature | Ovaries small, undeveloped, clear, jelly-like or glassy, grey-pink | Only unyolked and non-atretic oocytes |  |
| **2** Developing | Ovaries small, opaque, light yellow in colour; individual oocytes not discernible | Mainly unyolked and a few partially yolked oocytes, with no major atresia |  |
| **3** Developed | Ovaries relatively large and quite turgid, yellow-orange; individual oocytes discernible | Oocytes at several phases: unyolked, partially yolked, but dominated by advanced. Maybe some minor atresia of advanced yolked oocytes |  |
| **4** Gravid or running ripe | Ovaries large, orange, clear hydrated oocytes visible among opaque oocytes. Oocytes may be ovulated | Oocytes present at all stages from unyolked to hydrated. Some atretic oocytes and post-ovulatory follicles may be present, but generally dominated by advanced yolk and hydrated oocytes. |  |
| **5** Regressing or resting | Varies small to medium, mustard yellow/orange/reddish. More flaccid than previous stages, and with granular appearance | Oocytes of all stages may be present; however, there is a high incidence of atresia suggesting the end of spawning. Post-ovulatory follicles not found |  |

### *Female weight ( $p_w^N$ )*

To account for variations in female body weight that are not normally distributed, adult samples were grouped into eight weight classes ranging from <100 – 800 grams. The proportion of fish in each weight bin was then included as an input into the size-based spawning biomass equation as described in McGarvey et al. (in review). A multinomial error distribution was applied to determine the uncertainty for the proportion of fish in each weight bin (McGarvey et al. in review).

### *Batch Fecundity ($F_w$)*

Formalin preserved oocyte samples taken 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 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 1:

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

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 using an allometric function with residual error that increases with $Wt$. and used to estimate the batch fecundities of mature females in all samples. The allometric function for fecundity against weight was taken as a continuous variable.

$$\hat{F}(W) = \alpha \cdot W^{\beta}. \qquad \text{[Equation 2]}$$

where $\alpha$ and $\beta$ are allometric coefficients.

A maximum likelihood estimator that accounted for heteroscedasticity in the spread of the residuals was used in the model fit to estimate the parameters $\alpha$ and $\beta$ (McGarvey et al. in review). Weight-dependent batch fecundity estimates were calculated for the mid-point of each weight bin using this allometric relationship with normally distributed error

$$F_w = \alpha \cdot \breve{w}_w^{\beta}.$$

These are also included into the size-dependent estimation of spawning biomass (McGarvey et al. in review)

### *Sex ratio (R)*

Sex ratio was determined using two datasets: 1) fish sampled during DEPM surveys, and 2) using fish collected from SAFCOL fish markets as part of a continuing sampling program performed by SARDI. SAFCOL data only included fish that were caught during April in 2017 and 2018 to align with the timing of the egg surveys. Two datasets were used to ensure that biased sex ratios by weight did not occur during DEPM surveys where more females by number were caught in a short sampling period.

Quantities of mature (Gonad stage > 2) males and females in each sample were used to estimate the sex ratio $(\bar{R}_i)$ according to Equation 3:

$$\bar{R}_i = \frac{F_i}{(F_i + M_i)}. \qquad \text{[Equation 3]}$$

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 4:

$$R = \sum \left[ \bar{R}_i \times \frac{n_i}{N} \right]. \qquad \text{[Equation 4]}$$

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 (McGarvey et al in review).

### *Spawning fraction (S)*

The estimates of spawning fraction (*S*) should be calculated as the mean proportion of females that were in spawning condition during the survey period. This should be determined using post-ovarian follicles (POFs) which demonstrate whether a female fish has spawned on a given day. However, due to issues with adult sampling, histology could not be performed on available gonads to determine whether POFs were present in sampled fish. To facilitate the development of DEPM techniques, a range of spawning fraction sensitivities was further tested based on the results of Fowler et al. (1999) who demonstrated that as much as 100% of fish could be spawning in individual samples but the likely spawning frequency was over 2 – 3.6 days. This equates to a range of spawning fractions of 0.28 – 1.

In future applications of the DEPM, the mean spawning fraction of the population should be calculated from the average of the sample means weighted by the proportional sample size according to Equation 5.

$$S = \sum \left[ \overline{S}_i \times \frac{n_i}{N} \right]. \qquad \text{[Equation 5]}$$

Where, $\overline{S}_i$ is the mean spawning fraction of each sample (number of spawning female fish divided the total number of mature females), *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 (McGarvey et al., 2018).

## Ichthyoplankton Surveys

### *Sampling location*

Two DEPM surveys were undertaken from *RV Ngerin*, one each in April 2017 and 2018. Each survey was conducted to align with the peak of spawning (late April) for King George Whiting in southern Gulf waters and Investigator Strait (Fowler et al., 1999). Sampling was undertaken prior to the recently established seasonal closure for King George Whiting (1 – 31 May). The focal survey area extended from Wardang Island across to the Sir Joseph Banks Group and southern Spencer Gulf, through Investigator Strait and north to Tapley Shoal in southern Gulf St. Vincent (Figure 2). The first survey was carried out from 26 April – 1 May 2017 and consisted of 126 stations. The stations were spaced in a 2 x 4 nm grid pattern where possible. Station 80 was included to sample Orcades Bank Sanctuary Zone, which is a marine park implemented in 2014 to protect key King George Whiting habitat. The second survey was carried out from 24 – 28 April 2018 and replicated the 126 stations sampled in 2017. Oceanographic profiles were recorded at 62 predetermined stations in both 2017 and 2018 using a SBE 19plus V2 SeaCAT Profiler CTD™ (conductivity-temperature-depth) recorder (Figure 2). The CTD recorder was lowered to 1 m subsurface and allowed to pump water for three minutes, and it was then deployed vertically to within 5 m of the bottom depth of the station.

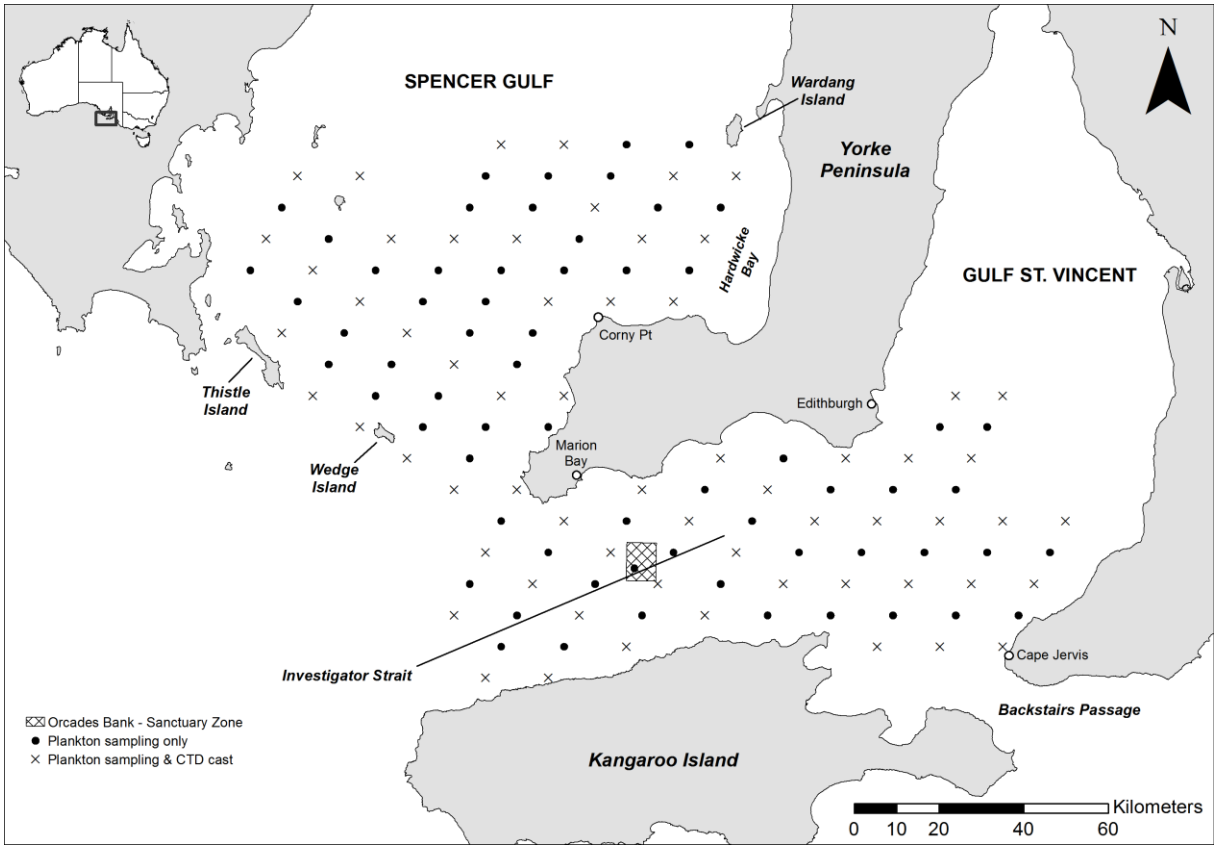



**Figure 2.** Plankton sampling map for the King George Whiting DEPM surveys in both 2017 and 2018.

### *Plankton sampling*

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 obliquely deployed and retrieved to within 5 m of the seabed and at a speed of ~1 m.s$^{-1}$. Each net was fitted with a General Oceanics ™ 2030 flow-meter, 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 meter using either a digital counter (General Oceanics) or marked rope mainline. The desired maximum depth was based on a 45° calculation and validated with a Sensus ultra ™ depth and temperature logger. Where there were discrepancies of >500 propeller rotations between paired flow-meter readings, the reading was considered erroneous, and the relationship between wire length released and flow-meter reading was used. The correction for flow meter discrepancy occurred 23 times over both surveys. Upon retrieval of the nets, they were washed down, and the plankton samples were rinsed from the two cod-ends and combined into a 1 L sample container. All samples were preserved in 95% ethanol and refrigerated at 4°C prior to sorting.

Plankton samples were sorted using a modified Sedgwick-Rafter sorting tray under a stereo dissecting microscope. All teleost eggs and larvae were removed from each plankton sample, eggs were separated

into two categories: ‘possible’ and ‘unlikely’ King George Whiting eggs. The main diagnostic features used to classify ‘possible’ King George Whiting eggs were described in Fowler (2000). King George Whiting eggs were spherical (size range 0.84 – 0.93 mm) and had a smooth chorion; narrow perivitelline space; a single oil globule that is approximately 30% of egg diameter (size range 0.22 – 0.34 mm), which was located opposite to the blastodisc or developing larvae; yolk that is unsegmented and yellowish in colour (Fowler, 2000). During the mid and later stages of development pigmentation spots on the oil globule appeared, and the embryo develops as long and thin with pigment spots distributed dorsally and laterally (Fowler, 2000, Ham and Hutchinson, 2003). All eggs and larvae were retained in fresh 95% ethanol and refrigerated at 4°C prior to species validation through molecular analysis.

## King George Whiting egg validation

To discriminate King George Whiting eggs from mixed species environmental samples, a molecular ISH approach developed earlier for Snapper by Oxley et al. (2017) was implemented. This technique uses a horseradish peroxidase (HRP) enzyme conjugated oligonucleotide probe that binds specifically to the target species’ mitochondrial 16S ribosomal RNA and generates a blue colour through oxidisation with a HRP reactive substrate (namely 3,3,5,5 tetramethylbenzidine, TMB) (Figure 3). As validated for use in an earlier DEPM program for Snapper (Steer et al., 2017), this technique requires the mechanical piercing of the chorion of each egg to expose the internal embryonic tissue for allowing the effective binding of the molecular probe. Although the structural integrity of the eggs is compromised, the developmental stage of the eggs can still be assigned.

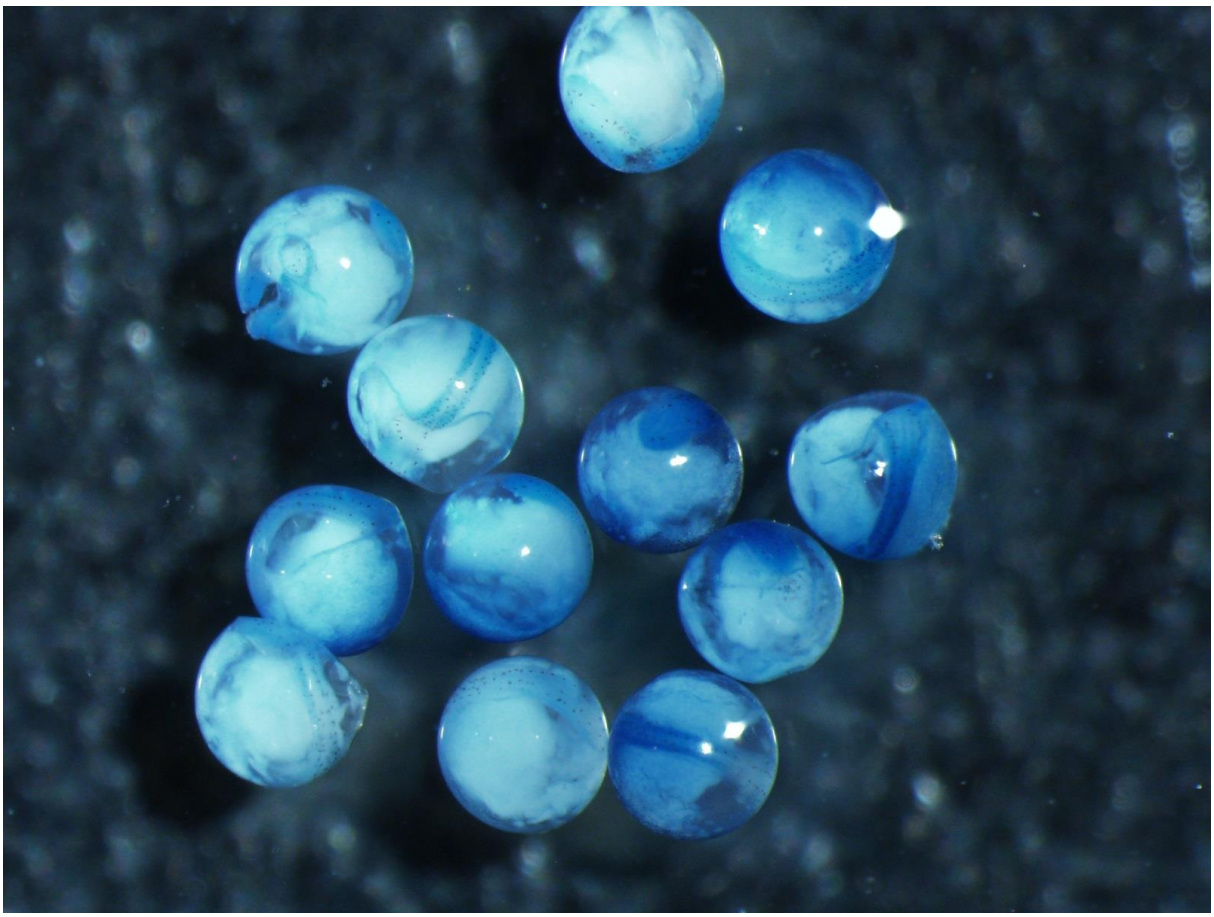


**Figure 3.** Validated King George Whiting eggs post *in-situ* hybridisation technique sampled during the 2017 DEPM survey.

In developing this technique for King George Whiting, four candidate molecular probes were initially designed against sequences from closely related co-spawning and/or co-occurring species (Figure 4). Of these, three probes (i.e. KGW_5P391, 3P61 and 3P395) were selected for further evaluation based on their overall molecular structural ratings and numbers of nucleotide mismatches with non-target species (as an indicator of their likelihood of producing false-positives through the cross-reaction of non-target species). The universal *Eukarya* probe (EUK516) (Amann et al. 1990) was used as a positive control for the reactions and the nonsense probe (NonEUB338) (Wallner et al. 1993) as a negative control for nonspecific background hybridisation (data not shown). Specificity were assessed using larvae from King George Whiting (with 100% complementarity to the probe sequences as the target taxa), and Southern School Whiting – SSW (*Sillago bassensis*) larvae as the closest genetic representative with the smallest number of nucleotide mismatches (2-6 nt) to the target.

The three selected probes were subsequently assessed using fresh (ethanol-preserved) and taxonomically verified King George Whiting and Southern School Whiting larvae, as the target and most closely related co-spawning species respectively. Mean density of blue pixel density per larvae (%) was evaluated in

ImageJ 1.50i (http://imagej.nih.gov/ij) with a hue setting of 120–190. ISH sample images were captured on an Olympus SZX7 stereo microscope using default exposure settings. Evaluation of these probes over a range of stringencies from 0–30% (based on the % formamide added to the reactions, v/v) revealed that all probes were specific to King George Whiting, with no cross-reaction observed with Southern School Whiting at the lowest possible stringency (i.e. 0%) (Figure 5). Whilst this indicates that all of the designed probes could be used with a high-level of confidence (with no or minimal occurrence of false positives), probe 3 (KGW_3P61) had the greatest signal (based on the observed colour intensity) over a greater range of stringencies (i.e. from 0–10%). Given this, and the comparably smaller range of nucleotide (nt) mismatches with co-occurring and/or co-spawning non-target species (from 4–8 nt, see Figure 4), this probe was selected for use in assessing the mixed species egg samples at a stringency of 5%.

After species validation, the eggs were assigned to 1–10 developmental stages to align with the universal fish egg stages in Ward et al. 2018 (Table 2). The egg-stage based age relationship was derived from previous King George Whiting DEPM by Fowler (2000). A modified stage-age relationship was developed from the induced spawning temperature related egg development trials (Fowler et al., 1999, Ham and Hutchinson, 2003). The egg development stages in Fowler (2000) were adjusted to align with the 10 egg stage development series.

**Table 2**. The description of the ten 'universal' stages from Ward et al. (2018) used to describe King George Whiting embryo development in this study. * indicates the equivalent developmental egg stages for King George Whiting from Fowler (2000).

| ***Stage*** | ***** | ***Description*** |
|---|---|---|
| 1 | (1–2) | Cells ≤ 64 |
| 2 | (3–7) | Cells ≥ 64 |
| 3 | (8–9) | Blastoderm covers > ½ of yolk; no blastopore |
| 4 | (10) | Blastopore present; head distinct; tail undefined; optic vesicles begin to differentiate |
| 5 | (10–11) | Blastopore closed; optic cups form; somites appear |
| 6 | (11) | Embryo ~1/2 around yolk; tail bulbous & just beginning to separate from yolk in late stage |
| 7 | (12) | Embryo ~2/3 around yolk; tail fully separated from yolk and becomes pointed, tail still straight (no bend ('kink') in tail) |
| 8 | (13) | Embryo ≤ 3/4 around yolk, head structure and caudal fin fold becoming more defined, tail 'kinked' or bent at angle |
| 9 | (14) | Embryo ≥ 3/4 around yolk, head structure and caudal fin fold well developed, tail near snout |
| 10 | (15–16) | Embryo fully developed, tail near snout (almost touches or past snout), twisted off embryonic axis just prior to hatching |

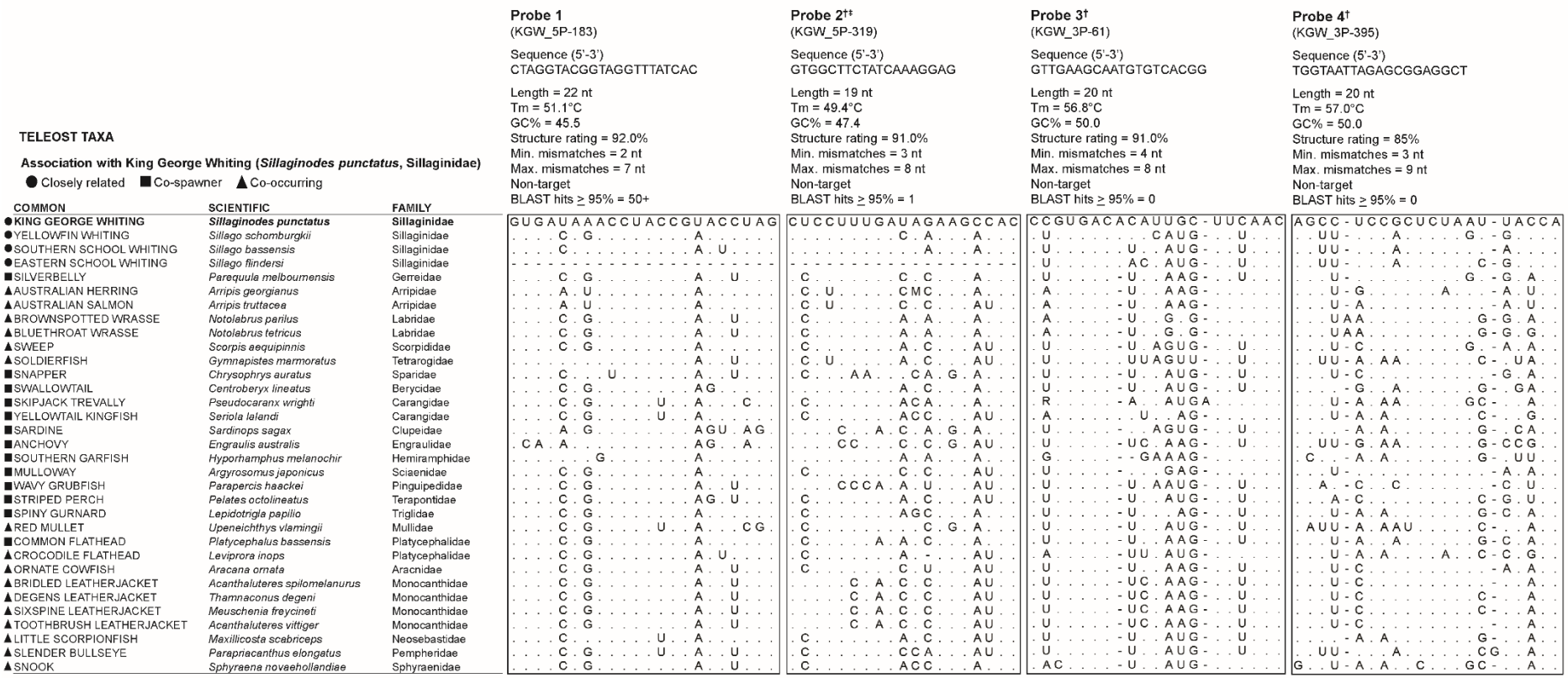



**Figure 4.** Oligonucleotide probe sequences specific for King George Whiting – KGW (*S. punctatus*) and their corresponding target sequence regions in King George Whiting and closely related, co-spawning and co-occurring non-target fish species. The structural characteristics and the numbers of nucleotide (nt) mismatches and positive BLAST hits with non-target taxa (≥ 95%) are indicated for each of the designed *in-situ* hybridisation (ISH) probes; †Probes 2, 3 and 4 were selected as candidates for experimental validation. ‡Occurs within the same region as the ISH probe designed and implemented for Snapper (Oxley et al. 2017).

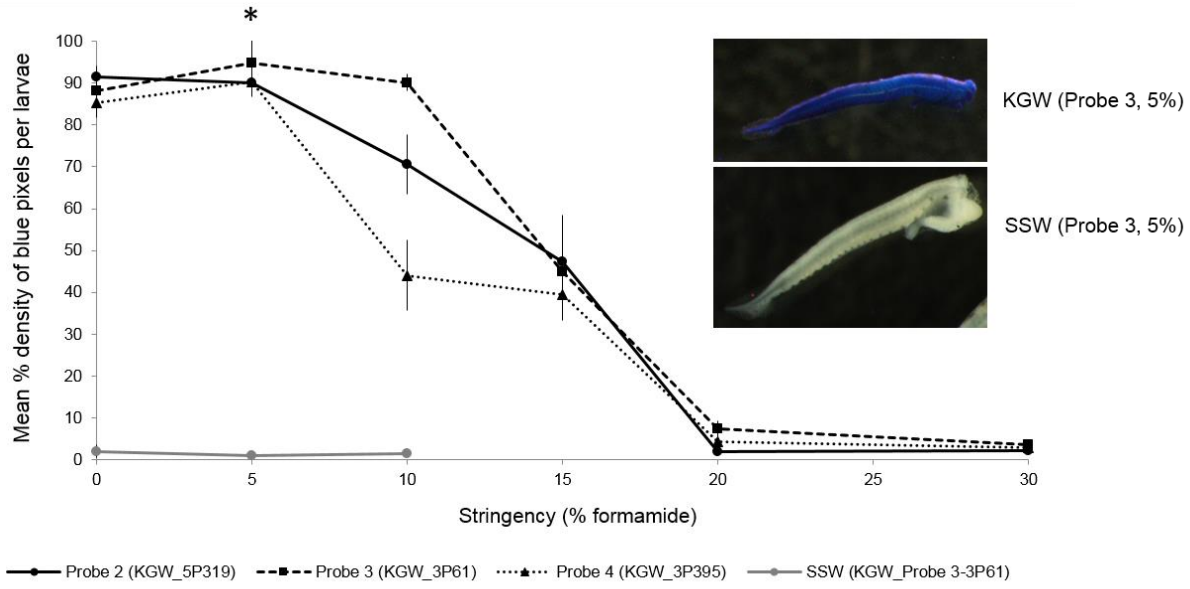



**Figure 5.** Evaluation of mean density of blue pixels per larvae (%) for each of the hybridisation stringency conditions (based on % formamide) and specificity of King George Whiting – KGW (*S. punctatus*) molecular probes 2 (KGW_5P391), 3 (KGW_3P61) and 4 (KGW_3P395). Southern School Whiting – SSW larvae was used as the closest genetic representative (SSW KGW_Probe 3). Probe 3 (KGW_3P61) was used in assessing the mixed species egg samples at the optimal stringency of 5% (*); representative ISH images of King George Whiting and Southern School Whiting larvae with Probe 3 are presented to the right of the graph.

## Daily Egg Production Method (DEPM)

### *Egg density (*$D_{t,s}$*)*

Egg density of stage *s* ($D_{t,s}$) under one square metre of water was estimated at each sample (i.e. station) *t* as

$$D_{t,s} = (1/net.area) * (1/distance_t) * N_{t,s}^{eggs} * depth_t \quad \text{[Equation 6]}$$

where $N_{t,s}^{eggs}$ is the number of eggs stage *s* in each sample *t*, *net.area* is the surface area of the next ($m^2$), $distance_t$ is the volume of water measured using the flowmeters ($m^3$) and $depth_t$ is the maximum depth (m) to which the net was deployed at sample *t*.

The spatial extent of egg densities was determined using geostatistical kriging. 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 spatial extent of spawning activity for each survey.

### *Estimates of spawning area (A)*

The Voronoi natural neighbour (VNN) method (McGarvey et al., 2018, 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 ($m^2$) of each polygon where eggs were present were summed to determine *A*.

### *Daily egg production ($P_0$)*

The timing of egg release for King George Whiting has been previously identified to occur during the afternoon, potentially in conjunction with the rising tide (Fowler et al., 1999). The stage-based egg density estimator developed by McGarvey et al (2018) was used to determine mean daily egg production. This method is an improved approach for demersal species rather than least squares regression approaches (Mosek and Lynn, 1996) applied for small pelagic species. This is due to the much lower egg densities that are spawned by demersal fish. The advantage of this approach is that egg mortality (*Z*) is specified *a priori* rather than estimated (McGarvey et al., 2018). This benefits estimates of $P_0$ as *Z* can be difficult to estimate when egg densities are naturally low. However, estimates of $P_0$ are not strongly influenced by different values of *Z* in relation to parameter uncertainties introduced in the DEPM equation (for example spawning fraction *S*). To avoid biasing $P_0$ estimates, a range of *a priori* *Z*'s were applied, ranging from 0.2 $day^{-1}$ to 0.6 $day^{-1}$ with a mid-point of 0.4 $day^{-1}$ used in further analyses. This *Z* range is further included in sensitivity analyses that includes all DEPM parameters.

Using *a priori* estimates of *Z*, $P_0$ (mean daily egg production) was calculated as

$$P_0 = \frac{1}{n_t} \sum_{t=1}^{n_t} * \sum_s D_{t,s} * e(Z * Age_{t,s}) / H_{t,s} \qquad \text{[Equation 7]}$$

where $n_t$ was the number of tows, the age in days of each stage *s* in sample *t* is calculated as

$$Age_{t,s} = \frac{9.82 * e(-0.11 * Temp_t) * S^{1.35}}{24} \quad \text{[Equation 8]}$$

where $Temp_t$ is the measured temperature of each sample *t* (°C) and *S* is the stage number. Hatching time in days for stage *s* in each sample *t* ($H_{t,s}$) is calculated as

$$H_{t,s} = Age_{t,10} + (Age_{t,10} - Age_{t,9}) / 2 \quad \text{[Equation 9].}$$

The standard error of $P_0$ ($\sigma_{P_0}$)was estimated as

$$\sigma_{P_0} = \sqrt{\text{var}(\sum_s D_{t,s} * e(Z * Age_{t,s}) / H_{t,s}) / \frac{1}{n_t}} \quad \text{[Equation 10]}$$

### *Application of the size-based spawning biomass estimator (DEPMWt)*

A size-based spawning biomass estimator was applied for King George Whiting which provides three outputs: 1) spawning biomass that are more precise than a traditional DEPM; 2) the number of spawning females; and 3) the number of spawning females in each weight bin. The DEPMWt approach uses the parameters described previously $\left\{P_0, A, S, R, \{p_w^{\mathrm{N}}\}, \{F_w\}\right\}$ as well as their associated errors. The estimated number of females ( $N^{\mathrm{fem}}$ ) were estimated as

$$N^{\mathrm{fem}} = \frac{P_0 \cdot A}{S \cdot \sum_{w=1}^{w} F_w \cdot p_w^{\mathrm{N}}} \quad \text{[Equation 11]}$$

The spawning biomass was estimated as

$$B = \left( \frac{P_0 \cdot A}{S \cdot R \cdot \sum_{w=1}^{\omega} F_w \cdot p_w^{\mathrm{N}}} \right) \cdot \sum_{w=1}^{\omega} p_w^{\mathrm{N}} \cdot \breve{w}_w \,. \quad \text{[Equation 12]}$$

Lastly, the number of females ( $N_w^{\mathrm{fem}}$ ) in each weight class was estimated as

$$N_w^{\mathrm{fem}} = N^{\mathrm{fem}} \cdot p_w^{\mathrm{N}}, \quad w = 1 \ldots \omega.$$

The variance of each of these quantities is estimated using delta approximation, where the overall variance of the spawning biomass estimate is written as

$$V(B) = \frac{1}{R^4 \cdot S^4 \left( \sum_{w=1}^{\omega} F_w \cdot p_w^{\mathrm{N}} \right)^4} \cdot \left\langle \begin{array}{l} \left[ P_0^2 \cdot R^2 \cdot S^2 \cdot V(A) + A^2 \cdot R^2 \cdot S^2 \cdot V(P_0) + A^2 \cdot P_0^2 \cdot S^2 \cdot V(R) + A^2 \cdot P_0^2 \cdot R^2 \cdot V(S) \right] \cdot \\ \left\{ \left( \sum_{w=1}^{\omega} F_w \cdot p_w^{\mathrm{N}} \right)^2 \cdot \left( \sum_{w=1}^{\omega} p_w^{\mathrm{N}} \cdot \breve{w}_w \right)^2 \right\} + \\ A^2 \cdot P_0^2 \cdot R^2 \cdot S^2 \cdot \left\{ \left( \sum_{w=1}^{\omega} V(F_w) \cdot \left( p_w^{\mathrm{N}} \right)^2 \right) \cdot \left( \sum_{w=1}^{\omega} p_w^{\mathrm{N}} \cdot \breve{w}_w \right)^2 \right\} + \\ A^2 \cdot P_0^2 \cdot R^2 \cdot S^2 \cdot \left\{ \sum_{i=1}^{\omega} \left[ V(p_i^{\mathrm{N}}) \cdot \left( \breve{w}_i \cdot \sum_{w \neq i}^{\omega} F_w \cdot p_w^{\mathrm{N}} - F_i \cdot \sum_{w \neq i}^{\omega} p_w^{\mathrm{N}} \cdot \breve{w}_w \right)^2 \right] \right\} \end{array} \right\rangle \quad \text{. [Equation 13]}$$

In similar fashion, variance formulas for the estimates of $N^{\text{fem}}$ and $N_w^{\text{fem}}$ were derived and are given as follows:

$$V(N^{\text{fem}}) = \frac{1}{S^4\left(\sum_{w=1}^{\omega} F_w \cdot p_w^{\text{N}}\right)^4} \cdot \left\{ \begin{array}{l} \left[V(P_0)\cdot A^2 \cdot S^2 + P_0^2 \cdot V(A)\cdot S^2 + P_0^2 \cdot A^2 \cdot V(S)\right]\cdot\left(\sum_{w=1}^{\omega} F_w \cdot p_w^{\text{N}}\right)^2 + \\ P_0^2 \cdot A^2 \cdot S^2 \cdot \left[\left(\sum_{w=1}^{\omega} V(F_w)\cdot\left(p_w^{\text{N}}\right)^2\right) + \left(\sum_{w=1}^{\omega} V(p_w^{\text{N}})\cdot\left(F_w\right)^2\right)\right] \end{array} \right\} \quad \text{[Equation 14]}$$

$$V(N_w^{\text{fem}}) = \frac{1}{S^4\left(\sum_{w'=1}^{\omega} F_{w'} \cdot p_{w'}^{\text{N}}\right)^4} \cdot \left\{ \begin{array}{l} \left(p_w^{\text{N}}\right)^2\left[V(P_0)\cdot A^2 \cdot S^2 + P_0^2 \cdot V(A)\cdot S^2 + P_0^2 \cdot A^2 \cdot V(S)\right]\cdot\left(\sum_{w'=1}^{\omega} F_{w'} \cdot p_{w'}^{\text{N}}\right)^2 + \\ P_0^2 \cdot A^2 \cdot S^2 \cdot \left[V(F_w)\cdot\left(p_w^{\text{N}}\right)^4 + V(p_w^{\text{N}})\cdot\left(\sum_{w'\neq w}^{\omega} F_{w'} \cdot p_{w'}^{\text{N}}\right)^2\right] + \\ P_0^2 \cdot A^2 \cdot S^2 \cdot \left[\left(p_w^{\text{N}}\right)^2\left\langle\left(\sum_{w'\neq w}^{\omega} V(F_{w'})\cdot\left(p_{w'}^{\text{N}}\right)^2\right) + \left(\sum_{w'\neq w}^{\omega} V(p_{w'}^{\text{N}})\cdot\left(F_{w'}\right)^2\right)\right\rangle\right] \end{array} \right\} \quad \text{.[Equation 15]}$$

Full details of how these equations were derived and specifics of variance estimation for individual parameters are available in McGarvey et al. (in review). The estimation formulas for $B$, $N^{\text{fem}}$, and $N_w^{\text{fem}}$, and their estimate variances are coded as functions in the new R package 'DEPM', downloadable from https://github.com/jonathansmart/DEPM. The repository contains the current executable 'DEPM' package, as well as descriptive vignettes. This package also includes functions that implement the stage-based estimation method for $P_0$ (McGarvey et al., 2018).

These quantities were determined for the entire survey area (both gulfs) for 2017 and 2018. However, due to adult sampling limitations, sex ratio was estimated using data for both years combined. A range of spawning fractions were included based on Fowler et al. (1999).

### *Visual comparison to standard DEPM approach*

To demonstrate the increased precision attained through the DEPMWt approach, the standard DEPM methods using the Parker equation (Piquelle and Stauffer 1985) was also applied:

$$B = \frac{P_0 \cdot A}{R \cdot F \cdot S / W}.$$ [Equation 16]

The number of females ( $N^{\text{fem}}$ ) was estimated as

$$N^{\text{fem}} = P_0 \cdot A / (S \cdot F)$$ [Equation 17]

where the same values for $P_0$, $A$, $R$ and $S$ were applied and $F$ and $W$ were estimated as the mean fecundity and total weight of females, respectively. The variance of $B$ was estimated as

$$V(B) = B^2 \cdot \left( \frac{V(P_0)}{P_0^2} + \frac{V(R)}{R^2} + \frac{V(S)}{S^2} + \frac{V(\bar{W})}{\bar{W}^2} + \frac{V(\bar{F})}{\bar{F}^2} \right)$$ [Equation 18]

and the variance of $N^{\text{fem}}$ was estimated as

$$V\left(N^{\text{fem}}\right) = \left(N^{\text{fem}}\right)^2 \cdot \left( \frac{V(P_0)}{P_0^2} + \frac{V(S)}{S^2} + \frac{V(\bar{F})}{\bar{F}^2} \right).$$ [Equation 19]

#### *Sensitivity of biomass estimates to individual parameter uncertainty*

Several DEPM parameters can often have some level of uncertainty around them and the consequences of these uncertainties need to be considered with regards to final estimates of spawning biomass. As part of a sensitivity analysis, different values of $P_0$, $A$, $R$ and $S$ were included in the DEPMWt biomass estimator. This was performed for one of these parameters at a time while holding all other parameters at their estimated value. The range of sensitivities for $P_0$ were based on varying the *a priori* value of $Z$ in its estimation. The range of $A$ extended from 6000 – 14000 km$^2$. The range of $R$ extended from 0.45 – 0.7 which accounts for females growing to larger sizes than males. Therefore, the sex ratio by weight is likely to be larger than 0.5. The range of $S$ extended from 0.28 – 1 based on the historical sampling of Fowler et al. (1999).

## Source to sink connectivity

### Bio-physical models

#### *Regional oceanographic dynamics*

Spencer Gulf and Gulf St. Vincent are south facing, shallow inverse estuaries which have a positive salinity gradient from mouth to head (35 – 50 ppt), and moderate fluctuations of seasonal water temperatures (~12 – 24 °C).

During the austral summer, temperature and salinity increase in both gulfs which leads to the formation of thermohaline frontal systems at the entrance to Spencer Gulf and in Investigator Strait. The fronts act as environmental barriers that impede water exchange, and subsequent plankton transport, between the gulfs and the continental shelf throughout the austral summer and autumn. Water temperatures in the gulfs decrease in late autumn and early winter, which leads to low density shelf water being drawn into southern Spencer Gulf and Investigator Strait, and the resumption of shelf/gulf exchange. The seasonality of the frontal systems coincides with the peak spawning period for King George Whiting in South Australia.

### *Bio-physical model description*

The larval transport model coupled a hydrodynamic model to an offline Lagrangian particle tracking model to simulate the dispersal of larval King George Whiting from the DEPM survey station locations. Parameters of the hydrodynamic model were derived from the physical oceanographic characteristics of Spencer Gulf, Gulf St. Vincent and Investigator Strait. The particle tracking model simulated larval dispersal which included behaviour and settlement parameters that were developed from published biological data.

### *Hydrodynamic model*

Ocean circulation within the study area was simulated using the Regional Ocean Modelling System (ROMS). ROMS is a high resolution, three-dimensional, free-surface oceanic model that uses topography-following coordinates in the vertical direction, and orthogonal curvilinear coordinates in the horizontal direction. The ROMS developed for this region corresponds with the Two Gulfs Model (TGM) available through eSA-Marine (https://pir.sa.gov.au/research/esa_marine). The model resolution is 1,500 m in the horizontal with 15 sigma levels in the vertical, which is run at a 200 s time step to solve for the tidal currents that dominate the gulfs. Conditions for temperature, salinity, sea level and currents at the open ocean boundaries were prescribed daily by the Bluelink Reanalysis. The model was forced by atmospheric data including pressure, wind, heating and evaporation at a sub-daily time step provided by the NCEP Climate Forecast System Reanalysis v. 2 , and tidal forcing was provided by TPXO8 (Egbert and Erofeeva, 2014). Predictions of the TGM were compared against the measured sea level height, tidal current velocity and residual current velocity at a buoy (SAM8SG) of the South Australian Integrated Marine Observing System (SAIMOS). For comparison, tidal currents were resolved along a principal axis (i.e. major current direction). Residual currents, driven by short-term (3-10 d) weather events, were resolved along a principal axis after removing the tidal signal. To validate the hydrodynamic model we compared the tidal amplitude, phase, and current velocity predicted by the model to recorded observations at four locations in Spencer Gulf, Investigator Strait, and Gulf St. Vincent.

### *Particle tracking model*

Particle tracking was undertaken using the larval transport model (LTRANS). LTRANS uses outputs from the ROMS hydrodynamic model to track the trajectories of particles in three-dimensions, accounting for particle advection, vertical turbulent particle motion, reflective boundary conditions, larval swimming behaviour, and settlement. Hourly outputs (external time step) from the TGM were used to run LTRANS with an internal time step of 10 minutes. The influence of sub-grid scale turbulence on particle movement was simulated using a random displacement model with horizontal diffusion equivalent to 1 $m^{-2}$ $s^{-1}$. Boundary conditions were imposed on particle trajectories at each internal time step of the larval transport model. Particles that intersected a land boundary due to advection or turbulence were reflected at an angle equal to the angle of approach, and at an equal distance to which the particle had originally passed the boundary. Particles that passed through a vertical boundary due to behaviour were held just above or below the bottom and surface boundary, respectively. Particles that intersected an open ocean boundary were considered out of bounds and removed from further simulations.

To reflect the spatiotemporal variations in King George Whiting egg densities between sampling years, the number of particles released at each station was the estimated King George Whiting egg density multiplied by 100. To assess the model predicted spawning success, the seeding locations were grouped into 13 larger sub-regions, which was comprised of 9 – 11 stations.

### *Settlement areas*

Settlement areas for King George Whiting were identified by the presence of post-settled larvae found during intermittent recruitment surveys which have been conducted since 1977. Settled larvae have been found at 25 sites sampled within the boundaries of the hydrodynamic model (Figure 6). These settlement areas are commonly characterised by shallow (<5 m) intertidal *Zostera spp*. seagrass beds. The 25 settlement areas were divided into six regions based on their geographic locations: SWSG – south-west Spencer Gulf; NSG – northern Spencer Gulf; SESG – south-east Spencer Gulf; IS – Investigator Strait; GSV – Gulf St. Vincent; and KI – Kangaroo Island (Figure 6; Table 3).

**Table 3**. Post-settled larval King George Whiting survey sites located throughout the gulf and inshore coastal waters of South Australia. Locations are depicted in figure 6. Region: NSG = northern Spencer Gulf; SESG = south-east Spencer Gulf; IS = Investigator Strait; south-west Spencer Gulf GSV = Gulf St. Vincent; KI = Kangaroo Island.

| Site # | Site name | Region |
|---|---|---|
| 1 | Blanche Harbour | NSG |
| 2 | Chinamans Creek | NSG |
| 3 | Yatala Harbour | NSG |
| 4 | Port Pirie Creek | NSG |
| 5 | Port Davis Creek | NSG |
| 6 | Cowleds Landing | NSG |
| 7 | Fishermans Bay | NSG |
| 8 | Franklin Harbour | NSG |
| 9 | Tumby Bay | SESG |
| 10 | Boston Bay | SESG |
| 11 | Proper Bay | SESG |
| 12 | Port Davenport | IS |
| 13 | Hardwicke Bay | SWSG |
| 14 | Port Victoria | SWSG |
| 15 | Coobowie | GSV |
| 16 | Stansbury | GSV |
| 17 | Port Vincent | GSV |
| 18 | Pine Point | GSV |
| 19 | Ardrossan | GSV |
| 20 | Price Creek | GSV |
| 21 | Port Wakefield Creek | GSV |
| 22 | Barker Inlet | GSV |
| 23 | Bay Shoals | KI |
| 24 | Brownlow | KI |
| 25 | American River | KI |

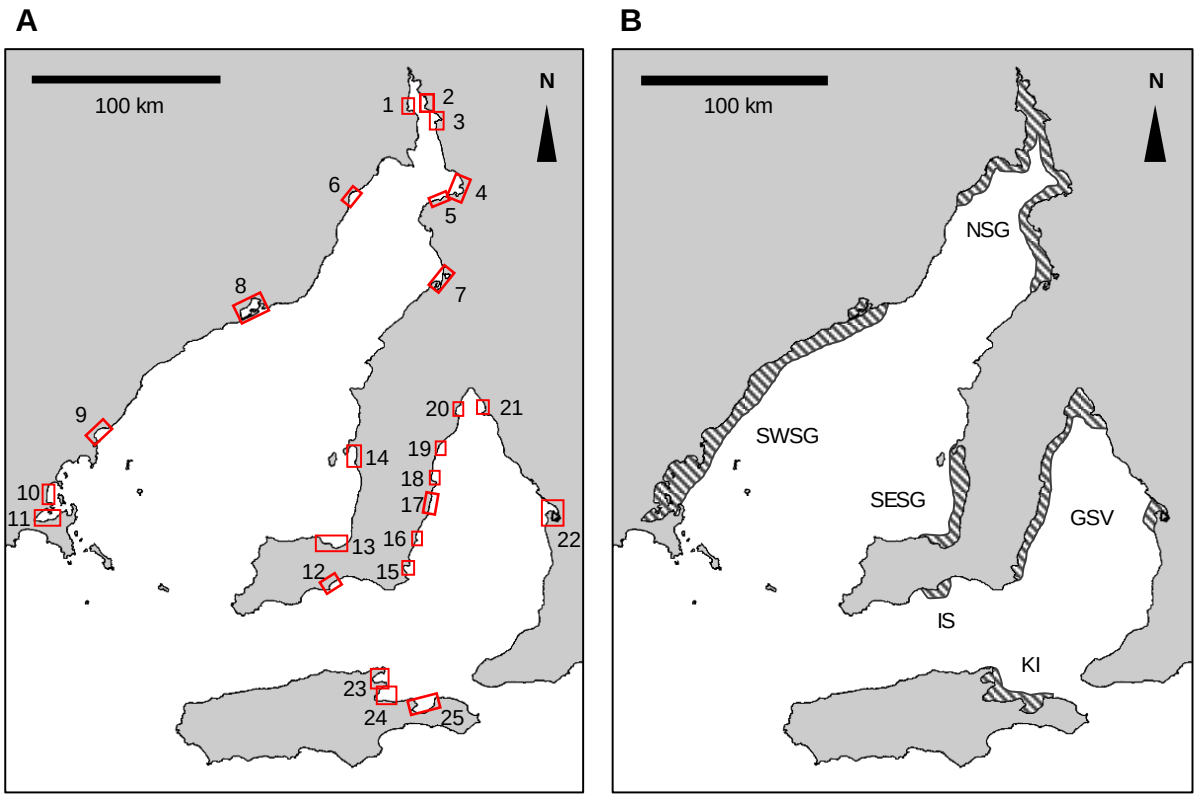



**Figure 6**. (A) Locations of King George Whiting settlement areas (1–25) incorporated into the larval transport model. Settlement areas were included based on the presence of recently-settled larvae from recruitment surveys since 1977. Station names are in Table 3. (B) Dashed areas show the broader settlement regions. SWSG = south-west Spencer Gulf; NSG = northern Spencer Gulf; SESG = south-east Spencer Gulf; IS = Investigator Strait; GSV = Gulf St. Vincent; and KI = Kangaroo Island

### *Larval development and behaviour*

King George Whiting eggs and newly hatched larvae are buoyant and remain at or near the surface during embryonic development and, therefore, were modelled as passive particles that remain in the surface layer (0 – 5 m) (Table 4). The duration of the egg phase was 2 days based on the mean time until hatching of reared eggs at 19 °C, which best reflected the water temperature at the time of collection (range 17.8 – 19.9 °C). Newly hatched larvae (2.1 mm SL) remain in the surface layer because they each carry a buoyant yolk sac and have no discernible swimming ability. After 5 to 8 days, yolk-sac absorption is complete and the swim bladder begins to develop as the fish enter the flexion stage.

During the flexion stage (24 days post hatch (dph)), larvae range in size from 5.5 to 6.5 mm SL, have a functioning swim bladder, and begin to develop caudal, pectoral and anal fins. Reared larvae begin vertical movement at this stage, and field observations of inflated swim bladders for larvae collected at night indicate that they undergo diurnal vertical migration (DVM). During the flexion stage, larvae were modelled as having DVM behaviour at 1 cm $s^{-1}$ and 92 dph (Table 4). The DVM scheme assumed that the larvae swim down when the light level exceeded a threshold of 0.0166 E $m^{-2}$ $s^{-1}$. To determine the

light level, the model first calculated the surface irradiance using estimates of day length, or the time since the sun started to rise, and irradiance at solar noon. Irradiance at the depth of the particle location was then calculated using the surface irradiance and the attenuation coefficient.

From the flexion stage onwards, larval movement was simulated to include biological inputs (i.e. larval stage duration and behaviour) on the modelled projections. Larval behaviour was directly related to length-at-age (Table 4), which was determined from the estimated growth rates of recently-settled larvae that recruited to Barker Inlet, a significant nursery area in Gulf St. Vincent, in 2017. Only larvae that hatched at the same time as the egg survey (late April) were considered for growth rate calculations ($n$ = 25). These larvae were captured on 28 July and 12 August 2017 and ranged in length from 17.7 to 20.0 mm SL and in age from 92 to 117 days.

The 'average growth rate' (mm day$^{-1}$) provided an estimate of mean daily growth during larval development, and was calculated as:

$$\frac{L_c - L_o}{a} \qquad \text{Equation 7}$$

Where $L_c$ is length at capture, $L_o$ is length at hatch (2.1 mm; Bruce, 1995), and $a$ is age (days). The mean average growth rate was 0.15 mm day$^{-1}$ (± 0.01), which was used to estimate length-at-age. The model was initialised at midnight of 1 May and ran until the modelled larvae were > 20.5 mm SL (approximately mid-September). The model used the average growth rate of recently-settled larvae to estimate length-at-age and the duration of each larval stage.

Larval King George Whiting experience a prolonged post-flexion stage before they are competent to settle. During the post-flexion stage, larvae range in size from 6.5 to 15.0 mm SL and were modelled to have DVM behaviour at 2 cm s$^{-1}$ (Table 4). The caudal, pectoral, anal and dorsal fins are almost fully developed at 15.0 mm SL.

Larvae were able to settle to the pre-defined settlement areas during the settlement stage (15.0–20.5 mm SL) (Table 3) for 39 day (92–131 dph). Larvae were considered competent to settle at > 15 mm SL because this is the smallest size that settled larvae have been collected from nursery areas. Similarly, very few pre-settlement larvae > 20.5 mm SL have been captured. Larvae exhibit reverse diurnal vertical migration (RDVM) behaviour during the settlement stage once in the vicinity of settlement grounds. They remain near the surface during the day and are distributed throughout the water column at night. Larvae were modelled as having RDVM behaviour during the settlement stage when the water depth was < 10 m. If the depth was > 10 m, larvae continued standard DVM behaviour. The model assessed the locations of larvae at each internal time-step during the settlement stage. If a larva was within the boundaries of a settlement polygon, it was considered settled and stopped moving. If a larva was outside

of the boundaries of a settlement polygon, it did not settle during the settlement stage and was considered dead.

**Table 4.** Development and behavioural characteristics of King George Whiting larvae incorporated into the bio-physical model. DVM: diurnal vertical migration; RDVM: reverse diurnal vertical migration. Summary of larval behaviour showing the predicted growth rate (mm $days^{-1}$), age (dph) and duration (days) of each development stage.

| Stage | Egg | Hatchling | Pre-flexion | Flexion | Post-flexion | Settlement |
|---|---|---|---|---|---|---|
| Size (mm) | 0.8 | 2.1-3.0 | 3.0-5.5 | 5.5-6.5 | 6.5-15.0 | 15.0-20.5 |
| Behaviour | Buoyant (float) | Buoyant (float) | Buoyant (float) | DVM | DVM | DVM |
| | Surface (0-5 m) | Surface (0-5 m) | Surface (0-5 m) | Water column | Water column | RDVM (<10 m) |
| | Passive (drift) | Passive (drift) | Passive (drift) | Active | Active | Settle |
| Vertical movement | N/A | N/A | N/A | 1 cm $s^{-1}$ | 2 cm $s^{-1}$ | 2 cm $s^{-1}$ |
| Growth (mm $days^{-1}$) | - | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Age (dph) | - | 0-6 | 6-24 | 24-31 | 31-92 | 92-131 |
| Duration (days) | 2 | 6 | 18 | 7 | 61 | 39 |

## Management Strategy Considerations

The King George Whiting spawning spatial closure was enforced from May 1 to 31 in 2017, 2018 and 2019. The initial closure (2017 and 2018) went through a process of review, and subsequently, a refinement in 2018 by the management authority in South Australia for Fisheries and Aquaculture (PIRSA). The effectiveness of these closures was assessed by comparing the percentage of overlap of both the initial closure and the newer refined closure with the interpolated egg density distributions and the spawning areas (1 – 13) that contributed to greater than 10% settlement success as predicted by the bio-physical model for both years.

The geographic information system package 'ArcGIS' was used to calculate the size of the spawning area and the spawning blocks which contained >10 % settlement success inside and outside of both the new and existing closure. The spawning area used for this comparison was defined as all areas with a minimum egg density 0.1 $eggs.m^{-2}$ derived from the interpolated geostatistical kriging layer of egg density. The spawning areas with greater than 10% settlement success were derived from the outputs of the bio-physical model and were grouped into 13 sub-regions which comprised of 9 to 11 plankton survey stations per sub-region based on similar predicted larval trajectories.

# Results

## Adult King George Whiting samples

### *Adult collection*

A total of 923 adult King George Whiting were collected from all sectors in April of 2017 and 2018 (Table 5). The recreational fishing sector contributed 562 frames, the highest number of samples; 255 whole fish came from the commercial fishing sector; 106 were collected during scientific cruises. A large size range of adult fish were collected (210 – 533 mm TL), with 60% of fish sampled between 330 and 400 mm TL (Figure 6). The total weights for female King George Whiting ranged from 48.27 to 809.89 g, with a mean weight of 288.22 ± 5.88 g, and for males weight ranged from 48.27 to 660.68 g, with a mean weight of 233.10 ± 5.78 g.

**Table 5**. Summary of adult King George Whiting samples obtained during April 2017 and 2018 for each region and fishing sector. IS = Investigator Strait; KI = Kangaroo Island; NGSV = northern Gulf St. Vincent; NSG = northern Spencer Gulf; SGSV = southern Gulf St. Vincent; SSG = southern Spencer Gulf; WC = West Coast

| | 2017 | | | 2018 | | | Total n |
|---|---|---|---|---|---|---|---|
| **Region** | **Commercial** | **Recreational** | **Research** | **Commercial** | **Recreational** | **Research** | |
| IS | | 33 | | | 36 | | 69 |
| KI | | 56 | | | | 1 | 57 |
| NGSV | 4 | 106 | | | 10 | | 120 |
| NSG | 3 | 63 | | 213 | | | 279 |
| SGSV | | 18 | 53 | | 28 | | 99 |
| SSG | 16 | 175 | 30 | 19 | 34 | 22 | 296 |
| WC | | 3 | | | | | 3 |
| **Total** | **23** | **454** | **83** | **232** | **108** | **23** | **923** |

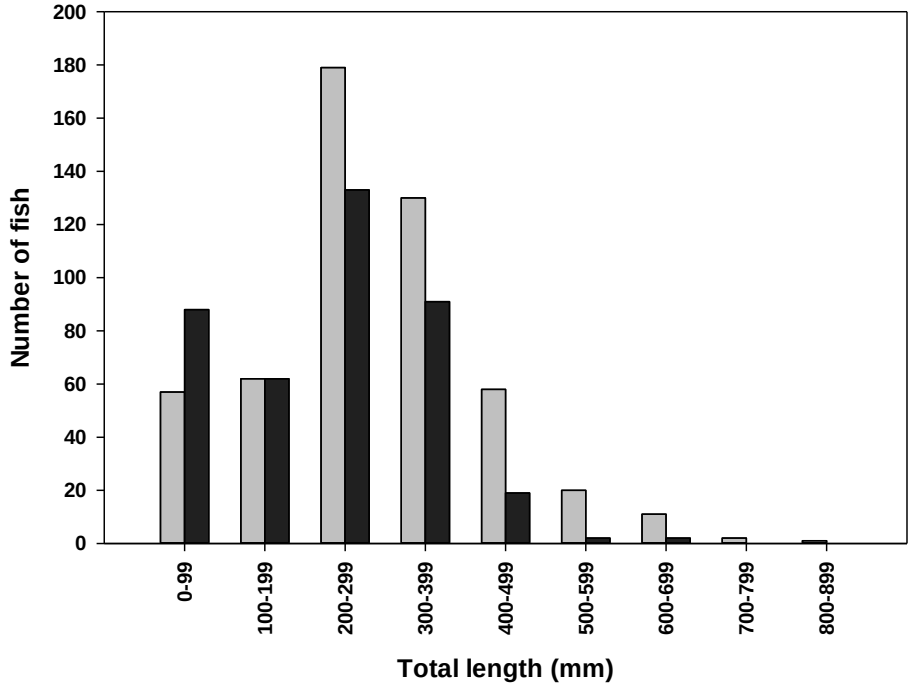



**Figure 7.** Length frequencies for all adult King George Whiting samples collected through 2017 (grey) and 2018 (black) from recreational, scientific and commercial fish sampling.

### *Sex ratio*

The sex ratio (female to male) by number for each region ranged from 0.46 – 0.71 with a mean of 0.58 ± 0.033 across all regions. The sex ratio of females by number was 0.60 ± 0.46 for the three regions (SGSV, IS and SSG) within the boundary of the plankton surveys (Table 6). The sex ratio by weight ranged from 0.49 – 0.76 per region and a mean of 0.62 ± 0.033 across all regions. Within the plankton survey area the sex ratio of females by weight was 0.64 ± 0.03 (Table 6).

As the sex ratio by number was skewed towards females for fish sampled as part of DEPM surveys the overall sex ratio by weight for surveyed fish was 0.64 (± 0.03 SE). To minimise the risk of this being an overestimation caused by more numerous females, sex ratio was also estimated in 2017 and 2018 using fish that were sampled at SAFCOL fish markets through ongoing SARDI sampling (n = 454). These data only included mature fish (Gonad stage > 1) collected during April in each year. The sex ratios by weight using these data were 0.57 (± 0.03 SE) in 2017 and 0.64 (± 0.03 SE) in 2018. A comparison of these results show that the sex ratio estimated by research surveys was appropriate for inclusion in the DEPMWt approach.

**Table 6.** Summary of adult King George Whiting sex ratio and spawning fraction results for 2017 and 2018 for each region and combined survey area. Total Wt. = grams; Female Wt. = grams; R count = ratio of females by number; R wt. = ratio of females by weight in grams ; IS = Investigator Strait; KI = Kangaroo Island; NGSV = northern Gulf St. Vincent; NSG = northern Spencer Gulf; SGSV = southern Gulf St. Vincent; SSG = southern Spencer Gulf; WC = West Coast.

| Region | Total Count | Count Females | Total Wt. | Female Wt. | R Count | R Wt. |
|---|---|---|---|---|---|---|
| IS | 68 | 48 | 28829.91 | 21847.15 | 0.71 | 0.76 |
| KI | 57 | 26 | 19195.90 | 9329.34 | 0.46 | 0.49 |
| NGSV | 120 | 72 | 32643.06 | 19638.57 | 0.60 | 0.60 |
| NSG | 283 | 146 | 41451.10 | 25727.43 | 0.52 | 0.62 |
| SGSV | 91 | 50 | 26778.81 | 15119.35 | 0.55 | 0.56 |
| SSG | 295 | 176 | 92781.06 | 57727.17 | 0.60 | 0.62 |
| WC | 3 | 2 | 735.68 | 484.60 | 0.67 | 0.66 |
| Survey area | 454 | 274 | 148389.78 | 94693.66 | 0.60 | 0.64 |

### *Spawning fraction (S)*

Spawning fraction could not be estimated from available adult data. Therefore, for the purposes of further sensitivity analysis, a 'base-case' estimate of 0.64 was used to estimate the uncertainty of remaining parameters. However, this is an assumed value representing the mid-point of the *S* range included in a sensitivity analysis. It should not be considered as a reliable value of spawning fraction beyond this analysis.

**Table 7.** Summary of adult King George Whiting parameters incorporating into the DEPM estimates for 2017 and 2018. $P_0$ = daily egg production; $P_0$ *SE* = daily egg production standard error; *Z* = egg mortality; *R* = sex ratio of females by weight; *R variance* = sex ratio variance; *A* = spawning area in $km^2$; *S* = assumed spawning fraction; *S variance* = spawning fraction variance.

| Year | $P_0$ | $P_0$ *SE* | *Z* | *R* | *R variance* | *A* | *S* | *S variance* |
|---|---|---|---|---|---|---|---|---|
| 2017 | 2.08 | 0.2075 | 0.4 | 0.6422 | 0.001568 | 8676 | 0.648 | 0.00596 |
| 2018 | 1.598 | 0.1843 | 0.4 | 0.6422 | 0.001568 | 10465 | 0.648 | 0.00596 |

### *Batch Fecundity*

The sample size of spawning stage 4 females collected during the scientific cruises in 2017 and 2018 were insufficient to accurately estimate batch fecundity (n = 3). Therefore, batch fecundity estimates were calculated from a combination of adult King George Whiting collected in this study and from Fowler et al. (1999). The combination of current and historical data resulted in batch fecundity estimates from 105 spawning King George Whiting. Batch fecundity estimates ranged from 5,250 to 152,190 eggs with a mean (and SE) of 58,870 ± 2,822.28 eggs (Figure 8). The relationship between batch fecundity and total fish weight was Fecundity = $42.13*W^{1.18}$.

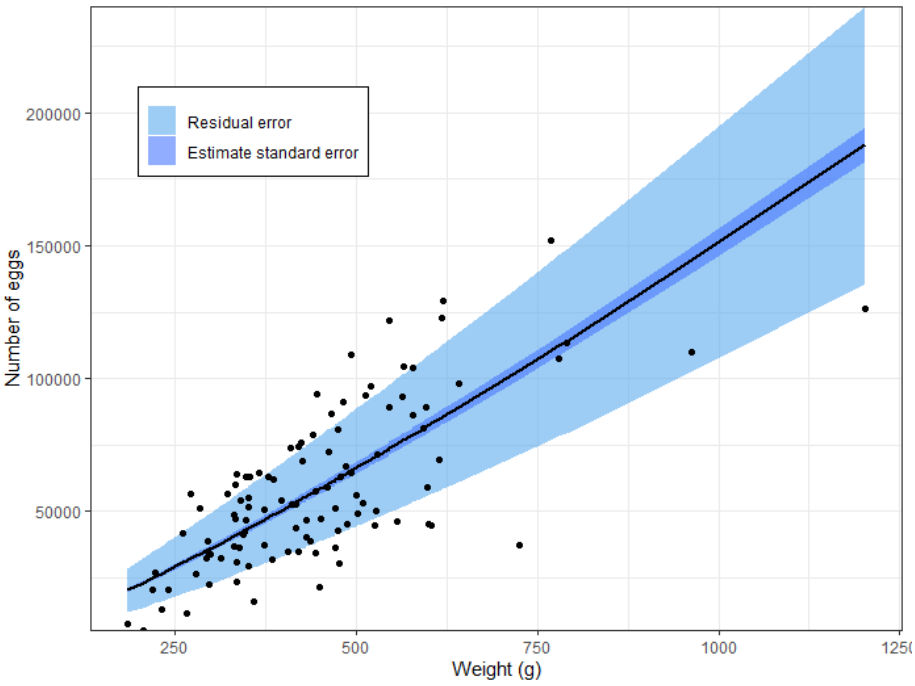



**Figure 8.** Relationships between batch fecundity and total weight for King George Whiting from samples collected in 2017 and 2018 and derived from Fowler et al. 1999.

## Key spawning areas

### *King George Whiting Egg Counts*

A total of 541 eggs were confirmed to be King George Whiting eggs after ISH validation from the 2017 plankton survey. King George Whiting eggs represented 9% of the total eggs collected and were found at 80 of the 126 (63%) stations sampled. The King George Whiting egg counts per station ranged from 0 to 28 eggs, with 21 stations containing >10 eggs per station.

In 2018, a refinement in the bongo net sampling technique resulted in an almost doubling of water filtered compared to 2017. In 2017, a total of 7,011.89 $m^3$ of water was filtered during the plankton tows, with a mean of 55.65 ± 1.36 $m^3$ per station. In 2018, 13,178.95 $m^3$ of water was filtered, with a mean of 104.59 ± 3.5 $m^3$ per station. The total number of King George Whiting eggs collected during the 2018 plankton survey was 944, which represented ~5% of the total eggs sampled. In 2018, King George Whiting eggs were found at 94 of the 126 (75%) stations sampled and ranged from 0 to 109 eggs, with 24 stations containing >10 eggs.

### *King George Whiting Egg Density*

In 2017, 70.5% of the stations within southern Spencer Gulf contained egg densities of >0.1 $egg.m^{-2}$, with 8.2% of stations containing >10 $eggs.m^{-2}$. Two key areas of increased spawning activity with

stations containing >10 eggs.m$^{-2}$ were identified within southern Spencer Gulf. The first area of increased egg density in southern Spencer Gulf was a ~40 nm$^{-2}$ area, situated north of Wedge Island (Figure 9). This area of spawning activity consisted of three stations of >10 eggs.m$^{-2}$, with a peak of 18 eggs.m$^{-2}$. The second peak of spawning activity in southern Spencer Gulf was a single station in Hardwick Bay, which contained 17 egg.m$^{-2}$. Over half (56.9%) of the stations in Investigator Strait and southern Gulf St. Vincent had egg densities of >0.1 egg.m$^{-2}$. However, only 3.08% of stations had relatively high levels of egg densities of >10 egg.m$^{-2}$. These stations of increased spawning activity were located in the west of Investigator Strait and along the north-western coastline of Kangaroo Island (Figure 9). This peak in egg density was at a single station which had 18.66 egg.m$^{-2}$, which represents the highest egg densities for the 2017 DEPM survey.

In 2018, the percentage of stations containing egg densities of >0.1 eggs.m$^{-2}$ in southern Spencer Gulf increased to 75.4%. However, no stations were found to have high levels of egg densities (>10 eggs.m$^{-2}$), the highest egg density identified in southern Spencer Gulf was 7.41 eggs.m$^{-2}$. Indicating a reduction in spawning activity during the 2018 DEPM survey in the southern Spencer Gulf.

Conversely, the number of stations with egg densities of >0.1 eggs.m$^{-2}$ increased to 75.38% during the 2018 DEPM survey in Investigator Strait and Gulf St. Vincent. A large area (~50 nm$^{-2}$) of increased spawning activity was identified in central Investigator Strait in 2018, with five stations containing high egg densities of >10 egg.m$^{-2}$. The peak in egg density for this area was 16.24 eggs.m$^{-2}$, which was the highest identified for the 2018 survey.

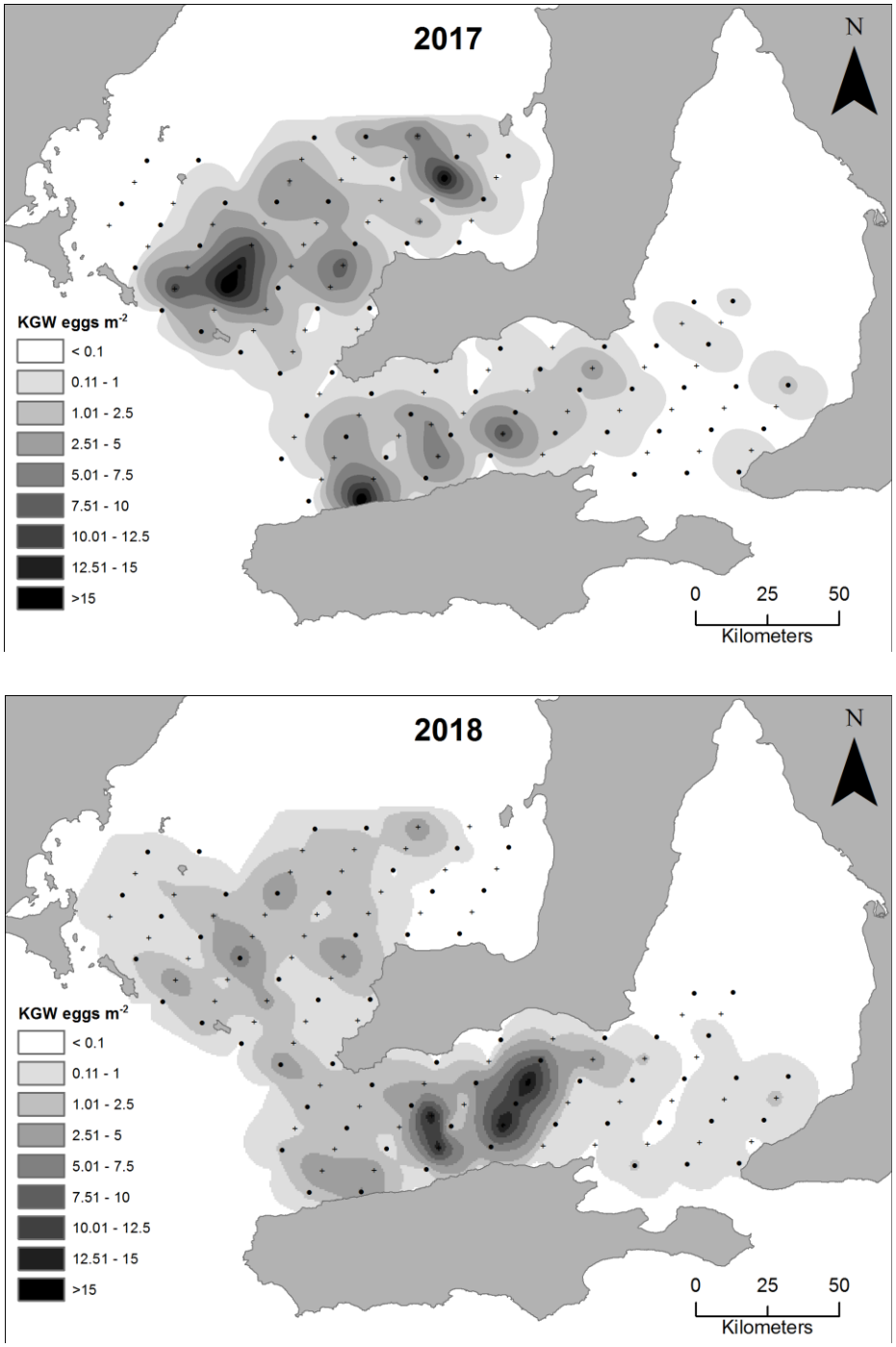



**Figure 9.** King George Whiting egg density distributions derived from DEPM plankton surveys from 2017 (top) and 2018 (bottom).

### *Egg Stage-Ages*

Egg stage-at-age information was adapted from egg development-temperature trials on King George Whiting by Fowler (2000), converting an initial 16 stages to the 10 universal egg stages presented in Table 2. Temperature ranges in the trial were 16 – 22°C, which was marginally outside of the observed temperature range (17.8 – 20.4°C) during the plankton surveys in 2017 and 2018. King George Whiting

Eggs developed faster at elevated temperatures, reaching stage 10 (hatching) between 38 to 40 hours at 22°C. Eggs development was almost twice as slow at 16°C, reaching stage 10 between 74 to 77 hours (Figure 10). The duration of each stage within temperature bands was consistent with the exception of stage 2. This stage can persist for up to a day at lower temperatures and can still persist for up to 14 hours within the temperature range where King George Whiting spawn (18 – 20°C). This poses an issue for accurately determining the true age of stage 2 eggs.

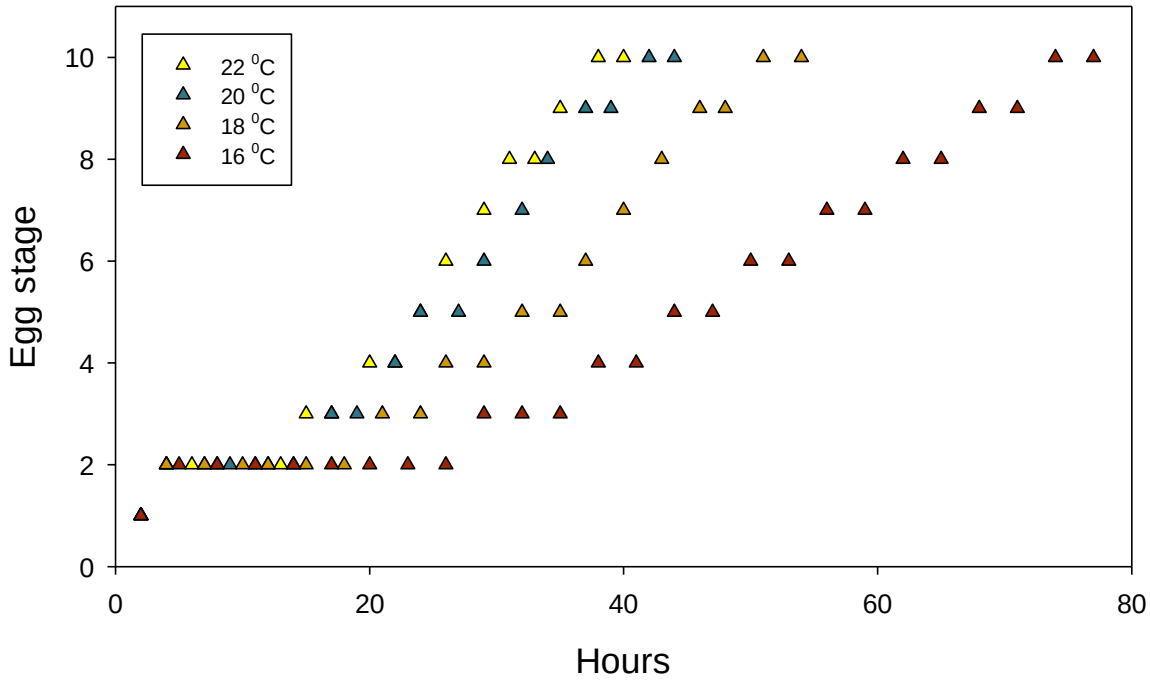



**Figure 10.** King George Whiting egg stage development in hours based on laboratory egg rearing trials in Fowler (2000).

### *Daily Egg Production*

Values of $P_0$ were very low throughout the study irrespective of egg mortality rate, ranging from 0.46 – 1.04 egg.day$^{-1}$.m$^{-2}$ (Figure 11). At an *a priori* egg mortality rate of 0.4 day$^{-1}$, mean daily egg production ranged from 0.52 to 0.70 egg.day$^{-1}$.m$^{-2}$ across years (Figure 11). Overall, the mean daily egg production was 21 – 32% higher for each increment of assumed egg mortality in 2018 when compared to the estimates in 2017. However, these small differences are negligible, with most mean daily egg estimates overlapping with the ranges of error variances.

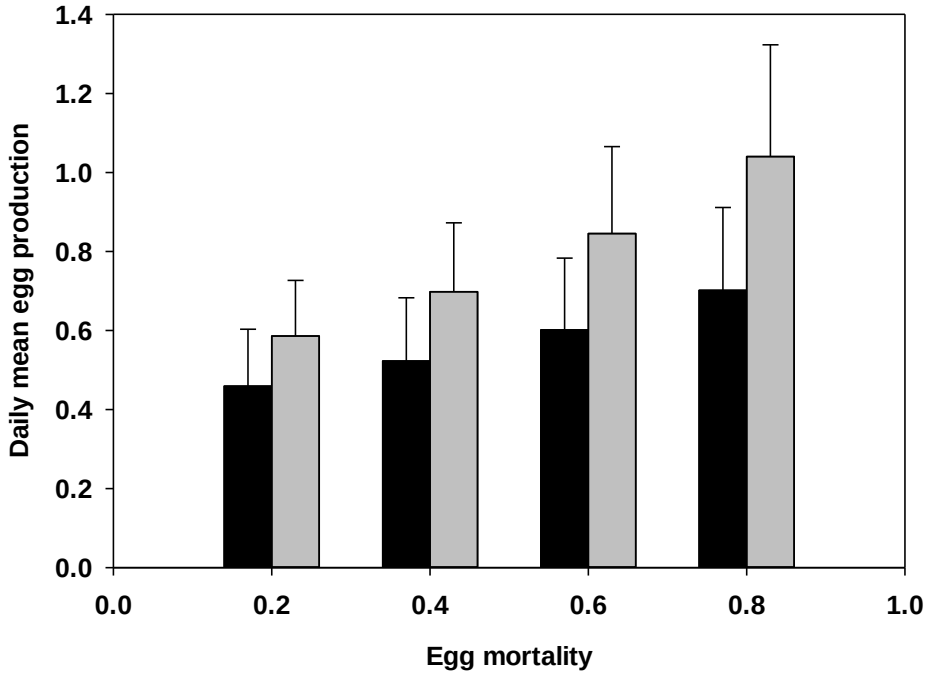



**Figure 11.** Estimates of daily egg production ($P_o$) (error bars = SE), the mean egg density at the time of spawning for 2017 (black) and 2018 (grey). Values of daily egg production are given for four *a priori* values of egg mortality (*Z*).

### *Spawning Area (A)*

Estimates of spawning area were derived from the sum of VNN areas for stations where King George Whiting eggs were present (Figure 12). The estimated spawning areas encompassed the majority of the plankton survey area in both years. In 2017, the spawning area was 8,676 km$^{-2}$, which represented 62% of the plankton survey area (13,876 km$^{-2}$). In 2018 the spawning area increased to 10,465 km$^{-2}$, which represents 75% of the survey area. The increase in spawning area in 2018 was the result of low densities of eggs being identified from the east of Investigator Strait and southern Gulf St. Vincent.

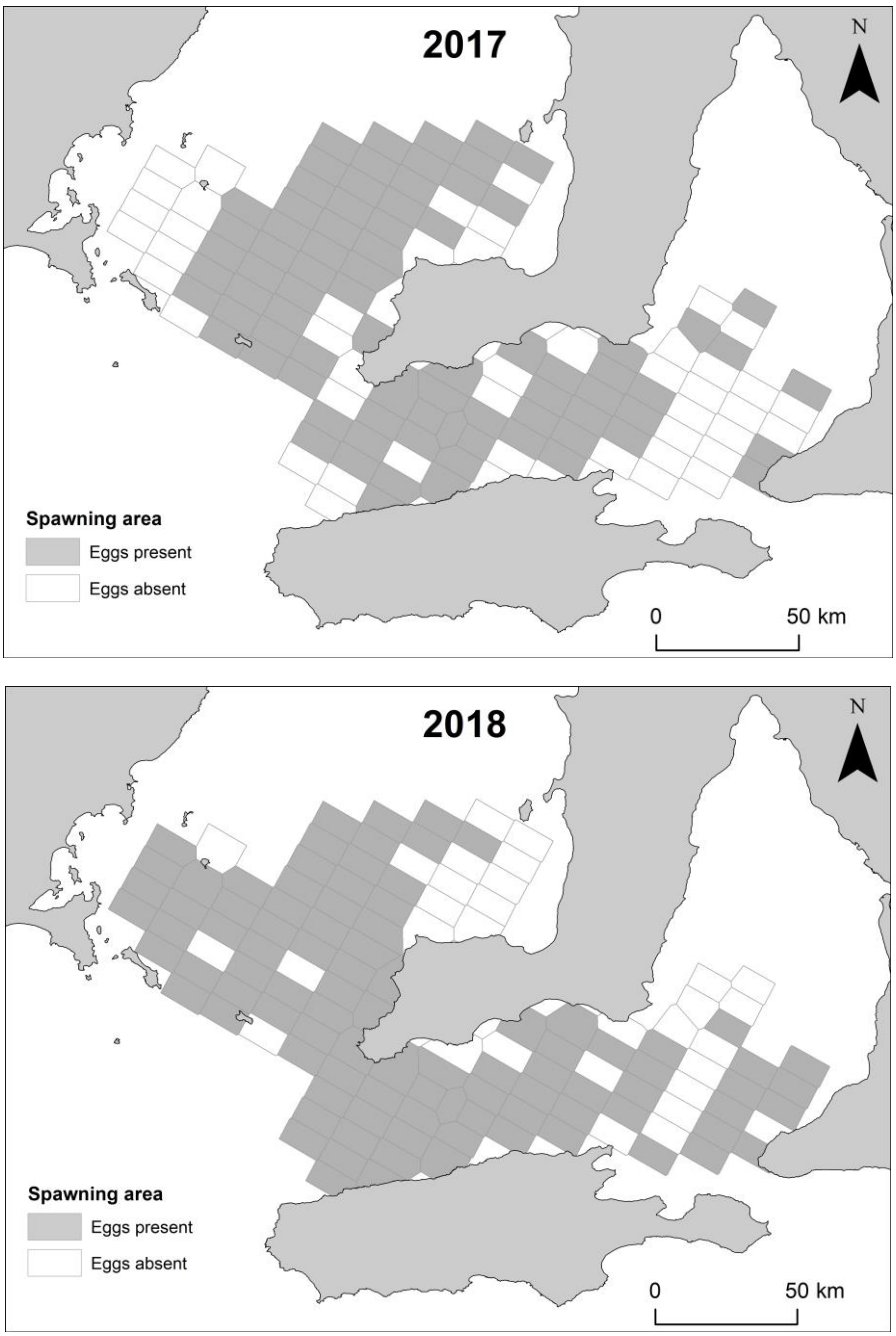



**Figure 12.** King George Whiting egg VNN areas derived from DEPM plankton surveys from 2017 (top) and 2018 (bottom). Grey polygons indicate stations where eggs were present.

### *Influence of DEPM parameters on estimating spawning biomass (B)*

### *Egg mortality (Z)*

Similar to the modified DEPM estimate derived for Snapper in Steer et al. (2017), this method incorporates an assumed value of egg mortality (*Z*). There were moderate differences in modelled estimates of spawning biomass under varying assumed egg mortality rates (*Z*) which ranged from 0.2 to

0.6 day$^{-1}$. Estimates of spawning biomass increased by 26,000 – 37,000 kg with each incremental rise in egg mortality, this was consistent for both years (Figure 16).

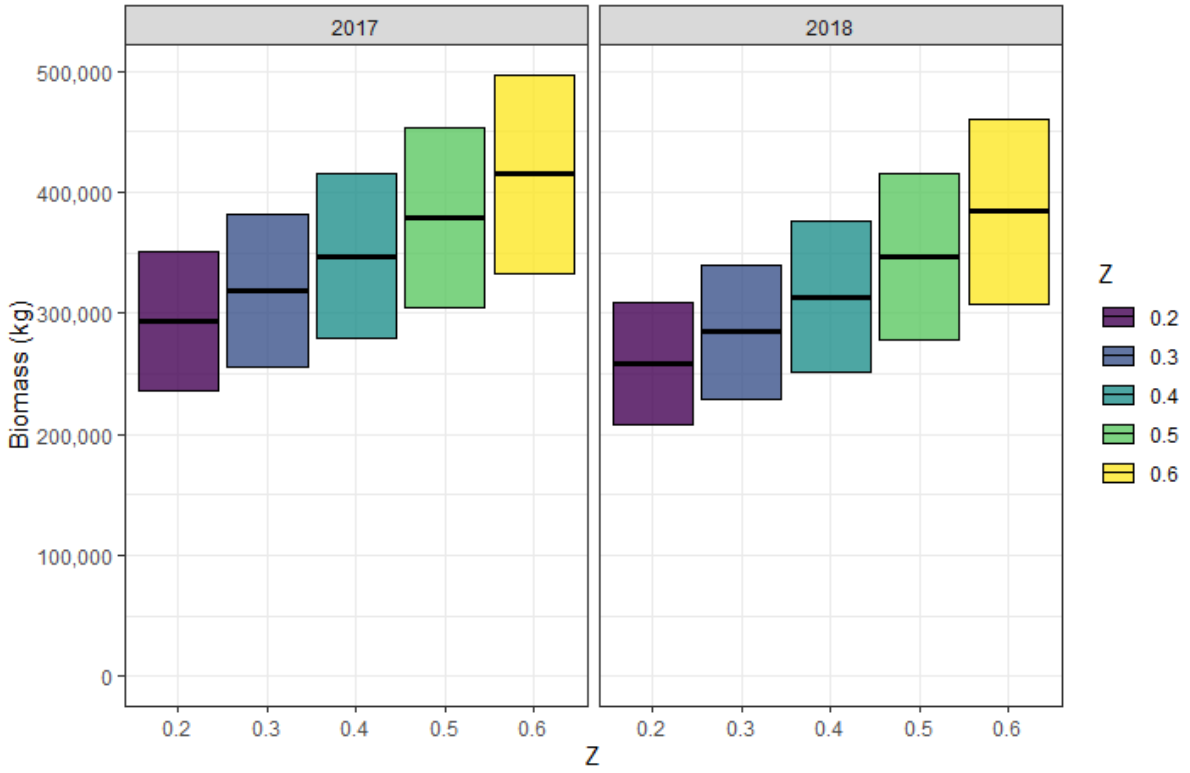



**Figure 13.** King George Whiting spawning biomass estimates for 2017 (left) and 2018 (right) computed assuming five different values for egg mortality rate (*Z*) and using an assumed spawning fraction (*S)* of 0.64. Error bars = Standard Error.

### *Biomass estimate sensitivity to $P_0$, A, R or S*

King George Whiting spawning biomass estimates were most sensitive to spawning area (*A*), sex ratio (*R*) and spawning fraction (*S*) (Figure 17). Biomass is linearly related to *A* as would be expected. However, it should be noted that the lower range of *A* that occur in this sensitivity test can only occur if other species eggs were misidentified as King George Whiting and caused additional stations to be included in the *A* estimate. However, this is unlikely. The method of estimating *A* based on the sum of station areas where eggs were present is a conservative approach as *A* can only be underestimated rather than overestimated.

Spawning fraction and sex ratio have exponential relationships to biomass as they are accounting for proportions of the population included in surveys. Sex ratio is unlikely to be underestimated for King George Whiting given that females grow larger than males. Both DEPM survey and SAFCOL datasets show that *R* should be above 0.57 for King George Whiting. Spawning fraction therefore remains the most influential parameter for biomass in DEPM methods, emphasising that it must be accurately

estimated to produce reliable estimates of biomass A low spawning fraction ($S$ = 0.28) produced a $B$ estimate of 725,000 kg while a high spawning fraction ($S$ = 0.95) produced a $B$ estimate of 214,000 kg. This represents a potential change in $B$ of more than three orders of magnitude depending on the true level of $S$.

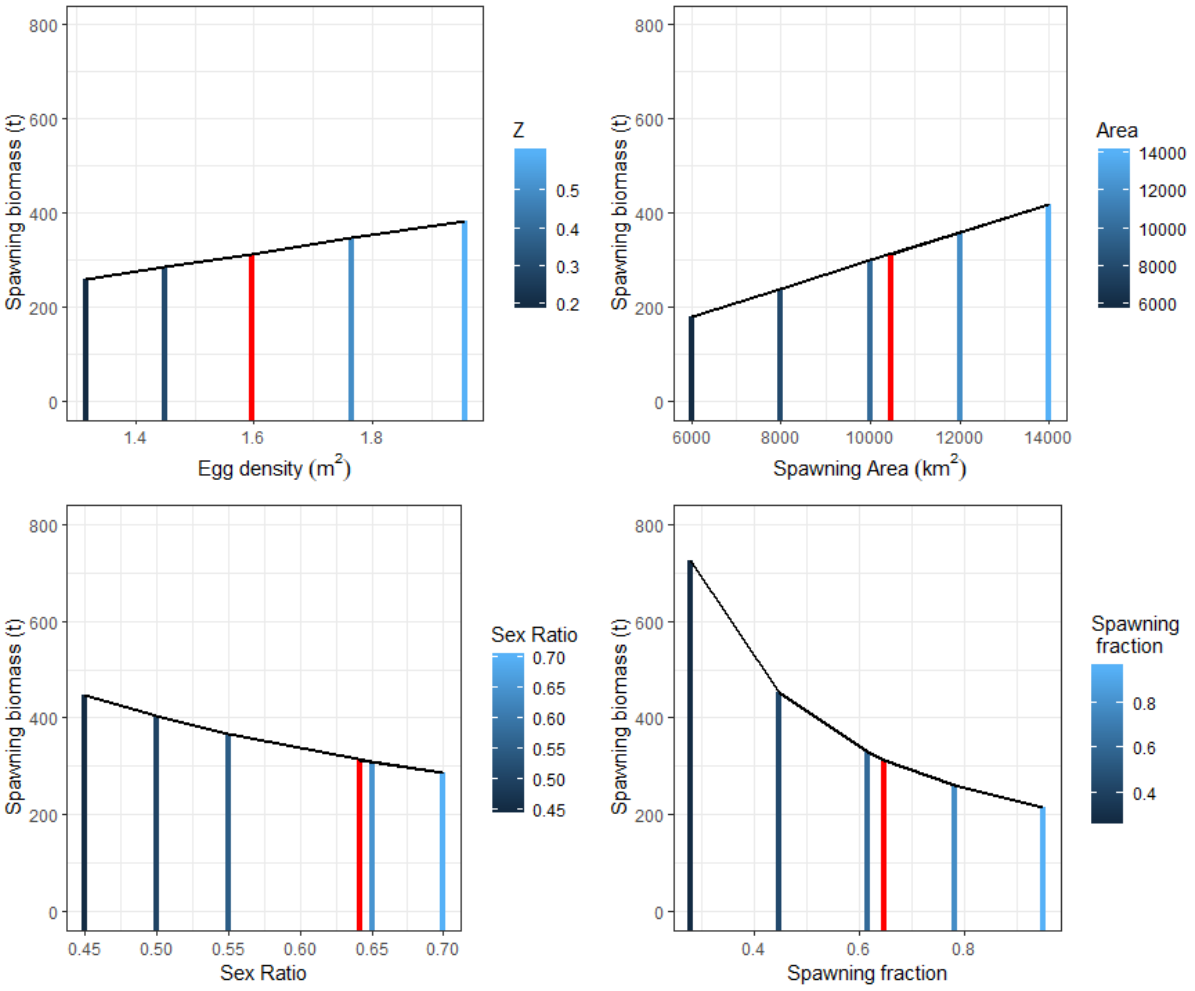



**Figure 14.** Sensitivity of King George Whiting spawning biomass estimates for 2018, using an assumed spawning fraction (*S)* of 0.64. In each panel different values of each parameter are applied while holding remaining parameters constant at estimated values. Steeper changes in biomass over plausible parameter ranges demonstrate greater sensitivity to a parameter. Red bars represent the value estimated for 2018.

### *Comparison to standard DEPM approach*

Both the DEPM results and size based DEPM (DEPMWt) results were produced using a single set of parameters for $A$, $P_0$, $R$ and $S$ in order to display the difference in variance between the two approaches. The DEPMWt produced identical estimates of biomass to the standard DEPM but with much narrower variance. The standard error for biomass in 2017 and 2018 were 68,100 kg and 62,039 kg, respectively

using the DEPMWt approach (Figure 17). Whereas the DEPM approach produced standard errors of 102,813 kg and 105,112 kg in 2017 and 2018 respectively. These are 51% and 69% larger than the DEPMWt standard errors in 2017 and 2018, respectively.

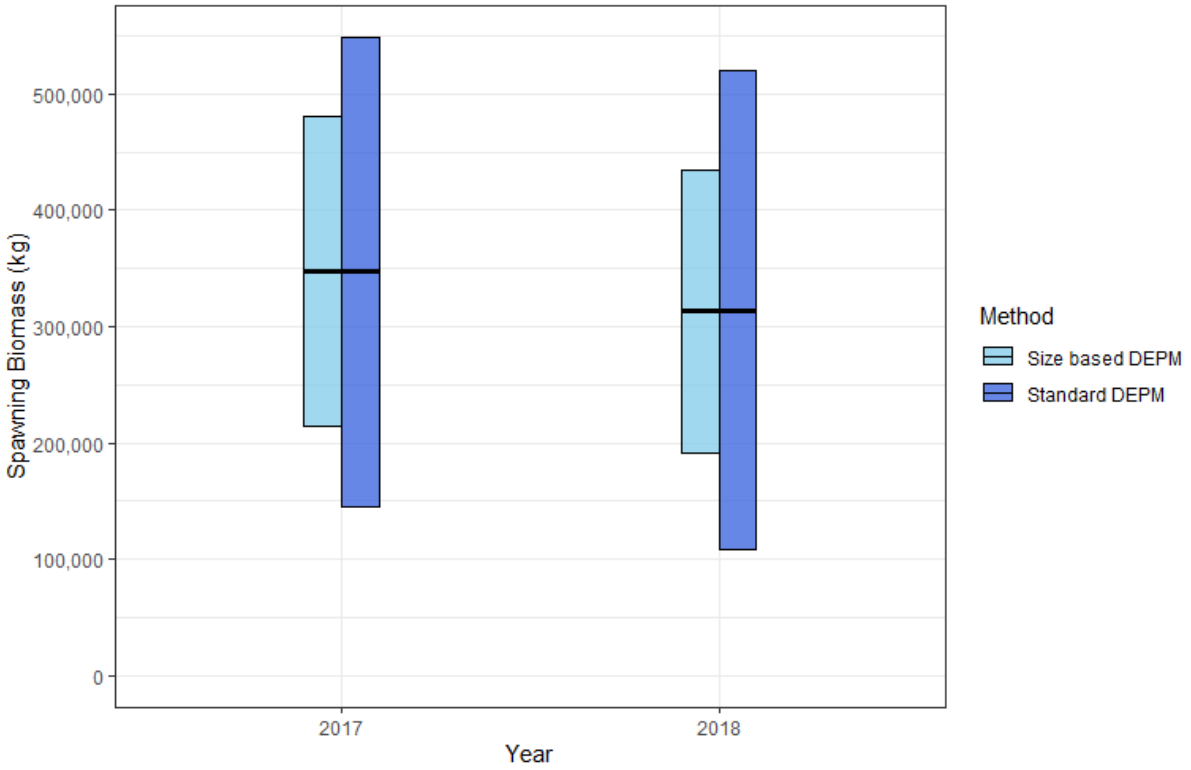



**Figure 15.** Comparison of the size based DEPM approach (DEPMWt) to the standard DEPM approach for King George Whiting in 2017 and 2018, using an assumed spawning fraction (*S)* of 0.64.

## Source to sink connectivity

### *Oceanographic observations*

Water temperature and salinity was recorded at 62 plankton survey stations evenly distributed across the study site. Water temperatures and salinities were calculated as the mean of the upper 5 m of the water column from the CTD cast profiles. Water temperature profiles differed between years during the 2017 and 2018 plankton surveys, with temperatures in 2017 ~1°C lower than observed in 2018 (Figure 18). In 2017, water temperatures ranged from 17.8 to 19.9°C with a mean of 18.8°C ± 0.05 SE, water temperatures were lowest in southern Spencer Gulf and western Investigator Strait (Figure 18). In 2018, water temperatures were notably warmer and ranged from 18.6 to 20.4°C with a mean of 19.4°C ± 0.04 SE (Figure 18).

Salinity profiles varied across the plankton survey area each year, with lower salinity waters coinciding with the cooler waters in the southern Spencer Gulf. Similar patterns in salinity profiles were observed

in both years. The salinity ranging from 35.6 – 37.5 PSU in 2017 and a mean of 36.5 PSU ± 0.04 SE. In 2018, similar salinity ranges were observed with 35.9 – 38.3 PSU with a mean of 36.6 PSU ± 0.05 SE.

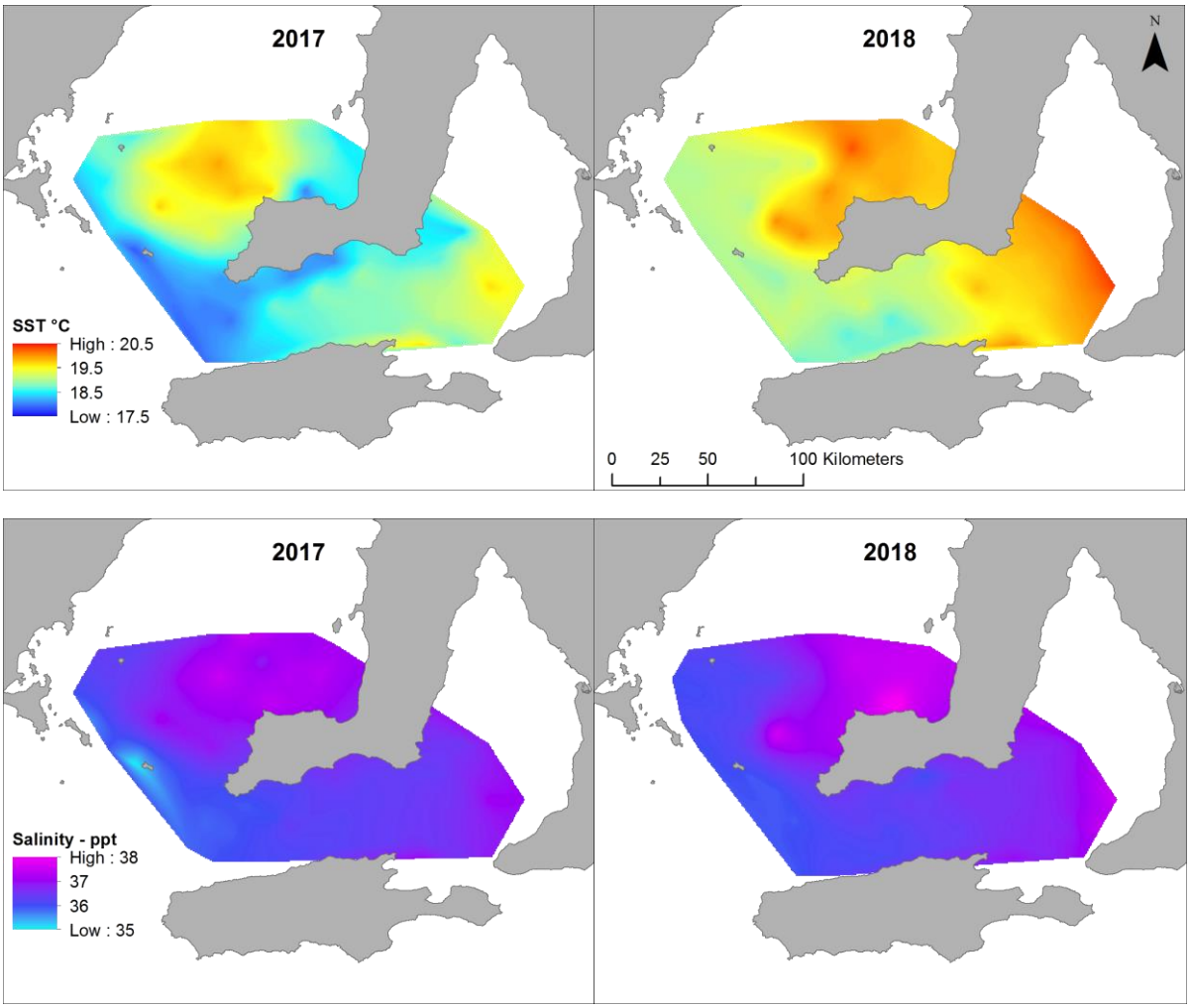



**Figure 16.** Interpolated mean water temperature (top left and top right) and salinity profiles (bottom left and bottom right) observed during the King George Whiting DEPM plankton surveys in 2017 and 2018.

## Hydrodynamic Model validation

There was strong agreement between the tidal amplitude, phase, and current velocity predicted by the hydrodynamic model and measurements recorded at the observational buoy. Tidal currents averaged ~0.3 – 0.4 m $s^{-1}$ in Spencer Gulf and Investigator Strait, and ~0.2 – 0.3 m $s^{-1}$ in Gulf St. Vincent. Tidal currents were up to 4 – 5 times stronger than residual currents. Residual currents within Spencer Gulf and Gulf St. Vincent averaged ~0.05 m $s^{-1}$ (0.0 – 0.2 m $s^{-1}$), whereas the residual current near the entrance of Spencer Gulf and in Investigator Strait averaged ~0.1 m $s^{-1}$ (0.0 – 0.4 m $s^{-1}$).

Residual circulation throughout the study area was consistent between years. In Spencer Gulf and Gulf St. Vincent, water entered along the western boundary and flowed northward towards the head, and then

flowed out along the eastern boundary (Figure 19). This resulted in a general clockwise circulation within each gulf. There was a strong eastward current that flowed from the western boundary of the model south of Eyre Peninsula, across the mouth of Spencer Gulf and through Investigator Strait. This current resulted in a strong outflow through Backstairs Passage and across the open ocean boundary to the east.

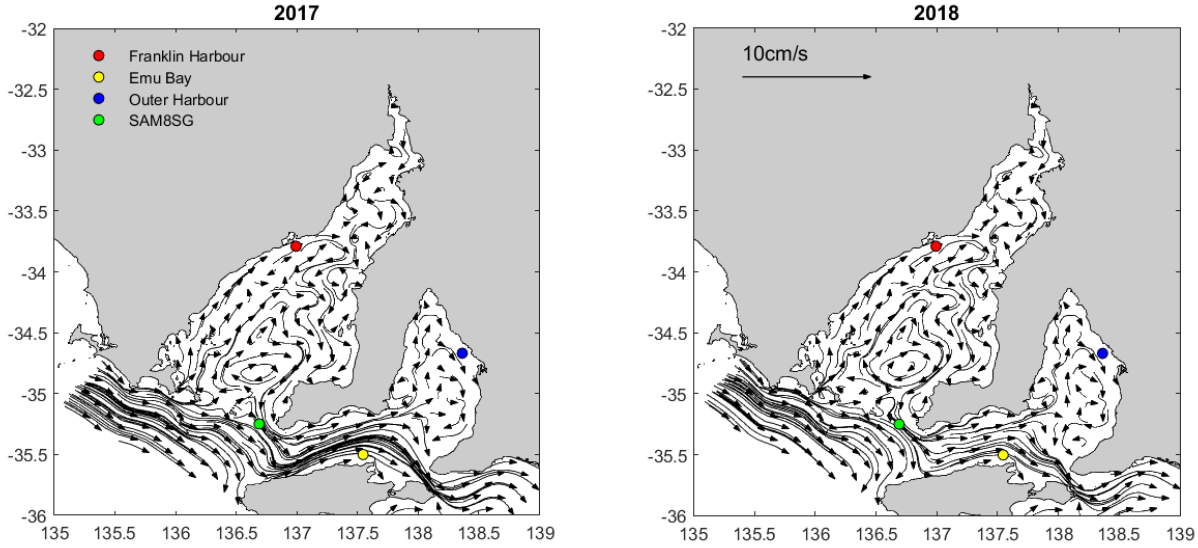



**Figure 17.** Mean depth-averaged residual circulation throughout South Australia's gulf systems predicted by the hydrodynamic model. Vectors show the direction and velocity of residual currents. Coloured circles identify the locations of the four observation sites used to validate the outputs of the hydrodynamic model.

## Loss of Larva

The bio-physical model predicted that 72.2% and 77.6% of particles in 2017 and 2018 would be transported outside of the open ocean boundaries, respectively (Figure 20). In both years, the majority of particles (60.6% and 57.3% in 2017 and 2018) were transported in an eastward current along the north Coast of Kangaroo Island, which resulted in a high egg and larval density outflow through Backstairs Passage and across the eastern model boundary (Figure 20). A further 11.6% and 20.3% of particles in 2017 and 2018 were dispersed within a southerly current that flowed out the eastern side of southern Spencer Gulf and across the boundary to the west of Kangaroo Island. The majority of particles that passed across the eastern boundary were originally seeded in Investigator Strait (84 – 94%), whilst particles that crossed the western boundary mostly originated from southern Spencer Gulf (65 – 91%) (Figure 20).

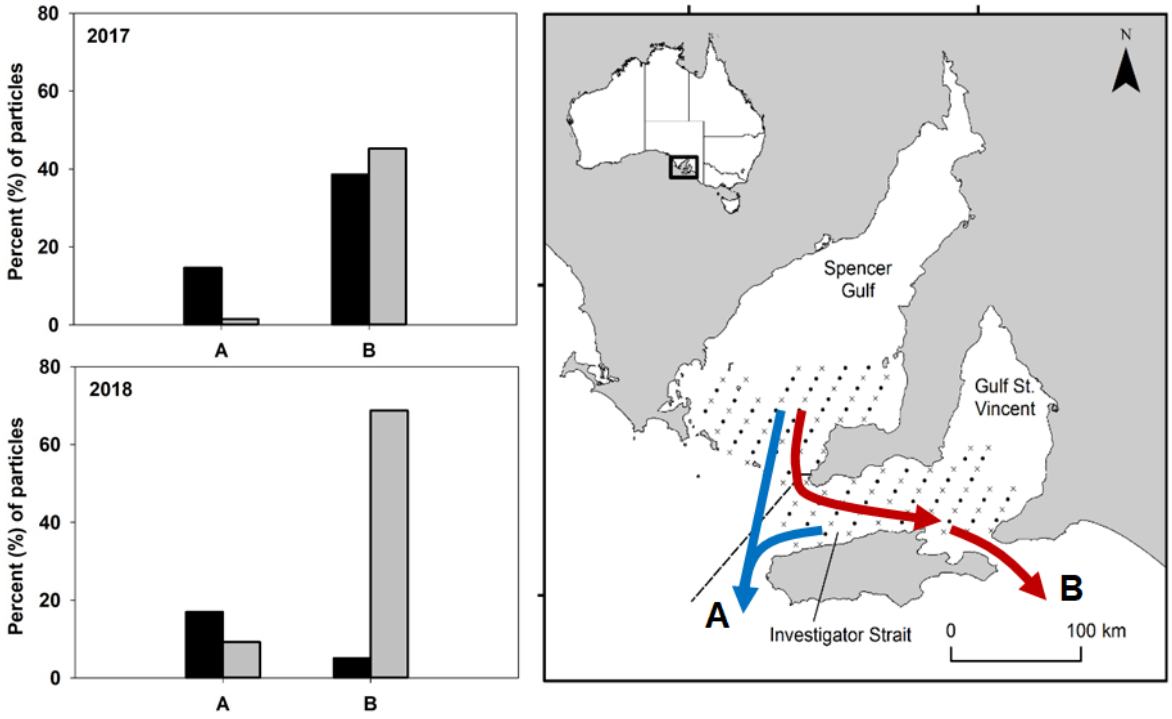



**Figure 18.** Percentage of overall particles released that intersected the open ocean boundaries of the larval transport model in 2017 (top left) and 2018 (bottom left), which were considered out of bounds and excluded from simulations. The dotted black line separates the two spawning regions: SSG – southern Spencer Gulf (black); IS – Investigator Strait (grey). (A) Percentage of particles that intersected the boundary to the west of Kangaroo Island; (B) percentage of particles that intersected the boundary to the east of Kangaroo Island. The arrows indicate the main direction of particle flow from within the model to the model boundaries.

## Areas of spawning success

In 2017, a total of 26,597 particles were released in the bio-physical model, with 326 (1.23%) particles predicted to reach one of the predetermined settlement locations that are anticipated to result in spawning success (Figure 6). The predicted spawning success was higher for Spencer Gulf (1.8%) than for Investigator Strait (0.3%) (Table 8). Southern Spencer Gulf had the highest spawning success with a combined 85% of settled particles originating from areas 2, 3, 4 and 6 (Figure 21). The modelled spawning success for southern Spencer Gulf correlated with the observed highest levels of King George Whiting egg densities from the 2017 plankton survey.

In 2018, the number of particles released in the model decreased to 22,581, with 294 (1.3%) particles projected to reach the settlement sites (Figure 6). Investigator Strait had a slightly higher predicted spawning success with 1.4% compared to southern Spencer Gulf with 1.2%. Areas of successful spawning shifted in 2018, with spawning areas 7 and 9 (18% and 37%) in Investigator Strait possessing the highest levels of spawning success. Low levels of spawning success was identified for southern Spencer Gulf, with all areas 2, 3 and 4 exhibiting <10% spawning success.

**Table 8.** Total number of particles released and overall settlement success (%) for each spawning area (1-13) predicted by the bio-physical model in 2017 and 2018. Settlement success values are the percentage of particles that settled from each spawning area ((number settled / number released) * 100). The number of particles released at each station was the calculated King George Whiting egg density derived from the plankton survey multiplied by 100. Stations – numbers of plankton stations in each area.

| | | 2017 | | 2018 | |
|---|---|---|---|---|---|
| **Region (area)** | **Stations** | **# Seeded** | **% Settled** | **# Seeded** | **% Settled** |
| **Spencer Gulf** | **61** | **17167** | **1.8** | **7671** | **1.2** |
| 1 | 9 | 0 | 0 | 622 | 2.3 |
| 2 | 10 | 6265 | 1.3 | 2134 | 1 |
| 3 | 9 | 2356 | 2.8 | 1491 | 1.5 |
| 4 | 11 | 4237 | 1.7 | 2175 | 1.3 |
| 5 | 11 | 1692 | 1.3 | 1226 | 0.3 |
| 6 | 11 | 2617 | 2.3 | 23 | 4.4 |
| **Investigator Strait** | **65** | **9430** | **0.3** | **14910** | **1.4** |
| 7 | 9 | 1410 | 0.4 | 3034 | 1.5 |
| 8 | 10 | 3619 | 0.1 | 2194 | 0.7 |
| 9 | 9 | 751 | 0.9 | 3662 | 3 |
| 10 | 10 | 3118 | 0 | 5053 | 0.5 |
| 11 | 9 | 139 | 7.9 | 157 | 2.6 |
| 12 | 8 | 158 | 0 | 277 | 0 |
| 13 | 10 | 235 | 0 | 533 | 0 |
| **Total** | **126** | **26597** | **1.2** | **22581** | **1.3** |

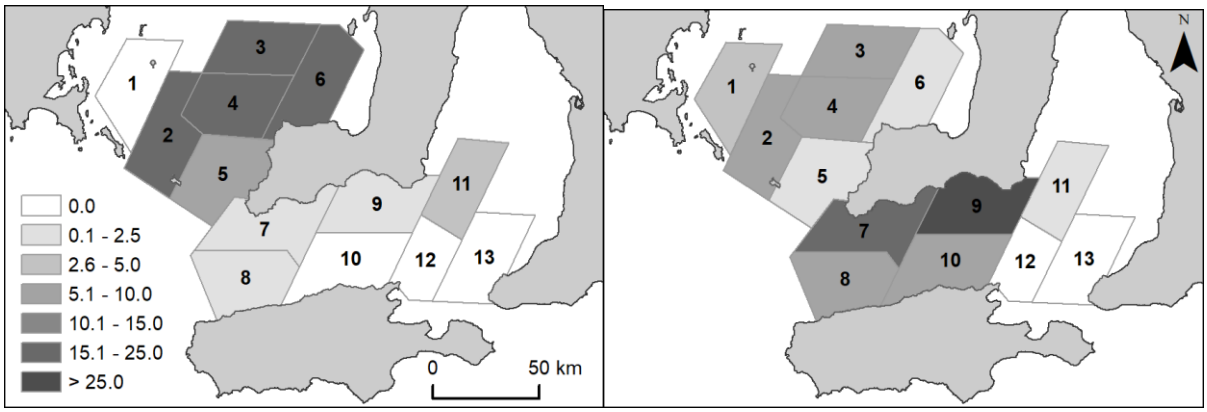



**Figure 19.** Percent contribution of each spawning area (1-13) to overall settlement as predicted by the bio-physical model for 2017 (left) and 2018 (right).

## Settlement success

As a result of the high numbers of particles which flowed out of bounds, the projected settlement success was very low for both 2017 and 2018. In 2017, the model predicted that of the 25 areas of known

settlement, 12 sites would have at least one particle settle. Seven sites had >10 particles settle, and six of these sites were within close proximity to the plankton survey area. The regions which had the highest settlement were the south-east Spencer Gulf (51%), Gulf St. Vincent (23%) and the northern Kangaroo Island bays (19%) (Figure 22). There was limited success for particles settling in Investigator Strait (7%), and no settlement predicted for northern Spencer Gulf. Hardwick Bay, within the southern Spencer Gulf region had the highest predicted settlement for 2017 at 37%. Within Gulf St. Vincent, Coobowie had 16% settlement and Barker Inlet had 5% settlement, which was the only settlement area outside of the plankton survey to exhibit successful settlement (Figure 22). The model predicted that the northern bays of Kangaroo Island were also important settlement areas, with American River (14%) and Bay of Shoals (4%) indicating successful settlement (Figure 22).

In 2018, particles were predicted to reach more settlement locations, with 8 sites having >10 particles reach settlement and 16 sites having >1 particle successfully settle. A shift in successful settlement was identified between regions, with Gulf St. Vincent increasing to 58% of particles settled and southern Spencer Gulf decreasing to 18% of successful settlement (Figure 22). The northern bays of Kangaroo Island maintained a similar 22% of success particle settlement (Figure 22). Very little settlement success was predicted for northern Spencer Gulf (1%) and for within Investigator Strait (1%).

The same settlement zones identified in the 2017 model as being the most important for settlement as were again identified in 2018. The settlement zone of Coobowie in Gulf St. Vincent had the highest percent of settlement for 2018 with 37%. In Barker Inlet in Gulf St. Vincent, settlement success increased in 2018 to 11.5%, This region continued to have the highest settlement success for any zone outside of the plankton survey, and has been historically identified as an important nursery for King George Whiting. Hardwick Bay continued to have the highest predicted settlement success within southern Spencer Gulf at 13%. The northern bays of Kangaroo Island had high predicted settlement for 2018, with American River and Bay of Shoals having 16% and 5% settlement success (Figure 22).

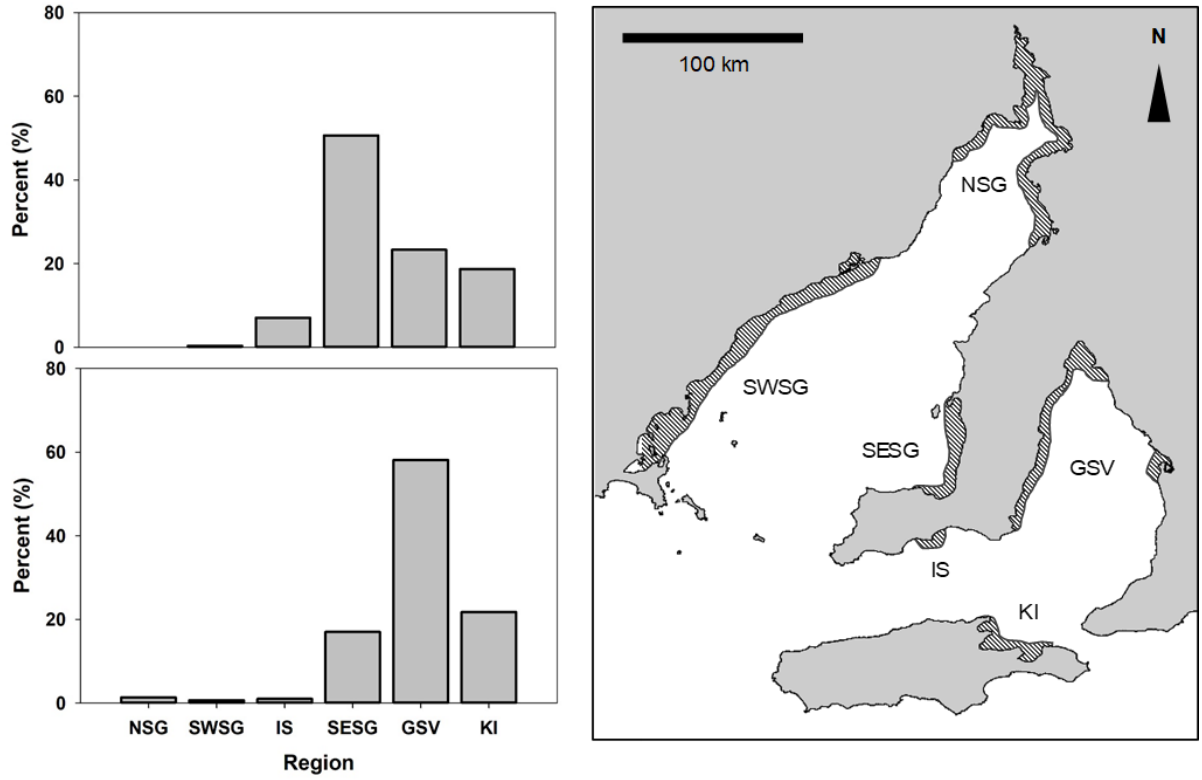



**Figure 20.** Percent contribution of each settlement region to overall settlement success in 2017 (top left) and 2018 (bottom left). SWSG – south-west Spencer Gulf; NSG – northern Spencer Gulf; SESG – south-east Spencer Gulf; IS – Investigator Strait; GSV – Gulf St. Vincent; and KI – Kangaroo Island.

## Connectivity between spawning and settlement

The bio-physical model predicted successful settlement for particle movements ranging from relatively localised self-seeding (<50 km) to large scale movements of up to 200 km. In 2017, 85% of the particles that settled originated from the southern Spencer Gulf spawning areas of 2, 3, 4 and 6 (Figure 23). Predicted settlement was highest to Hardwicke Bay (37%) in south-east Spencer Gulf, which involved relatively short, localised dispersal distances of 20 – 90 km (Figure 20). The next highest settlement was to Coobowie Bay (16%) in Gulf St. Vincent, and American River (14%) in the north Coast of Kangaroo Island (Figure 23). Particles that settled to both areas originated in southern Spencer Gulf and were entrained in an eastward current that flowed through Investigator Strait and travelled relatively large distances of up to 150 – 200 km. In 2018, the predicted settlement was highest to the same three settlement areas, but the source of particles and predicted settlement differed from 2017. The particles that settled to Hardwicke Bay (13%), similar to 2017 originated from spawning areas 1 – 4 in Southern Spencer Gulf (Figure 21). Predicted settlement was highest to Coobowie Bay (36%) for 2018, the particles that settled here were from spawning areas 7 and 9 in northern Investigator Strait and likely travelled 40 – 60 km (Figure 23). American River accounted for 16% of settlement, and these particles

originated from spawning areas 8 and 10 along the north Coast of Kangaroo Island (60 –100 km) (Figure 23).

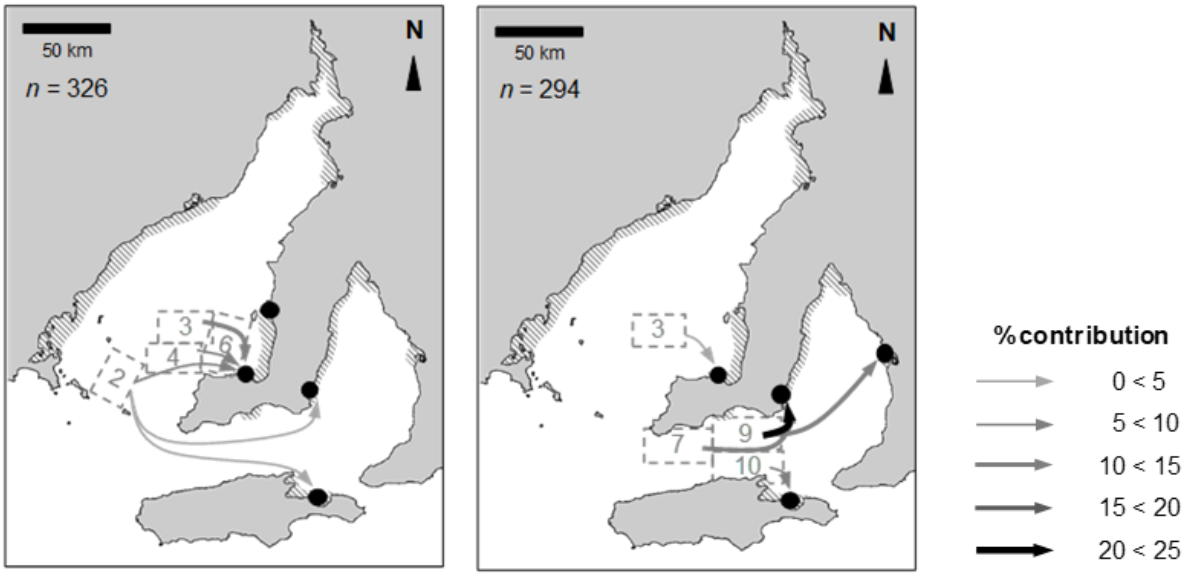



**Figure 21.** Relationships between spawning areas (1-13) and key settlement areas in (left) 2017 and (right) 2018. Only settlement areas that accounted for ≥ 10% of overall settlement were shown (black circles). The percent contribution of each spawning area to settlement is shown by the colour and width of the connecting arrow. *n* = total number of settled particles.

## Management Strategy Considerations

In 2017, there was a large amount of spawning area overlap within the initial closure (61.6%) (Table 9). However, a remaining 38.4% of spawning area occurred outside the closure, which included two areas of peak spawning activity. The largest of these peaks in spawning activity occurred in southern Spencer Gulf to the west of the newly implemented closure; the other peak in spawning was one station to the south of the closure, along the North Coast of Kangaroo Island in Investigator Strait (Figure 24). The percent of coverage of spawning area estimated from the 2017 survey reduced to 35.1% in the refined closure (Table 9) (Figure 25). The estimated spawning area in 2018 had a moderate amount of coverage (53.9%) inside the initial closure, with only low densities of King George Whiting eggs being identified outside (46.1%) of the initial closure to the east and west of its boundaries (Figure 25). The percent of the spawning area covered in the 2018 survey by the refined closure reduced to 26.8%. However, all peaks (>15 eggs.m$^{-2}$) in King George Whiting egg densities identified in the 2018 plankton survey were within the boundary of the smaller refined closure (Figure 25), predominately in Investigator Strait.

The key areas of spawning that contribute to >10% settlement success had extensive coverage by the initial closure for both years of predicted settlement success, which ranged from 63.0% in 2017 to 90.0%

in 2018 (Table 9 and Figure 25). The only spawning region of successful settlement which occurred outside of the initial closure was block number 2 in southern Spencer Gulf (Figure 25). The percent of coverage of the spawning area blocks with successful settlement for the refined closure reduced to 24.9% in 2017 and 66.3% in 2018. This reduction in closure coverage for 2017 is the result of the exclusion of the spawning area blocks 2, 3 and 4 (Figure 25). For 2018, the spawning area blocks with increased settlement success were predominately located in Investigator Strait which were largely covered by the refined closure. However, similar to what was observed in the 2017 bio-physical model predictions, the refined closure excluded the consistently important spawning area of block 3 (Figure 25).

**Table 9.** The percentage of overlap of the King George Whiting spatial closures with the estimated area of egg densities (> 0.1 eggs.m$^{-2}$) derived from the DEPM plankton surveys and the spawning areas with >10% successful settlement predicted by the bio-physical model.

| | Spawning Area | | | | >10% settlement success | | | |
|---|---|---|---|---|---|---|---|---|
| | Initial closure | | Revised closure | | Initial closure | | Revised closure | |
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| Inside closure | 61.6 | 53.9 | 35.1 | 26.8 | 63.0 | 90.0 | 24.9 | 66.3 |
| Outside closure | 38.4 | 46.1 | 64.9 | 73.2 | 37.0 | 10.0 | 75.1 | 33.7 |

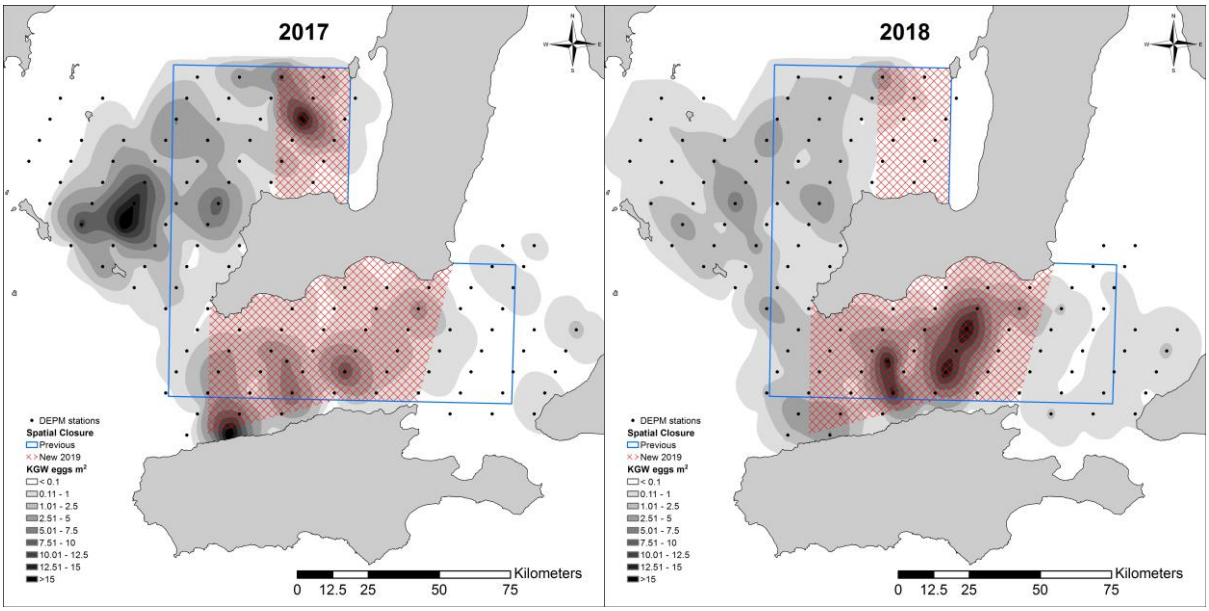



**Figure 22.** The King George Whiting spatial closures implemented in 2017 and modified in 2019, overlayed with the estimates of egg densities derived from the DEPM plankton surveys conducted in 2017 (top) and 2018 (bottom).

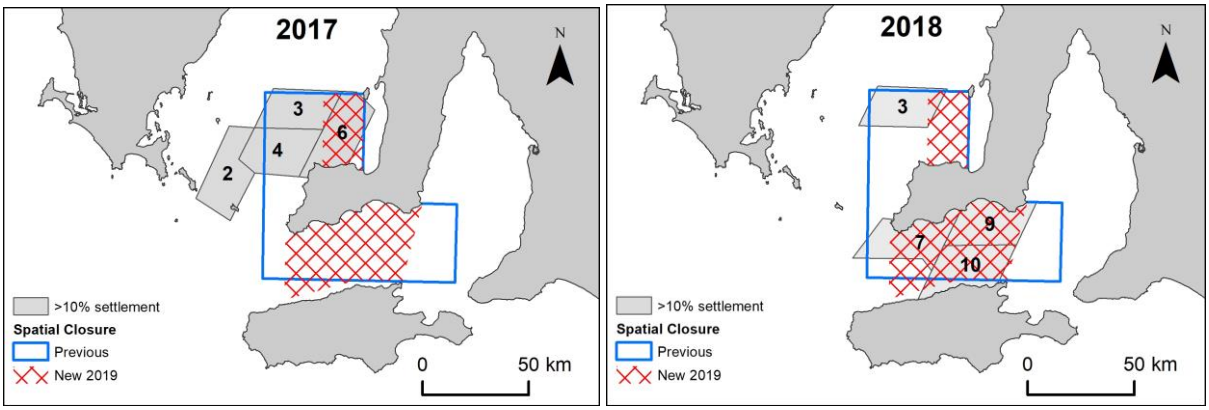



**Figure 23.** The areas of spawning that contribute to >10% successful settlement for King George Whiting larvae as predicted by the bio-physical model for 2017 (left) and 2018 (right).

# Discussion

### *Spawning dynamics of King George Whiting in South Australia*

King George Whiting eggs were broadly distributed over a large spatial range that contained 63% of sampling stations in 2017 and 74% of sampling stations in 2018. The relatively consistent distribution of eggs over both survey areas indicates that this species spawns throughout the southern Gulfs and Investigator Strait. This region has been previously identified as important habitat for King George Whiting reproduction in South Australia (Fowler et al., 1999, Fowler, 2000) and has underpinned the implementation of the Orcades Bank Marine Reserve. The spatial extent of spawning identified in this study contains the preferred habitat for adult King George Whiting, such as the scattered kunkarised shell beds, aeolianite reefs and low rising mounds that are found throughout southern Spencer Gulf and Investigator Strait (Shepherd and Sprigg, 1976). Despite the broad spawning area identified for both years, egg densities were relatively low, with most stations containing egg densities of <5 egg.m$^{-2}$. However, in both years 'hotspots' of increased spawning activity were identified, with limited stations in both surveys containing peak egg densities of >10 egg.m$^{-2}$.

An inter-annual spatial shift in peak spawning activity was identified between the two consecutive surveys, and it is unclear from this research whether the identified spatial shift in spawning activity is the result of the snapshot nature of a DEPM or an inter-annual shift in regional spawning activity. In 2017, peaks in egg densities of >10 egg.m$^{-2}$ were identified in southern Spencer Gulf, with three stations containing peak egg densities of >10 egg.m$^{-2}$ and one station in western Investigator Strait containing >10 egg.m$^{-2}$. A spatial shift in spawning activity occurred during the 2018 survey with the peak of egg density located in central Investigator Strait, with four stations containing egg densities of >10 egg.m$^{-2}$. The timing of both surveys were conducted to align with the peak of reproductive activity (late April) for King George Whiting in South Australia (Fowler et al., 1999). However, King George Whiting are a multiple batch spawner, and are capable of spawning every 1 – 3 days over a prolonged two month spawning period (mid-April to June) (Fowler et al., 1999, Fowler, 2000, Ham and Hutchinson, 2003). Consequently, it is plausible that fine-scale spatial variations in spawning activity could occur throughout the broader spawning area over a spawning period. However, it is difficult to determine whether the observed inter-annual differences in spawning locations are reflective of an annual shift in spawning activity or a within-season fine-scale variation in spawning. Therefore, an improved understanding of the temporal variation in spawning activity is required to confidently define the most important spawning areas.

Fine-scale spatial variations in spawning activity may also be influenced by localised fluctuations in environmental conditions such as water temperature. A difference in surface (<5m) water temperature of ~1 – 1.5°C was observed between plankton surveys (Figure 19), with cooler temperatures observed

in 2017, which may create fine-scale spatiotemporal variations in egg densities at the time of the surveys. It is unclear whether the observed differences in water temperatures between years had any influence on the spatial shift in peak egg densities. However, the peak in egg densities were predominately located in areas of cooler water (Figure 19). To resolve the uncertainty around spatial variations in peak spawning activity, multiple plankton surveys could be conducted across a single spawning season, potentially one survey every 2–3 weeks across the spawning period. However, the high cost of plankton surveys and subsequent laboratory work would likely be cost prohibitive.

### *Larval dynamics and connectivity between spawning grounds and nursery areas*

The bio-physical model over both years consistently predicted the highest larval settlement into four previously identified nurseries, Coobowie Bay and Barker Inlet in Gulf St. Vincent, Hardwicke Bay in south-east Spencer Gulf, and American River in northern Kangaroo Island. These model predictions are consistent with field observations that have identified settlement to these regions and, in particular, have confirmed high numbers of larvae settling in nursery areas of Gulf St. Vincent. Overall, the bio-physical model predictions emphasised the importance of southern Spencer Gulf and Investigator Strait as regions that are integral for spawning of King George Whiting. The predicted larval pathways indicate that both of these regions are fundamental for recruitment into both gulfs and that spatiotemporal variations in spawning and settlement success are likely across the prolonged spawning period and are primarily driven by regional oceanographic conditions.

Larval dispersal pathways and distances travelled differed between the spawning regions of Investigator Strait and southern Spencer Gulf. Larvae that originated in Investigator Strait were entrained in a strong eastward current which dispersed the majority (84 – 94%) of larvae in both 2017 and 2018 through Backstairs Passage and beyond the eastern boundary of the model. It is uncertain if these larvae eventually settle outside of the model boundaries. The remaining small proportion of larvae that originated along the northern boundary of Investigator Strait were dispersed into Gulf St. Vincent, where a small percentage of these were predicted to settle. The predicted dispersal distances of 40 to 100 km represent relatively short distances which supports the previously proposed hypothesis of recruitment to nursery areas for King George Whiting are locally supplied from spawning ground <50 km away (Fowler et al., 2000b, Rogers, 2019). In contrast, the bio-physical model predicted that larvae which originating in southern Spencer Gulf have a more varied dispersal pattern, with both localised seeding and long range distributions. The primary nursery area for larvae which originating in southern Spencer Gulf was Hardwicke Bay, in south-east Spencer Gulf. This represents dispersal over relatively short distances from 20 to 90 km and suggests that recruitment to this region primarily depends on localised population processes (Fowler et al., 2000b, Rogers, 2019).

Alternately, the bio-physical model predicted that some larvae from southern Spencer Gulf move southward and are then entrained into the eastward current that flows through Investigator Strait. Some

of these larvae followed the eastward current along the northern edge of Investigator Strait and were dispersed into Gulf St. Vincent, whilst others were transported to settlement areas along the northern Coast of Kangaroo Island. Both of these dispersal pathways transported larvae over 150 to 200 km and provides evidence of mixing from two different spawning hot-spots. These findings are consistent with previous projections of dispersal for larvae seeded in southern Spencer Gulf by Fowler et al. (2000b). The long range dispersal of larvae from southern Spencer Gulf is also liked to the breakdown of the thermohaline frontal systems which dissipate in late autumn. The breakdown of the thermohaline fronts allows the resumption of gulf water exchange, which likely supports the transport of larvae between gulfs. Furthermore, temporal variations in peaks of settlement for post-larval King George Whiting in northern Gulf St. Vincent indicates a bi-modal settlement pattern which is hypothesised to originate from alternate origins (Rogers, 2019). The observed bi-modal settlement pattern in post-larvae provides support for the predicted model projections of long-range larval dispersal from southern Spencer Gulf. The consistent importance of a small number of nurseries distributed in the south-east Spencer Gulf and Gulf St. Vincent identified by the bio-physical model suggest that these nurseries have the potential for further periodic post-larval settlement surveys. Overtime, data from the post-larval surveys could show success or failure of a spawning year, which could be incorporated into the fishery-dependant stock assessment model as a pre-recruitment index.

Over 60% of larvae were simulated to disperse outside of the eastern model boundary through Backstairs Passage as a result of a strong eastward current flowing through Investigator Strait. A previous hypothesis suggests that recruitment of King George Whiting in Victoria depends on seasonal wind forcing to disperse larvae from a western spawning ground, potentially in South Australia, to the Victorian nursery areas. Studies have predicted that larvae spawned in southern Spencer Gulf and Investigator Strait could potentially be transported up to 400 km to nurseries in the south-east of South Australia (Fowler et al., 2000b). It is likely a two-step process for the larvae that originate from the recognised spawning grounds in South Australia to reach settlement grounds in Victoria. Larvae supplied from the gulfs and Investigator Strait would settle in the south-east of South Australia and the adults of this population produce the larvae that advect eastward and settle to Victorian nursery areas. Furthermore, the influence or location of spawning grounds to the west of Spencer Gulf is unknown. During Austral autumn and winter the Leeuwin Current drives a warm water mass which flows eastward across the Great Australian Bight over the continental shelf waters of South Australia (Lenanton et al., 1991). The influence of this current and the potential for transported larvae spawned to the west of the study area is unknown. However, there is potential for the mixing of populations through these oceanographic processes.

### *Applicability of a DEPM to assess King George Whiting stocks*

A challenge for conducting a DEPM on demersal fish species has been obtaining adequate numbers of adult samples to accurately define the biological parameter inputs for the model (Fowler, 2000, Steer et al., 2017). A previous attempt of adapting the DEPM for King George Whiting found that a reliance on commercial fishery sampling introduced a bias in sample structure, and limited sample sizes due to the high costs in accessing adult samples was a major drawback in attempting to apply this method (Fowler, 2000). In an attempt to mitigate this issue, this study sourced adult samples from a combination of recreational fishers, commercial fisheries and scientific sampling. In collaboration with RecFish SA, a recreational fisher frame donation program was implemented to engage with the estimated 277,000 recreational fishers of South Australia. The objective was to request the donation of filleted King George Whiting frames to local fishing tackle stores in April of 2017 and 2018. Overall, the level of public engagement was positive with a total of 562 donated from 39 recreational fishers. Citizen science recreational catch sampling programs have been successfully implemented across Australia, including the NSW Research Angler program and the Western Australian, Send Us Your Skeletons program, and the Tassie Fish Frame Collection Program (Fairclough et al., 2014). Citizen Science based programs, are known to enable access to sampling over broader spatial scales than relying on fishery-dependent sources. This can also be beneficial for understanding the population biology of many non-commercially targeted, rare and cryptic species (Fairclough et al., 2014). In South Australia, engagement of the recreational sector with research has been limited to date. Small-scale, species specific programs have been conducted since the 1980s, such as, King George Whiting recreational fisher frame donations, the restocking of fresh water systems, and beach Mulloway (*Argyrosomus japonicus*) fishing frame deposit bins on the west coast and south-east of South Australia. The relative success of this recreational fisher frame donation program shows there is further possibility to extend this sampling method to other recreationally targeted species, such as, Snapper, Kingfish (*Seriola lalandi*), Black Bream (*Acanthopagrus butcheri*) and Yellowfin Whiting (*Sillago schomburgkii*).

Adult King George Whiting samples were also sourced from the commercial fishing sector through a routine market sampling program, and the collection of bycatch during the Spencer Gulf and Gulf St. Vincent Western King Prawn (*Melicertus latisulcatus*) observer surveys. A total of 255 samples were collected from both commercial fisheries over the two years; however, the majority of these fish (216 fish) were immature sub-adults captured in northern Spencer Gulf, which was outside of the plankton survey area. A further 106 large adult fish were collected during the plankton sampling cruise over both years within the spatial boundary of the DEPM.

Future DEPMs for King George Whiting should focus on obtaining a greater number of mature and, in particular, spawning females during the plankton sampling program. The total adult sample size of 454 fish (Table 5) collected within the boundary of the plankton survey area was inadequate to robustly define some biological parameters required to estimate a spawning biomass such as, batch fecundity and

spawning fraction. Limited numbers of late-stage spawning females were sampled during the plankton survey; therefore, the estimates of batch fecundity were primarily based on a previous study by Fowler in 1999 (Fowler et al., 1999). The estimates of spawning fraction was also constrained by the limited number of mature females sampled during both surveys, in particular during the scientific cruises. Due to the poor condition of gonads, histology was unable to be conducted on samples collected from the recreational fishers frame donation program and the commercial market sampling. An attempt to estimate spawning fraction through macroscopic analysis of King George Whiting gonads was unsuccessful. While the proportion of females in spawning condition can be determined by macroscopic analysis, the proportion of females spawning on a given day cannot be determined from these methods. Therefore a reliable estimate of spawning biomass could not be determined from the data available as they do not allow spawning condition to be determine accurately at a fine scale.

Further work is required to refine the inputs into the DEPM analysis which should involve a concentrated effort in obtaining a greater number of adult samples during the next DEPM to improve the accuracy of the spawning biomass. Obtaining more adult samples could be achieved by the continuation and expansion of the recreational catch sampling program and by enlisting commercial fishers to target King George Whiting during the plankton sampling. Furthermore, there was a clear spatial disparity identified in fish maturity between the northern and southern gulfs, with no spawning activity identified in fish outside of the southern gulfs and Investigator Strait. This supports the previous evidence and the results of this study that spawning only occurs in the southern gulfs and Investigator Strait. Therefore, efforts should be concentrated in obtaining maturity information for adult King George Whiting captured within this broader southern Spencer Gulf, Gulf St. Vincent and Investigator Strait regions.

This study attempted to maximise the likelihood of sampling King George Whiting eggs by utilising oblique plankton net tows rather than vertical tows. This method of plankton sampling was utilised due to the relative rarity of eggs in the water column identified in Fowler (2000). A refinement in plankton net towing technique between the 2017 and 2018 surveys resulted in a near doubling increase of average net tow distance from 109 m in 2017 to 205 m in 2018. As egg density is calculated to include distance travelled by the net through individual net flow metre readings, this improvement in technique did not affect the overall egg density estimates between years. However, it is probable that the longer net tow will increase the likelihood of encountering eggs in the water column. The egg counts from this study, similar to other demersal fish species are an order of magnitude lower than compared to a small pelagic species for which method DEPM was created (Steer et al., 2017). This may, in part, be due to the discrete and disparate spawning aggregations associated with the spawning strategies of demersal fish species. Many populations of demersal fish have a strong affinity to a particular habitat which then creates ‘hotspots’ of spawning activity as opposed to wide spread spawning activity over large spatial scales, as can be seen with small pelagic species. The rarity of King George Whiting eggs in the water column was considered as a major concern and precluded an attempt to estimate spawning biomass in the

previous trial of a DEPM for this species (Fowler, 2000). Furthermore, the rarity of eggs also further exacerbated by the difficulties in differentiating King George Whiting eggs based on morphological characteristics (Fowler, 2000).

This study has overcome previous issues with egg identification as it designed and validated an *in-situ* hybridisation technique to identify King George Whiting and larvae from mixed ichthyoplankton samples. This ISH technique was modified to suit King George Whiting after successful development of a similar probe for Snapper in South Australian gulf waters (Oxley et al., 2017). This technique uses a horseradish peroxidase (HRP) enzyme conjugated oligonucleotide probe to bind to King George Whiting mitochondrial 16S ribosomal RNA and generates a blue colour through oxidisation with a HRP reactive substrate. This technique is a relatively non-destructive method, still allows for developmental stages and ages of validated eggs to be determined after the probe is used. The accuracy of the probe was validated through a series of trials which also included larvae from the closely related, co-occurring Southern School Whiting. The 100% accuracy for differentiating these two species was critically important as field observations during the plankton survey indicated that Southern School Whiting were spawning simultaneously.

The creation of this probe has allowed the confident identification of King George Whiting eggs to be identified in mixed egg samples. King George Whiting eggs are similar to the eggs of many co-occurring demersal fish in size, oil globule dimensions and embryo development. In the development of the Snapper specific probe, misidentification rates of 19% to 80% were estimated based on egg morphology alone. A further 2% of eggs that were originally being categorised as 'unlikely' were confirmed as Snapper after ISH (Steer et al., 2017). However, there are limitations to the application of this technique, due to the robust nature of the eggs extracellular casing ('chorion'). The eggs needed to be manually ruptured, to enable the eggs to fully hybridise which is labour intensive. A consequence of this is that fewer egg development stages can be morphologically distinguished as some characteristics of egg development are affected through this process. For other species such as Snapper (*Chrysophrys auratus*), this was not an issue as these developmental stages lasted for similar durations. However, the second developmental egg stage for King George Whiting can last for an extended period, complicating age estimation for this stage. Future DEPM applications should consider separating this stage into more discrete stages that last for similar periods to others.

Although *in-situ* hybridisation is time consuming, its accuracy in egg identification alleviates any influence of error from misidentification and provides an unbiased validation technique. The successful development and implementation of this technique highlights the importance of 1) using a molecular based approach to accurately validate egg identification, and 2) applying ISH techniques to future DEPM studies where ambiguity in egg morphology exists.

### *Evaluation of DEPM for estimating Spawning Biomass (B)*

This study provides the most comprehensive application of DEPM to date for King George Whiting. As a demersal species, a tailored approach was required in order to apply the DEPM as this is an approach typically applied for small pelagic species. The most difficult issue to overcome for applying the DEPM to King George Whiting was the development of *in situ* hybridisation method to identify fertilised eggs, which overcame previous issues with egg identification. Additionally, the application an updated DEPM that includes a better description of population size structure (DEPMWt) provided much more precise estimates of preliminary biomass; demonstrating the continuing effectiveness this new technique has for demersal species (McGarvey et al. in review).

Despite overcoming the issue of egg identification and increase biomass precision achieved via the DEPMWt, there were several parameters of the DEPM that could not be resolved within this study. Therefore, no reliable estimate of biomass could be provided. The primary issue preventing biomass from being accurately estimated was the inability to estimate spawning fraction. Previous research has shown that King George Whiting can spawn up to every 3.6 days which corresponds to a spawning fraction of 0.28. However, sampling occurring at spawning events returned samples where every fish was actively spawning, providing a spawning fraction of 1 for that sample (Fowler et al., 1999). The consequence of this broad range of values and an inability to estimate an accurate spawning fraction during this study means that this parameter remains unresolved. This is an issue as the sensitivity analysis conducted here demonstrates that the low end of this range would estimate biomass as 213,914 kg in 2018 whereas the upper end of this range would estimate biomass as 725,782 kg in 2018. This is a difference of more than a factor of three – further demonstrating the importance of the accuracy of this parameter.

While a biomass estimate cannot be provided from the data available, there is opportunity for estimates of $P_0$, $A$, $R$, $F_w$ and $p_w^{\mathrm{N}}$ to be used to estimate biomass in 2017 and 2018 once a more robust estimate of $S$ becomes available. Recent research on the South Australian Sardine Fishery, where DEPM has been conducted for 15 years, shows that parameters such as spawning fraction can remain relatively stable over time with small variations from year to year (Ward et al., 2020). Therefore, if spawning fraction could be estimated robustly in future years for King George Whiting, it would be possible to retrospectively apply these values (with a high level of caution) if they are stable over time. This would likely require several years of data to determine but would be worthwhile if DEPM surveys were to be conducted for future assessments.

The DEPM can be continued in future King George Whiting assessments, as long as adult sampling can be undertaken comprehensively. This should include the sampling of adult females on the spawning grounds as well in other regions within Spencer Gulf and Gulf St Vincent. Ideally this would be undertaken by research surveys or dedicated commercial sampling where all available samples are

provided for histology. This would allow for updated estimates of batch fecundity and spawning fraction as well as providing sufficient data to describe the population weight structure for females. These estimates of spawning biomass would be valuable inclusions into the King George Whiting integrated stock assessment (WhitEst) as they provide a fishery-independent estimate of biomass, relieving the reliance on commercial catch and effort data which only contribute to half of the total catch. Therefore, while biomass could not be estimated in this study, there is sufficient evidence to demonstrate that the DEPM is a viable assessment option. Additionally, this study provides advice on how to overcome sampling limitations and accurately estimate spawning biomass for King George Whiting. This provide would provide important information for the continuing management of the resource.

### *Management Strategy Considerations*

Following the 2014 King George Whiting gulf stocks status assessment of 'transitional-depleting', a suite of management regulations were enhanced in December 2016 (Fowler et al., 2014). These changes included the implementation of a spatial closure in to protect the spawning King George Whiting stocks in southern Spencer Gulf and Investigator Strait from 01 to 31 May 2017. Although the spawning spatial closure was implemented as a management tool to promote the recovery of the resource, the relative benefit of its size and location in protecting spawning productivity was poorly understood. Therefore, an objective of this study was to evaluate the effectiveness of the spatial closures by assessing their overlap with the observed spawning activity and the predicted areas of spawning with increased settlement success from the bio-physical model.

The initial King George Whiting spawning spatial closure that was first implemented in 2017 provided the most expansive coverage. This closure covered over half the interpolated spawning area in both years and most peaks in egg densities, aside from two peaks outside of its boundary in the 2017 (Figure 25 and Figure 26). This larger closure covered 63% in 2017 and 90% in 2018 of the spawning areas which had greater than 10% total successful settlement for particles released. The spatial closure for spawning King George Whiting went through a process of review and was subsequently spatially refined for 2019. This refined closure was then implemented from the 1 to 31 of May 2019 and was reduced at both the eastern Gulf St. Vincent and western Spencer Gulf ends of the closure. This refined spatial closure covered the peaks in spawning activity and key areas of settlement success which occurred in Investigator Strait in 2018. However, the spatial variability in spawning activity between plankton surveys saw the refined closure exclude the areas of increased spawning activity and settlement success in southern and south-east Spencer Gulf. The south-east of Spencer Gulf region has been previously identified as an important area for spawning activity for King George Whiting in Spencer Gulf (Fowler et al., 1999).

Although this study improved on our understanding of the spawning dynamics of King George Whiting in the southern Gulfs and Investigator Strait, the snapshot nature of the egg surveys does not allow for the interpretation of spatiotemporal variations in spawning activity. It is unclear if this shift in peak

spawning activity was an annual shift or a reflection of fine-scale spatiotemporal variability. Following the results of this study, it is clear that the broader southern Spencer Gulf and Investigator Strait are important spawning grounds and are likely to be integral to the replenishment of the gulfs King George Whiting stocks. Therefore, due to the limited understanding of how spawning activity changes within this broader area over the prolonged spawning period, a conservative approach to implementing an effective closure may provide the greatest benefit to King George Whiting stocks. The resumption of the initial larger closure which offered the most protection to important spawning grounds for King George Whiting could potentially be the best option until further resolution of the spatiotemporal variability in spawning in established.

Additionally, the southern gulf regions are exposed to large-scale oceanographic variations, as the breakdown of the thermohaline frontal systems allows for the resumption of gulf and open water exchange during the King George Whiting spawning period. It is unclear how the temporal changes in environmental conditions impacts spawning and further work is required to resolve these issues. Furthermore, spawning closures should coincide with peak spawning activity. This time band is challenging to identify for King George Whiting as historically, the peak in spawning has occurred in April (Fowler et al., 1999). However, in such a dynamic oceanographic environment it could potentially vary annually depending on the prevailing environmental conditions. To resolve this uncertainty, increasing the frequency of DEPM surveys over a single spawning season and conducting the bio-physical model incorporating the observed egg densities from each survey would likely reveal the shifts in spawning activity and settlement success across the broader southern gulfs and Investigator Strait. The periodic sampling of mature adult King George Whiting across the greater spawning region, during the peak of spawning, would enable the identification of the peak in spawning activity. This could be an extension of the adult sampling program initiated by this study, with greater emphasis on the engagement of charter, recreational and commercial fishing sectors.

Future developments of the fishery-dependent WhitEst model could incorporate the provisional spawning biomass estimates derived from future DEPMs. However, the DEPM spawning estimates were calculated for the entire spawning area, which encompasses both Spencer Gulf and Gulf St. Vincent/ Investigator Strait stocks. Currently, the gulf stocks are assessed separately in the fishery-dependent WhitEst model as they are considered to be discrete stocks due to limited fish movement between the regions as determined from tag recaptured (McGarvey and Feenstra 2002). The predicted larval pathways estimated from the bio-physical model suggest that there is low levels of connectivity between southern Spencer Gulf and Gulf St. Vincent stocks. This may require a reconsideration of the scale to which these stocks are assessed, which will be dependent on the relevance and magnitude of connectivity between stocks.

The implementation of the initial spawning spatial closure as a management strategy was successful in the consideration of its scale, overlap with key spawning locations, and areas of increased settlement

success. The refined closure, omitted several key areas of increased egg densities and spawning areas of increased settlement success. A reconfiguration of the existing spatial closure for King George Whiting to include the relevant spawning dynamics information identified through this study would likely lead to increased protection of this stock. Incorporating the southern and south-eastern Spencer Gulf would potentially benefit the spawning success, as both of these areas were consistently identified as important areas for spawning and settlement success. There are numerous flow-on benefits for the protection of a spawning stock through the peak of spawning activity. Ideally, the protection of a stock during peak spawning time would increase recruitment back into the gulf systems. Increased recruitment would likely boost the replenishment of stocks and improve levels of fishable biomass. What is not considered is the influence of environmental conditions on larval advection, in particular for a species with a long larval pre-settlement phase such as King George Whiting. Irrespective of the implementation of a spawning closure, unfavourable environmental conditions at the time of spawning can potentially negate any increase in spawning activity during a short-term spatial closure, potentially resulting in a reduction in recruitment.

# Conclusion

This study improved our knowledge of the spawning dynamics and larval connectivity of King George Whiting in South Australia's southern gulfs and Investigator Strait. The development and application of a molecular *in-situ* hybridisation technique enabled the accurate identification of King George Whiting eggs and larvae. This method removed the potential for bias through misidentification, and demonstrated the importance of molecular techniques when morphological identification is ambiguous. King George Whiting eggs were distributed in low densities across a broad spawning area, and peaks in spawning activity was identified in southern Spencer Gulf and Investigator Strait over alternating years. However, very low levels of spawning activity was identified for southern Gulf St. Vincent in both years, indicating that the majority of spawning occurs in southern Spencer Gulf and Investigator Strait. This was further supported by the results of the bio-physical model, which predicted that the south-east of Spencer Gulf supplied both gulfs stocks with larvae and that spawning in Investigator Strait fed directly into the Gulf St. Vincent stock and potentially the south-east of South Australia. The culmination of these results emphasises the important role that the southern Spencer Gulf and Investigator Strait play in the supply of recruits and replenishment of both gulfs King George Whiting stocks. This increased knowledge of the spawning dynamics for this species has enabled the evaluation of strategic management options to enhance the protection of the spawning stock. Spatial closures were implemented and assessed to offer adequate protection for the spawning stock, based on key spawning locations, larval trajectories and regions of settlement success. This study suggests the continuation of spawning closures at the peak of spawning activity could aid in the replenishment of recruits into the gulf stocks. The DEPM can certainly

be applied for King George Whiting. However, this research highlights the importance of key considerations (adult sampling and spawning fraction) that must be addressed in future applications and further addressed as a priority if this technique was to be continued and integrated into future stock assessments. If an estimate of spawning fraction can be determined in future research that is appropriate for 2017 and 2018, then the combination of these data can be used to retrospectively estimate spawning biomass in these years. This could be attained through the future application of the DEPM for these stocks, which this study demonstrates as a viable assessment method.

# Implications

The implications from this research will be wide spread, extending from charter boat operators, commercial and recreational fishers to fisheries managers. Following on from the development of the species-specific molecular probe that was created for Snapper (FRDC 2014/019) (Steer et al., 2017), this study adapted and developed an *in-situ* hybridisation technique for positively identifying King George Whiting eggs and larvae out of mixed plankton samples. The successful adaption of this technique to King George Whiting enabled the positive identification of eggs, which are difficult to distinguish from co-spawning species. This study has highlighted the capability of molecular techniques in providing an unbiased approach to the identification of a target species, which should be applied when egg or larvae identification is ambiguous.

Key spawning locations for King George Whiting were identified in southern Spencer Gulf and Investigator Strait through the combination of egg abundance information and oceanographic modelling. Predicted larval pathways and modelled particle settlement success indicated that these regions of increased spawning activity play an integral role in the replenishment of both gulfs King George Whiting stocks. These results will be beneficial for fishery management as they provide critical early life history information required to assess the importance of these regions when implementing management strategies. As King George Whiting in South Australia is a highly valued shared resource by all fishers, the flow on effect of management decisions, such as, spatial closures, can have large ramifications for all (commercial, recreational and charter boat) sectors. The initial spatial closure, which was implemented in May 2017 and continued in May 2018, encompassed the majority of key spawning locations, modelled areas of settlement success, and prohibited any commercial, charter boat and recreational fishing within this zone. In 2019, the closure was further refined and omitted areas which were identified to be important for spawning King George Whiting. The combined results of this study can be used to inform the current and future management arrangements for South Australia's King George Whiting stocks.

# Recommendations

It is recommended that the results of this research be broadly distributed to PIRSA Fisheries and Aquaculture; Marine Fishers Association (commercial fishery representatives); The Gulf St. Vincent and Spencer Gulf Prawn associations; the commercial, Recreational and Charter Boat sectors of the South Australian Marine Scalefish Fishery; Victorian Fisheries Authority; WA Fisheries; national and international fisheries scientists; general public.

## Further development

The impact of spatiotemporal variations in spawning dynamics is currently unknown. A notable shift in peaks of spawning activity was identified between years. However, it is uncertain if this shift in spawning activity reflects short-term variations or a seasonal shift in activity. This uncertainty in spawning locations makes setting spatial closures challenging for fishery managers. In future DEPM assessments for King George Whiting, this uncertainty could be resolved by conducting multiple surveys across the spawning period to identify peaks and spatiotemporal shifts in spawning activity.

The only methodological refinement required to apply the DEPM to King George Whiting is the refinement of the second developmental egg stage. Presently, this development stage is far longer than other stages and therefore needs to be separated into multiple stages. Addressing this will then provide methods to estimate spawning biomass of King George Whiting if a more comprehensive data on adults can be collected. This is required to better estimate spawning fraction. If subsequent estimates of spawning fraction are determined over several years, and these estimates remain similar over times, then these can be used as surrogate values to retrospectively determine estimates of spawning biomass in 2017 and 2018, based on the present study.

# Extension and Adoption

There will be considerable engagement and extension of results from this project with PIRSA Fisheries and Aquaculture and commercial and recreational fisheries regarding the relevance of future spatial spawning closures and the potential for the integration of the DEPM into future stock assessments for King George Whiting.

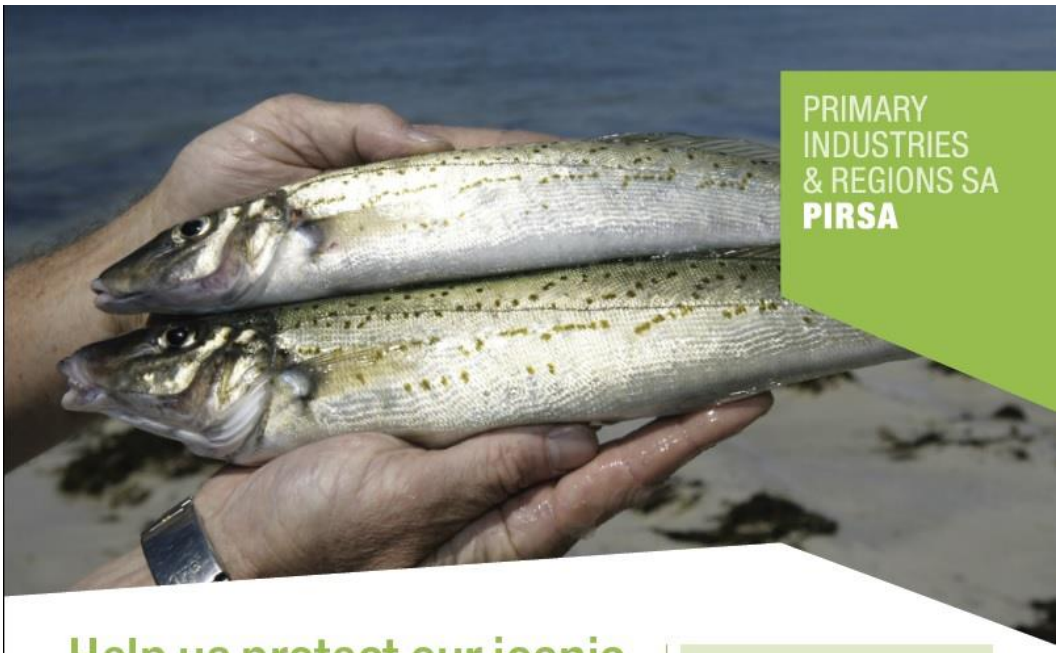
## Project coverage

2 August 2017: Chanel 10 Scope TV series featuring King George Whiting citizen science program

2017: ABC Radio program. King George Whiting spatial closure information.

# Appendix 1 Project materials developed

Flyers and posters were developed for the engagement of the recreational sector for both years in 2017 and 2018.




## Help us protect our iconic King George Whiting in SA

King George Whiting stocks are under pressure in SA waters.
Take part in new research to better understand this premium species and ensure a sustainable King George Whiting fishery for the future.

- **Collect** a form from your local tackle shop
- **Catch** and fillet your King George Whiting between 1-30 April 2018
- **Save/freeze** the frame (skeleton including head, tail and guts intact)
- **Return** the frame and completed form in a bag to your local tackle shop

Participating tackle shops: **www.pir.sa.gov.au/kgwresearch**

| FISHING LIMITS | Min Size (cm) | Bag Limit (per person) | Boat Limit (2 or more people onboard) |
|---|---|---|---|
| King George Whiting | | | |
| East of 136°E (including all Gulf waters) | 32 | 10 | 30 |
| West of 136°E | 30 | 10 | 30 |

**King George Whiting spatial closure: 1 - 31 May 2018**

An area of southern Spencer Gulf, southern Gulf St Vincent and Investigator Strait will be closed to protect this key spawning area during a critical reproductive period.

## WIN!

Return your King George Whiting frames for research to WIN great prizes.

Supported by:

WILSON

SHIMANO

Strike 1

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

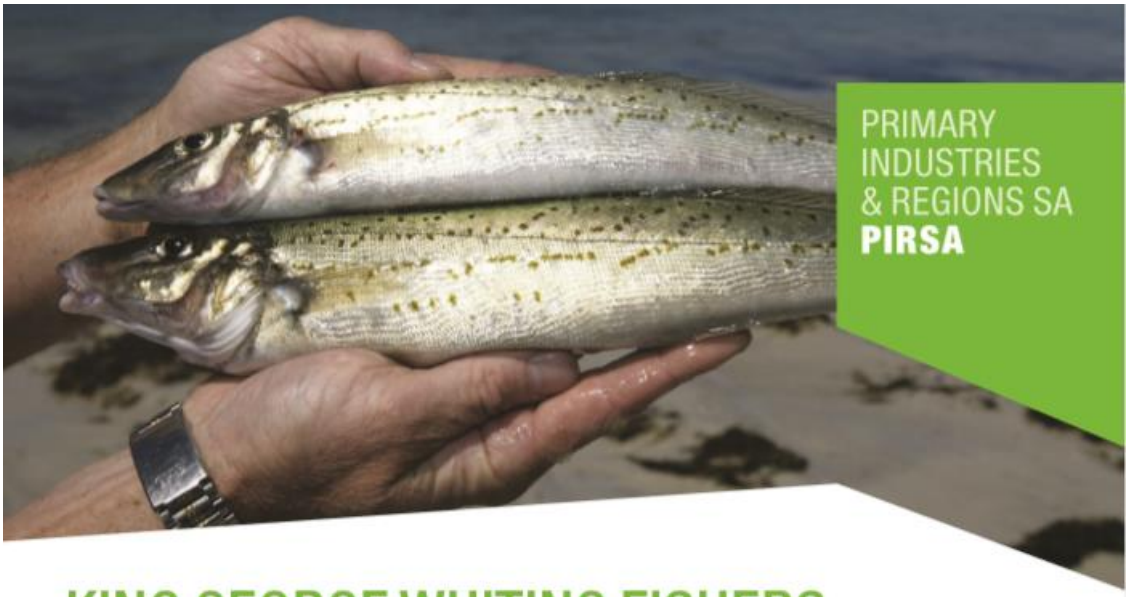
RecFish SA

FRDC Fisheries Research & Development Corporation

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

SARDI Research and Development




# KING GEORGE WHITING FISHERS – WE NEED YOU!

- Primary Industries and Regions SA (PIRSA) and RecFish SA need your filleted King George Whiting (KGW) frames in April 2018
- The project is being led by the South Australian Research and Development Institute, a division of PIRSA, in partnership with RecFish SA. The major funder is the Fisheries Research and Development Corporation
- Participants will go into a draw to win prizes courtesy of Strike 1 Fishing Charters, Wilson, and Shimano
- Your donated frames will help to improve biological knowledge on KGW and inform future management decisions
- KGW frames must have the **gut intact** with catch and contact details in the bag
- Use a lead pencil to fill out all personal details and mark the catch location on the map

For drop off locations go to **www.pir.sa.gov.au/kgwresearch**

WILSON SHIMANO Strike 1

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT RecFish SA FRDC SOUTH AUSTRALIA Government of South Australia Primary Industries and Regions SA SARDI RESEARCH AND DEVELOPMENT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**King George Whiting spatial closure: 1- 31 May 2017**
An area of southern Spencer Gulf, southern Gulf St Vincent and Investigator Strait will be closed to protect this key spawning area during a critical reproductive period.

## WIN!

Return your King George Whiting frames for research to WIN great prizes.

Supported by:

ENGEL
A LEGEND IN RELIABILITY

SHIMANO

Strike1

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

RecFish SA

FRDC

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

SARDI RD&E

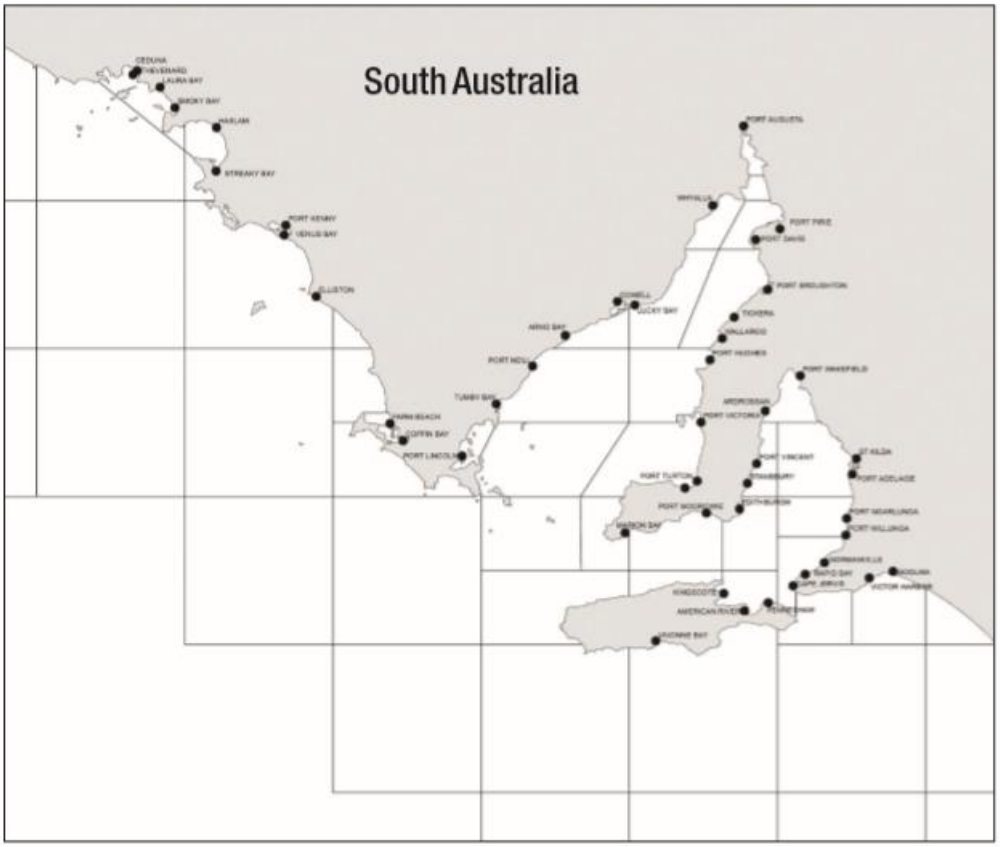
## Use a lead pencil to fill out all personal details and mark the catch location on the map of South Australia.

Name: _____

Date captured: _____

Email or phone: _____

Location: _____



**The supported PhD thesis and four scientific papers have been produced from a related PhD which was based on this work and are listed below.**

Rogers, T. A. (2019). The early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) in South Australia's gulf system. Doctor of Philosophy, University of Adelaide, Adelaide, South Australia, Australia. 201 pp.

Rogers, T. A., Fowler, A. J., Steer, M. A., & Gillanders, B. M. (2019). Resolving the early life history of King George whiting (Sillaginodes punctatus: Perciformes) using otolith microstructure and trace element chemistry. *Marine and Freshwater Research*.

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# Appendices

## APPENDIX 2: References


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## APPENDIX 2: Staff

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- Dr Andrew Oxley (Co-Investigator) (SARDI Molecular Ecology Sub-program Leader)
- Dr Mark Doubell (Co-Investigator) (SARDI Oceanography Sub-program Leader)
- Dr Tony Fowler (Co-Investigator) (SARDI Principle Scientist Finfish)
- Dr Michael Drew (SARDI Senior Research Officer)
- Dr Ana Redondo Rodriquez (SARDI Senior Research Services Officer)
- Dr Sarah Catalano (SARDI Senior Research Services Officer)
- Mr Damian Matthews (SARDI Research Services Officer)
- Dr Troy Rogers (SARDI Senior Research Officer)
- Dr Jonathan Smart (SARDI Research Scientist)
- *FV Ngerin* crew: Mr Darren Nohlmans, Mr Jason Nichols, Mr Andrew Sellick, Mr Chris Small