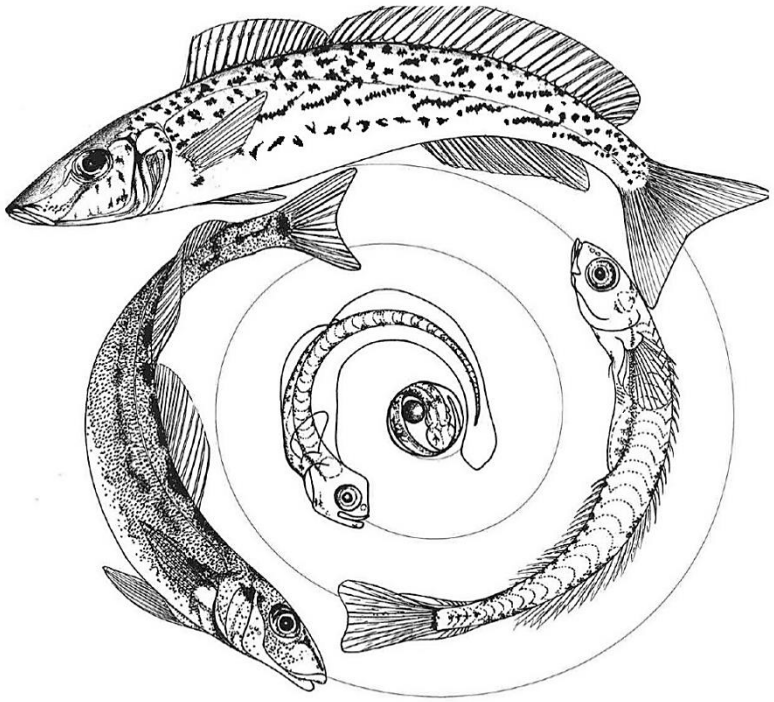
THE EARLY LIFE-HISTORY OF KING GEORGE WHITING (*Sillaginodes punctatus*: Perciformes) IN SOUTH AUSTRALIA'S GULF SYSTEM

TROY ADAM ROGERS



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DECLARATION

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Troy Adam Rogers
Submitted for examination 19 November, 2019.

Cover image: Life-history stages of King George whiting (*Sillaginodes punctatus*: Perciformes), by Paul Jennings. Reproduced with permission.

FRONTISPIECE

I dedicate this work to my parents, Paul and Meredith Rogers. The lifestyle that they have provided for my brother and I has undoubtedly resulted in my passion for the marine environment and the animals that occupy it. The countless family holidays, fishing trips and time spent around the South Australian coastline have shaped who I am today. I am eternally grateful for the sacrifices that they have made throughout their lives to give me the best opportunities to succeed.

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And finally, I dedicate this work to the 6,509 recently-settled King George whiting larvae that survived the gauntlet of physical and ecological pressures during larval dispersal to undergo settlement, only to end up in my sampling net. Their otoliths have been very valuable.

Photo: Meredith Rogers

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ABSTRACT

The life-history of many marine fish species involves a pelagic larval stage that connects spatially segregated spawning grounds and nursery areas. The dispersal of larvae in marine ecosystems is heavily influenced by physical oceanographic processes, which provide the potential for large-scale transport and mixing between groups of larvae that originated from different spawning grounds. Understanding the connectivity between these different populations identifies the spatial scale over which the life-history operates, and is necessary to delineate populations into the appropriate stock structure. This biological information underpins the development of effective fishery management strategies.

King George whiting (*Sillaginodes punctatus*; Perciformes) is a demersal marine finfish species endemic to temperate coastal waters of southern Australia, and conforms to the general bi-partite life-history cycle of most demersal fishes. South Australia is at the geographic centre of its distribution and supports the highest abundances and its most significant fishery. However, in recent years, commercial catches and estimated biomass in South Australia's gulfs have declined to record lows, and the populations were subsequently classified as 'transitional depleting'. Despite extensive research into the life-history of this species, there remains considerable uncertainty about the spawning sources, population connectivity and early life-history processes that ultimately culminate in recruitment. As such, the aim of this study was to understand the early life-history of King George whiting in South Australia's gulf system, and specifically, to investigate the connectivity between coastal spawning grounds and inshore nursery areas.

King George whiting is a multiple batch spawning species that produces large numbers of pelagic eggs throughout a protracted spawning season (ca. 4 months). Larvae that hatch at different times are exposed to different physical and ecological conditions during ontogeny that influence survivorship and subsequent recruitment. To investigate the temporal nature of recruitment throughout the settlement season, recently-settled larvae were collected fortnightly between July and November at a significant nursery area in Gulf St. Vincent. These larvae hatched between March and July, although a three week spawning period in May was responsible for >50% of recruitment. Throughout the settlement season, the recently-settled larvae progressively decreased in size (range: 16.1-25.3 mm SL) but increased in age (range: 92-184 d). As such, smaller, slower-growing larvae that experienced a longer pelagic phase were responsible for the majority of recruitment. In addition, otolith chemistry related to the natal origin of these larvae differed significantly between those that hatched from March to May, and those that hatched from May to July. There are two primary hypotheses to explain this: Either (1) within-season environmental change at a single spawning ground; or (2) the contribution of two different spawning grounds to recruitment at different times of the settlement season.

For demersal fish species, understanding connectivity during the larval phase is necessary to determine the spatial scale over which the life-history operates, as this is the spatial scale at which populations are considered ecologically discrete. To evaluate spatial connectivity and stock structure, recently-settled larvae were collected from nursery areas throughout Spencer Gulf and Gulf St. Vincent. Regional differences in the natal otolith chemistry of larvae that hatched at the same time indicated that the two regions are replenished by different spawning populations, and provide empirical support for the hypothesis that the populations of King George whiting in Spencer Gulf and Gulf St. Vincent represent discrete sub-populations.

The only recognised spawning area for King George whiting in south-eastern Australia is throughout southern Spencer Gulf and Investigator Strait. The otoliths of larvae collected throughout the recognised spawning area were examined to determine if the large spawning area represented a single spawning population or multiple discrete spawning grounds. The spatial distribution of larvae was broadly divisible into two groups – those in southern Spencer Gulf and those in Investigator Strait. There were no spatial differences in the sizes (3.0-5.0 mm SL), ages (5-21 d), hatch dates (7-24 Apr) or growth rates (0.09-0.21 mm d^{-1}) of larvae. However, otolith chemistry differed significantly between the two groups, providing empirical evidence that southern Spencer Gulf and Investigator Strait represent two independent spawning grounds.

Having determined that larvae which settled to nursery areas in Spencer Gulf and Gulf St. Vincent had originated from different spawning grounds, and that the recognised spawning area is comprised of two discrete spawning grounds, connectivity between them was investigated by simulating larval dispersal using a biophysical model. The model was seeded with particles according to the distribution and abundance of eggs throughout the spawning area and dispersal was simulated using three increasingly complex behavioural models. Predicted settlement was highest to nursery areas only short distances from regional spawning grounds, which indicated that population processes were localised within each gulf. However, the model also predicted that later in the spawning season, larvae originating in southern Spencer Gulf contributed to recruitment in Gulf St. Vincent. The within-season change in dispersal pathways corresponded to the breakdown of a thermohaline frontal system at the entrance of each gulf in early May, which is consistent with spatial and temporal patterns in the otolith chemistry of larvae. Consequently, the most parsimonious explanation is that the populations of King George whiting in South Australia's gulf system constitute a single, panmictic stock. The population connectivity identified in this study has implications for the understanding of stock structure, and subsequently, the spatial scale at which fishery management should be applied.

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PUBLICATIONS AND CONTRIBUTIONS

Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry

Authors: [1]Rogers TA, [2]Fowler AJ, [2]Steer MA and [1]Gillanders BM.

Status: Published in *Marine and Freshwater Research*, **70**: 1659-1674.

Spatial connectivity during the early life history of a temperate marine fish inferred from otolith microstructure and geochemistry

Authors: [1]Rogers TA, [2]Fowler AJ, [2]Steer MA and [1]Gillanders BM.

Status: Published in *Estuarine, Coastal and Shelf Science*, **227**: 106342.

Discriminating natal source populations of a temperate marine fish using larval otolith chemistry

Authors: [1]Rogers TA, [2]Fowler AJ, [2]Steer MA and [1]Gillanders BM.

Status: Published in *Frontiers in Marine Science*, **6**: 711.

Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish

Authors: [1]Rogers TA, [2]Redondo-Rodriguez A, [2]Fowler AJ, [2]Doubell MJ, [2]James C, [2]Drew MJ, [2]Steer MA, [2]Matthews D and [1]Gillanders BM.

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Chapter 1 – General Introduction

1.1 GENERAL LIFE-HISTORY OF MARINE FISHES

Many marine fish species conform to a bi-partite life-history that involves two distinct phases (Blaxter 1969, Houde & Hoyt 1987, Cowen & Sponaugle 2009). The first is the egg and larval stage. This life-history phase covers the development of an embryo inside of an egg that hatches as a larva, and includes all stages of larval development until the larva attains full external meristic characters (including fins and scales) and undergoes settlement (Neira et al. 1998, Leis & Carson-Ewart 2004) (Fig. 1.1). Throughout this early life-history, which can vary in duration from weeks to months, the developing larvae occupy the pelagic environment and reside within a dynamic planktonic community (Blaxter 1969, Houde & Hoyt 1987, Cowen & Sponaugle 2009). In general, larvae of marine fish species are very small and vulnerable, and at least initially, have poor swimming and behavioural capabilities that gradually develop during ontogeny (Leis 2006, Houde 2008). As such, the movement of larvae can be heavily influenced by physical oceanographic processes that include tidal currents, eddies, and wind-driven circulation, which provides the potential for dispersal over large spatial scales (Norcross & Shaw 1984, Cowen et al. 2000, Leis 2006).

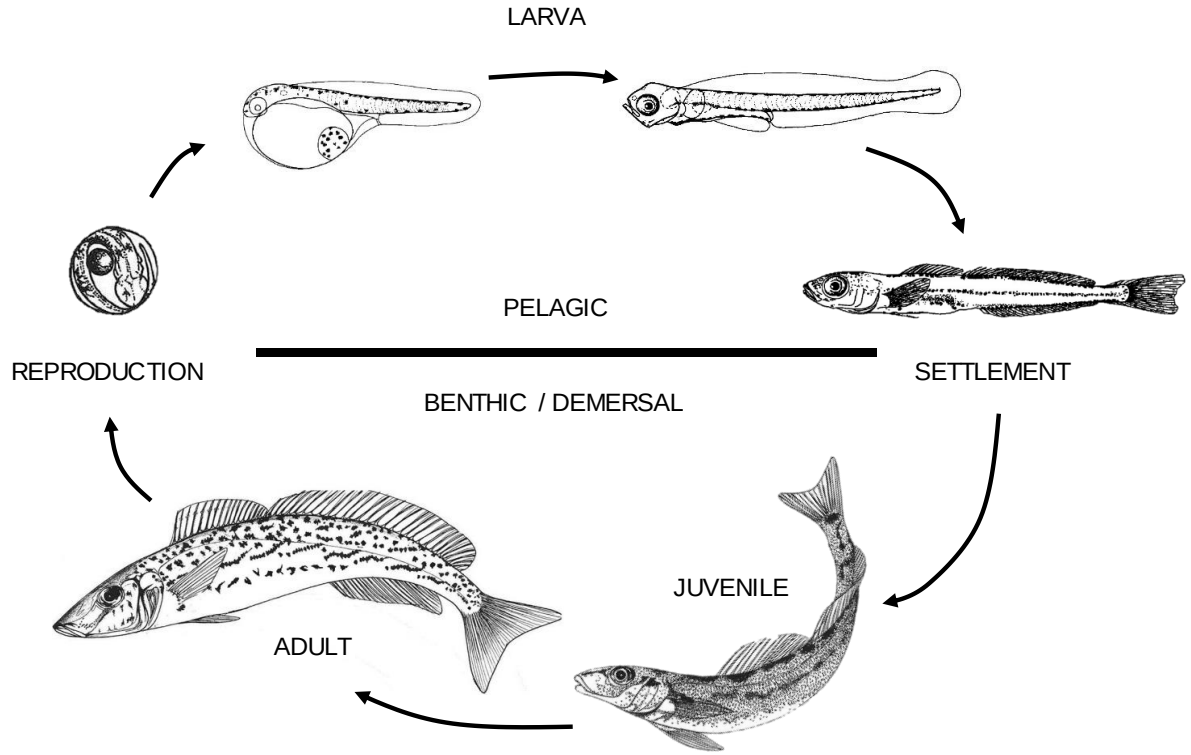

Fig. 1.1. General bi-partite life-history cycle of many marine teleost fish species. The early life-history phase involves the egg and developing larval stages which occupy the pelagic environment, and can range in duration from weeks to months. When adequately developed and in a suitable habitat, larvae settle to the benthic environment and develop into juveniles. The juveniles can reside in the nursery areas for some time, and eventually grow into adults which contribute to future generations.

Once adequately developed and in an appropriate habitat, the surviving larvae undergo settlement, which involves a transition from the pelagic to benthic environment as they continue to develop as juvenile fish (Blaxter 1969, Houde & Hoyt 1987, Cowen & Sponaugle 2009) (Fig. 1.1). This environmental transition often involves significant metamorphic change to body form and pigmentation, as juveniles begin to develop the morphological characteristics of their adult phenotype (Neira et al. 1998, Leis & Carson-Ewart 2004). The benthic environments suitable as nursery habitats for settlement are species-specific, but in general, offer abundant larval prey and protection from predators. As such, the life-history can involve ecological separation between life-history stages, including geographic separation between spawning grounds and nursery areas. Juvenile fish can remain in these nursery areas for some time as they develop into sub-adults, and eventually grow into adults that contribute progeny to future generations. For some species, particularly temperate marine fishes, the development of juveniles to adults to complete the life-history cycle can involve considerable movement to a benthic environment suitable for spawning (Fowler et al. 2000b, Jenkins et al. 2000). For other species, such as tropical reef fishes, the entire life-history can operate over a much smaller spatial scale (Swearer et al. 1999, Swearer et al. 2002, Jones et al. 2005).

1.2 FACTORS INFLUENCING RECRUITMENT OF FISHES

The pelagic larval stage is a critical period in the life-history of marine fishes (Hjort 1914, May 1974, Houde 2008). This life stage is characterised by large numbers of very small and vulnerable propagules that experience extremely high mortality rates. As such, small variations in larval survivorship can result in significant variation in recruitment, and the subsequent abundances of juvenile and adult fish (Hjort 1914, Houde & Hoyt 1987, Doherty & Fowler 1994, Houde 2008). Consequently, recruitment, defined in this study as the addition of recently-settled larvae to a population (Cowen & Sponaugle 2009), can vary drastically at different spatial and temporal scales. The relationship between larval abundance and adult population size was first proposed by Hjort (1914). He hypothesised that larvae must commence exogenous feeding before a ‘critical period’ soon after yolk-sac absorption, otherwise they would reach a point of no-return and subsequently die. As such, Hjort proposed that larval survivorship was likely to be poor when feeding conditions were not suitable, which was then reflected in the size of the adult population. Over the last century, Hjort’s ‘critical period’ hypothesis has formed the basis of many related prey-driven hypotheses concerning recruitment variability. For example, Cushing’s match-mismatch hypothesis proposed that strong recruitment would occur when the peak abundance of larvae coincided with high primary production (Cushing 1969, 1990). Others have suggested a series of meteorological and oceanographic processes that lead to concurrently high primary production and larval fish abundance, which in turn, results in improved larval survivorship and strong recruitment (e.g. Lasker 1978, Cury & Roy 1989).

Since the publication of Hjort's 'critical period' hypothesis over 100 years ago (Hjort 1914), recruitment variability and the factors responsible have been a central topic of fisheries science (Houde 2008). Throughout this time, an extensive body of research has demonstrated that recruitment is not strictly controlled by larval abundance and prey availability (Anderson 1988, Leggett & Deblois 1994, Cowan & Shaw 2002), but that other factors including predation (Bailey & Houde 1989, Leggett & Deblois 1994), environmental conditions (Blaxter 1992, Myers 1998, Humphries et al. 1999, King et al. 2003), and biological characteristics (Houde & Hoyt 1987, Pepin & Myers 1991, Bergenius et al. 2002) can also considerably influence recruitment. Consequently, it is now widely accepted that recruitment variability in fish populations reflects the complex interactions of numerous intrinsic and extrinsic factors throughout the egg and larval stages, which influence survivorship and subsequent recruitment (Leggett & Deblois 1994, Cowan & Shaw 2002, Houde 2008). In a review of larval recruitment processes, Houde (2008) summarised that recruitment variability in fish populations was largely regulated by five dominant mechanisms: (1) water temperature; (2) physical processes and features (i.e. circulation patterns, hydrographic variability); (3) larval food availability; (4) predation; and (5) individual biological characteristics (i.e. sizes and growth rates).

An understanding of the complex relationships between the physical environment and the biological processes that influence recruitment is necessary to interpret changes in fish populations. This is because temporal variation in recruitment becomes manifested in the age structures of populations (Victor 1986, Doherty & Fowler 1994, Berkeley et al. 2004), whilst spatial variation in recruitment influences population dynamics and the persistence of sub-populations (Caley et al. 1996, Cowen et al. 2000, Kerr et al. 2010). As such, fluctuations in population size and biomass largely reflect variation in the survivorship of eggs and larvae, which is ultimately mediated by the prevailing physical and ecological conditions (Houde 2008). Sinclair's member-vagrant hypothesis.

1.3 LARVAL TRANSPORT

The distribution and abundance of fish larvae is heavily influenced by transport from physical oceanographic processes (Norcross & Shaw 1984, Cowen et al. 2000, Houde 2008). These physical processes, which include tidal and residual currents, frontal systems, up- and down-welling, and wind stress, can act to disperse larvae away from where they were spawned, or retain them near the site of their natal origin (Norcross & Shaw 1984, Caley et al. 1996, Jones et al. 2005). For many demersal species, coastal spawning grounds and inshore nursery areas are spatially segregated, and therefore larval dispersal is an obligate process that connects life-history stages (Houde & Hoyt 1987, Cowen & Sponaugle 2009). For such species, recruitment variability is not only governed by larval survivorship relating to numerous biotic and abiotic factors, but also depends on physical processes to transport larvae to suitable habitats for settlement (Norcross & Shaw 1984, Jenkins et al. 1997, Houde 2008).

Eggs and early-stage larvae are predominantly passive, and therefore there is the potential for these life stages to be transported considerable distances from where they were spawned by the prevailing physical environment (Norcross & Shaw 1984, Houde 2008, Cowen & Sponaugle 2009).

Fish larvae develop swimming, sensory and behavioural capabilities during ontogeny that enable them to actively influence dispersal (Atema et al. 2002, Fisher 2005, Leis 2010). Late-stage larvae of many marine species have well-developed swimming abilities, with most capable of sustained swimming at speeds which exceed the prevailing currents (Fisher 2005, Leis 2006, 2010). Furthermore, late-stage larvae can also use visual, auditory and olfactory cues to establish orientation and travel towards settlement areas (Leis 2006). In doing so, larvae are capable of using physical oceanographic processes to reach settlement areas. Examples of such behaviour include utilising boundary layers to move in and out of currents (Norcross & Shaw 1984, Breitburg et al. 1995), vertical movement to increase food availability (Munk et al. 1989, Neilson & Perry 1990), using tidal fronts and streams to promote dispersal or retention (Kingsford et al. 1991, Lough & Manning 2001), and selecting tidal currents to optimise ingress into estuaries and nursery areas (Luettich et al. 1999, Hare et al. 2005, Teodosio et al. 2016). Large-scale larval transport from offshore spawning grounds to coastal areas is largely driven by oceanographic currents and wind stress which disperse early-stage larvae. Once near the coast, late-stage larvae use a suite of sensory cues to detect potential nursery areas and undergo settlement (Leis 2007, Teodosio et al. 2016). As such, the settlement of fish larvae has been correlated with numerous physical environmental parameters that include: wind stress (Shenker et al. 1993, Jenkins et al. 1997, Bergenius et al. 2005, Schlaefer et al. 2018); river discharge plumes (Kingsford & Suthers 1994, Grimes & Kingsford 1996); the lunar and tidal cycle (Jenkins & Black 1994, Findlay & Allen 2002, Saunders 2009); and solar radiation (Bergenius et al. 2005).

1.4 POPULATION CONNECTIVITY AND STOCK STRUCTURE

Connectivity, defined in this study as the exchange of individuals among populations (Cowen & Sponaugle 2009), is a central topic in marine ecology and is fundamental to understanding the stock structure of fish populations (Begg & Waldman 1999, Cowen et al. 2000, Cowen & Sponaugle 2009). Many marine fish species with a bi-partite life-history cycle conform to a meta-population structure, where relatively sedentary adults form spatially discrete sub-populations that are connected by a dispersive larval stage (Levins 1969, Harrison 1991, Bailey 1997, Secor & Rooker 2005, Cowen & Sponaugle 2009). The distribution of sub-populations within a meta-population is predominantly forced by the patchiness of suitable habitat (Harrison 1991, Hanski & Simberloff 1997), with the individuals that comprise each sub-population having similar biological characteristics (Begg & Waldman 1999, Cowen et al. 2000, Cowen & Sponaugle 2009).

For demersal fish species with pelagic larvae, quantifying the degree to which segregated sub-populations are connected through larval dispersal is necessary to determine the spatial scale over which the life-history operates (Cowen & Sponaugle 2009, Lowe & Allendorf 2010). An understanding of population connectivity is required to evaluate the resilience of a population to anthropogenic impacts such as fishing pressure (Hanski & Simberloff 1997, Carson et al. 2011), and identifies the most appropriate spatial scale for management (Begg & Waldman 1999). Historically, it was believed that populations of marine fish were demographically 'open' systems and that recruitment was essentially independent of local reproduction (Fig. 1.2A) (Hjort 1914, Caley et al. 1996, Cowen et al. 2000). This hypothesis stemmed from the potential for widespread dispersal of passive propagules in marine ecosystems, which would result in the extensive exchange of larvae between discrete sub-populations (Caley et al. 1996, Cowen et al. 2000, Cowen & Sponaugle 2009). However, in recent years, numerous empirical and theoretical studies have demonstrated that population processes can operate over much finer spatial scales (Swearer et al. 1999, Thorrold et al. 2001, Swearer et al. 2002, Jones et al. 2005, Jones et al. 2009). These self-recruiting populations are considered demographically 'closed' because of the lack of appreciable exchange of individuals amongst sub-populations (Fig. 1.2B) (Cowen & Sponaugle 2009). There is a high probability that most populations of marine fishes are maintained by a combination of both processes – i.e. a proportion of self-recruitment that is supplemented by larval production from other sub-populations (Fig. 1.2C) (Cowen et al. 2000, Swearer et al. 2002, Cowen & Sponaugle 2009). Consequently, understanding the demographic relationships between different sub-populations is necessary to determine the appropriate stock structure, which identifies the most appropriate spatial scale for management (Begg & Waldman 1999).

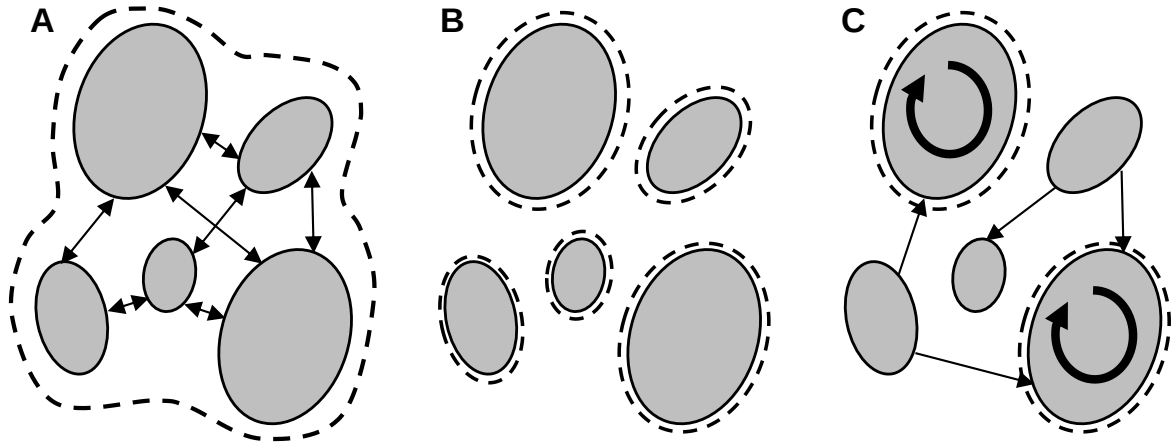

Fig. 1.2. Examples of theoretical meta-population structure of marine fish. Modified from Harrison (1991) and Bailey (1997). (A) a demographically 'open' population with extensive mixing between sub-populations; (B) a series of demographically 'closed' sub-populations with no appreciable exchange of individuals between them; and (C) a combination of both processes – local recruitment that is supplemented by larval production from elsewhere. Ellipses represent spatially discrete sub-populations, dashed lines indicate population boundaries and arrows indicate exchange between populations.

1.5 METHODS TO INVESTIGATE EARLY LIFE-HISTORY PROCESSES

Investigating the early life-history processes of fish populations and quantifying larval movement remain considerable challenges in marine ecology (Thorrold et al. 2002, Cowen & Sponaugle 2009, Swearer et al. 2019). This relates to the logistical difficulties involved in marking, and subsequently recapturing, high numbers of inherently small larvae that experience extremely high mortality rates. These challenges have led to a variety of molecular and phenotypic techniques to investigate early life-history processes. Molecular techniques depend on geographic and reproductive isolation over multiple generations that inhibit gene flow, and subsequently manifest as genetic differences between populations (Begg & Waldman 1999, Jones et al. 2009, Lowe & Allendorf 2010). However, molecular techniques commonly overestimate the degree of population connectivity and subsequently underestimate stock structure in wide-ranging species with a pelagic larval stage, such as for the Atlantic croaker (Lankford et al. 1999, Schaffler et al. 2009). This is because the exchange of only a few individuals per generation is sufficient to maintain genetic homogeneity among populations that are otherwise ecologically distinct (Bailey 1997, Cowen et al. 2000, Cowen & Sponaugle 2009). In contrast, phenotypic markers can identify relatively short-term, environmentally-induced differences between ecologically-separated populations (Begg & Waldman 1999, Thorrold et al. 2002, Cowen & Sponaugle 2009). As such, phenotypic and genetic techniques provide complementary biological information at ecological and evolutionary time-scales, respectively, for investigating population connectivity and stock structure.

Numerous methods have been used to assess population connectivity during the early life-history stages of fish. These include: marking larvae with fluorescent compounds (e.g. tetracycline; Jones et al. 1999, Jones et al. 2005, Secor et al. 2017), elemental tags (Pollard et al. 1999) or stable isotopes (Thorrold et al. 2006, Almany et al. 2009); quantifying natural environmental tags such as genetic markers (Planes et al. 2009, Almany et al. 2017) and geochemical signatures in calcified structures (Swearer et al. 1999, Thorrold et al. 2001, Swearer et al. 2002); and simulating larval dispersal using biophysical models (North et al. 2008, Swearer et al. 2019). Of the different techniques and structures considered, the biological information retained in fish otoliths provide unparalleled opportunities to retrospectively investigate the life-history of even the smallest fish. Otoliths, or ‘ear stones’, are paired crystalline structures that assist with hearing, orientation and balance which form during embryonic development and accrete continuously throughout the lives of teleost fishes (Campana & Neilson 1985). They form alternating opaque and translucent increments at a daily periodicity which can be interpreted to estimate age, and in turn, calculate hatch dates, growth rates and larval duration (Pannella 1971, Campana & Neilson 1985, Campana & Jones 1992). As the otolith grows, elements in minor and trace quantities from the surrounding aquatic environment can be incorporated into the calcium carbonate matrix at the precipitating surface. Once incorporated, these elements are permanently retained in the otolith and can

be interpreted along with age information to describe a chronological record of environmental history (Campana 1999, Elsdon et al. 2008). The geochemical signatures retained in otoliths can then be used to discriminate between populations of fish that have occupied different environments. An alternate approach for investigating early life-history processes is biophysical modelling. Biophysical models have become a leading approach for predicting potential larval dispersal patterns and population connectivity in marine ecosystems (Swearer et al. 2019). This method combines a physical hydrodynamic model, forced by oceanographic and atmospheric data, with a biological model which incorporates larval development, behaviour and swimming capabilities (Leis 2007, North et al. 2008). Theoretical predictions of larval dispersal from the model can be combined with empirical biological data, such as genetic parentage analysis or otolith chemistry, to best interpret population connectivity and stock structure (Bode et al. 2019, Swearer et al. 2019).

1.6 STUDY SPECIES - KING GEORGE WHITING

King George whiting (*Sillaginodes punctatus*; Cuvier, 1829) is a shallow-bodied, elongate, demersal marine finfish species of the order Perciformes. It is endemic to temperate coastal waters of southern Australia, ranging from Jurien Bay in Western Australia, around the southern coastline to Sydney in New South Wales (Kailola et al. 1993). South Australia is in the geographic centre of this distribution, and supports the highest abundances and the most significant fishery for King George whiting (Steer et al. 2018). It can grow to 72 cm in length and 2.5 kg in weight, and has longevity of up to 22 years (Fowler & Jones 2008). King George whiting conforms to the general bi-partite life-history of many demersal marine fish species. In South Australia, spawning occurs during the austral autumn and winter at low profile reefs and shoals in areas of moderate to high wave energy (Fowler et al. 1999, Fowler et al. 2000a). The only recognised spawning area for King George whiting in south eastern Australia is throughout southern Spencer Gulf and Investigator Strait (Fig. 1.3). This is the only area where reproductively active females with hydrated oocytes have been captured (Fowler et al. 1999, Fowler et al. 2000a), and simultaneously where eggs and early-stage larvae have been collected from plankton tows (Bruce and Short 1990, Fowler 2000). The developing eggs and larvae disperse over a long (80-130 d) pelagic phase, before the larvae settle to shallow seagrass beds in protected bays (Jenkins & May 1994, Fowler & Short 1996, Jenkins et al. 1997). In South Australia, these nursery areas are located in the bays along the West Coast of Eyre Peninsula and in the protected coastal waters of Spencer Gulf and Gulf St. Vincent (Jones et al. 1990, Fowler et al. 2000b, Fowler & Jones 2008). Juveniles develop for two to three years in the vicinity of the nursery areas to which they settled, before they move offshore to replenish the populations of spawning adults (Fowler et al. 2000b, Fowler et al. 2002).

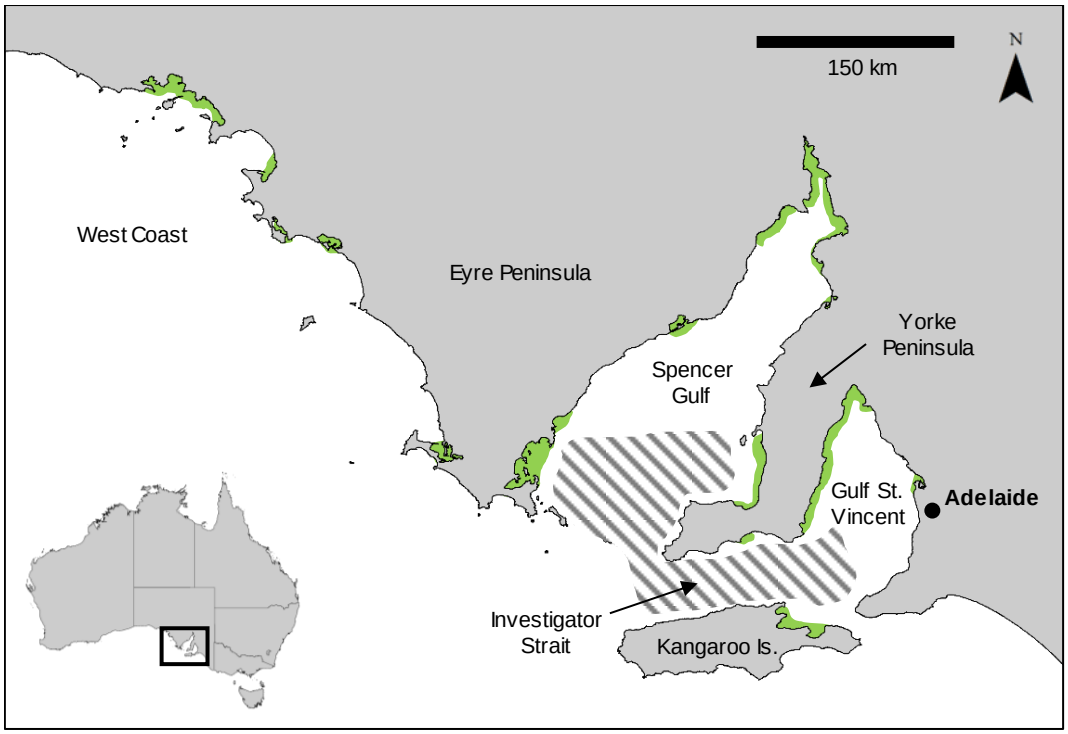

Fig. 1.3. Life-history of King George whiting in South Australia. Green shading represents the recognised nursery areas and the grey dashed area indicates the recognised spawning area. Inset – Map of Australia showing the study area along the southern coastline.

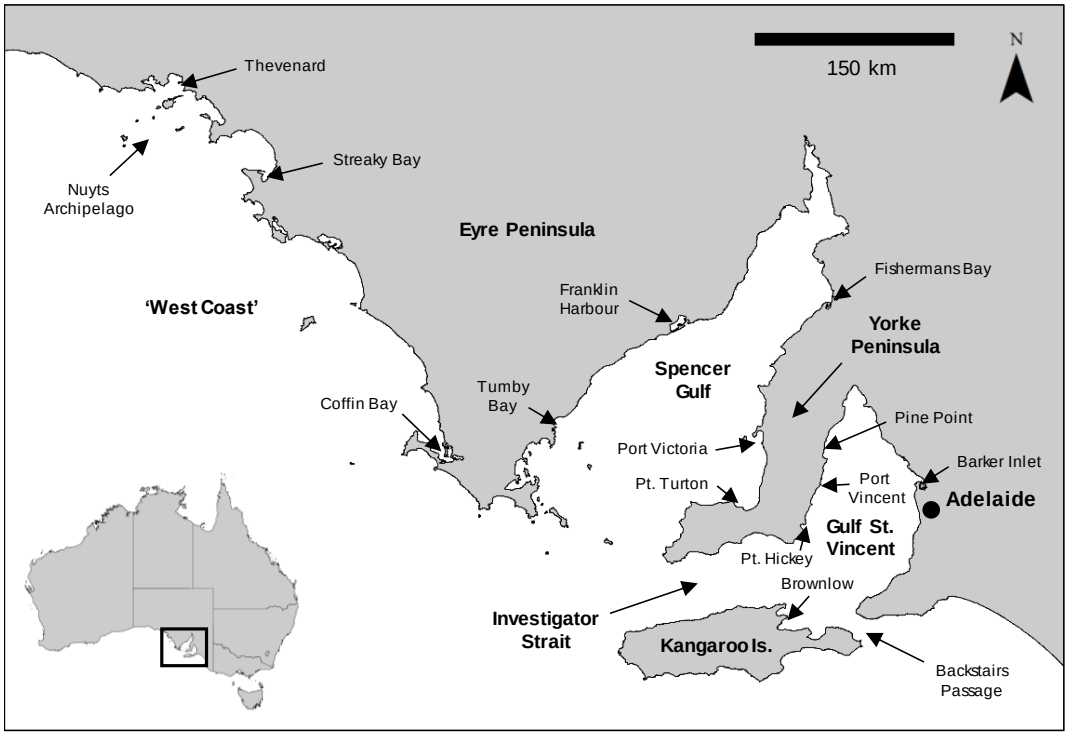

Fig. 1.4. Map of the study area in South Australia identifying the localities referred to throughout the thesis. Areas referred to as 'regions' are in bold and 'sites' are in normal font. Inset – Map of Australia showing the study area along the southern coastline.

Despite considerable research into the life-history of King George whiting (Scott 1954, Gilmour 1969, Robertson 1977, Jones et al. 1990, Fowler et al. 2000b, Jenkins et al. 2000, Fowler & Jones 2008, Jenkins et al. 2016), population connectivity and stock structure throughout southern Australia remains poorly understood. A recent molecular study indicated that the distribution of King George whiting was divisible into three genetic stocks – the Western Australian stock, the South Australia-Victoria stock, and the Tasmanian stock (Jenkins et al. 2016). Genetic homogeneity among the South Australian and Victorian populations indicates that there is at least some exchange of individuals between them (Kent et al. 2018). In Victoria, there is a long held hypothesis that recruitment of King George whiting is dependent on seasonal wind stress to disperse larvae from a western spawning ground, potentially in South Australia, to Victorian nursery areas (Jenkins et al. 2000, Jenkins 2005, Jenkins et al. 2016). However, differences in the otolith chemistry of recruits from South Australia and Victoria suggest that the two State-based populations are replenished by different spawning populations (Jenkins et al. 2016). Consequently, the South Australian and Victorian populations are considered separate biological stocks. The South Australian biological stock is divided into three regional populations for assessment and management purposes based on geographic separation and predicted relationships between spawning grounds and nursery areas (Fowler & McGarvey 2000, Fowler et al. 2000b). The regional populations are the West Coast of Eyre Peninsula, Spencer Gulf, and Gulf St. Vincent (Fig. 1.4) (Steer et al. 2018). However, such regional population connectivity and stock structure remains untested empirically.

1.7 BACKGROUND AND OBJECTIVES

King George whiting supports important commercial and recreational fisheries in South Australia and Victoria, where it is primarily targeted using handlines from small vessels (Steer et al. 2018). In South Australia, which supports the highest abundances and most significant fishery (Mobsby 2018), King George whiting is considered an iconic species and attracts the highest price per unit weight of the species taken in the Marine Scalefish Fishery (Steer et al. 2018). The fishery has traditionally targeted immature fish as they transition from the nursery areas in protected bays to the offshore spawning areas. Fishing effort towards the spawning population has historically been low, which has resulted in relatively stable recruitment into the population. However, recent trends in commercial catch statistics and modelled estimates of biomass suggest that the populations of King George whiting in Spencer Gulf and Gulf St. Vincent were in decline during the mid-2000s and reached record lows in the early 2010s (Fowler et al. 2014). As such, these populations were assigned the stock status of ‘transitional depleting’ (Fowler et al. 2014). This stock status means that the population is not yet recruitment overfished, but that management intervention is required to reduce fishing pressure and prevent the population from being depleted to an overfished state (Flood et al. 2016). These declines were attributed to a number of causes including increases in fishing effort towards the spawning population, improved fishing technology, and the absence of fishery-independent data to assess stock status (Fowler et al.

2014). An extensive consultation process involving all stakeholder groups acknowledged the need to identify the key spawning grounds and nursery areas, and the demographic relationships between them, in order to underpin the development of the most effective management options.

The aim of this study was to improve the understanding of the early life-history processes of King George whiting in South Australia's gulf system, and specifically, to investigate the connectivity between coastal spawning grounds and inshore nursery areas. The objectives of the study were:

(1) to describe the temporal nature of recruitment throughout the protracted settlement season. Specifically: (i) to determine the period of spawning that resulted in settlement; (ii) to assess temporal variation in the biological characteristics of larvae; and (iii) to interpret the otolith chemistry of larvae collected at different times in terms of potential spawning sources (Chapter 2);

(2) to identify the spatial scale over which the early life-history of King George whiting operates in South Australia. In doing so, differentiate between two hypotheses of stock structure (Chapter 3);

(3) to determine if recently-hatched King George whiting larvae in their natal waters of southern Spencer Gulf and Investigator Strait originated from a common source population (Chapter 4); and,

(4) to investigate the connectivity between spawning grounds and nursery areas of King George whiting in South Australia's gulf system by simulating larval dispersal using a biophysical model (Chapter 5).

In order to address the four objectives, four empirical studies have been completed that are detailed in Chapters 2 to 5. Each study had a specific objective that related to the overall aim. The overall study complements a concurrent research project that investigated the spawning dynamics of King George whiting in the context of identifying key spawning grounds and developing a fishery-independent estimate of spawning biomass (Steer et al. in prep.; FRDC 2016-003).

1.8 THESIS STRUCTURE

The thesis is organised as four data chapters (Chapters 2 to 5) that are written as stand-alone scientific manuscripts suitable for publication in a peer-reviewed journal. Consequently, there is some repetition in respective Introduction and Methods sections. Chapter 1 is a General Introduction that summarises and evaluates published literature relevant to the early life-history of marine fish species and the biology King George whiting. Chapter 6 is a General Discussion that highlights the key outcomes of the study, considers the implications for management and suggests directions for further research.

CHAPTER 1 - General Introduction

This chapter introduces background information and evaluates previous literature relevant to the life-history cycles of marine fishes, factors influencing recruitment, larval transport, population connectivity and the biology of King George whiting. I outline the overarching aim and objectives of the study, the thesis structure, and a synopsis of each chapter.

CHAPTER 2 – Resolving the early life history of King George whiting (*Sillaginodes punctatus*; Perciformes) using otolith microstructure and trace element chemistry

Many marine fish species are batch spawners that produce high numbers of pelagic eggs repeatedly throughout a protracted spawning season. For such species, the larvae that hatch at different times may experience different physical and ecological conditions during their ontogenetic development, and in turn, develop different early life-history characteristics which influence recruitment. In this chapter, recently-settled larvae were collected fortnightly from July to November at a significant nursery area in two consecutive years. The early life histories of larvae were reconstructed from the incremental microstructure and trace elemental chemistry of their otoliths. The early life-history characteristics of larvae that settled at different times throughout the season were compared to assess for temporal variation in settlement and the potential of multiple spawning sources contributing to recruitment.

This chapter is published in *Marine and Freshwater Research*, **70**: 1659-1674.

CHAPTER 3 - Spatial connectivity during the early life history of a temperate marine fish inferred from otolith microstructure and geochemistry

The life-history cycles of many demersal fish species involve a dispersive larval stage that connects spatially discrete spawning grounds and nursery areas. Understanding connectivity during dispersal is necessary to determine the spatial scale over which fish populations operate, which is the appropriate scale for management. In this chapter, I collected recently-settled larvae from 13 nursery areas across three regions in South Australia over two consecutive years. Otolith microstructure and trace element chemistry analysis were used to retrospectively investigate the early life-history of larvae. The underlying methodological approach was to compare the otolith chemistry of larvae that hatched at the same time to infer population connectivity at multiple spatial scales, and to evaluate two proposed models of stock structure.

This chapter is published in *Estuarine, Coastal and Shelf Science*, **227**: 106342.

CHAPTER 4 - Discriminating natal source populations of a temperate marine fish using larval otolith chemistry

Empirically quantifying larval movement among populations is necessary to establish connectivity and determine population dynamics, yet it remains one of the greatest challenges in marine ecology. This largely relates to analytical challenges involved in differentiating between source populations of dispersing larvae in open marine environments. In this chapter, pelagic larvae were collected throughout the only recognised spawning area in South Australia in two consecutive years. The distribution of larvae was divisible into two spatially-discrete groups. The incremental structure and elemental composition of otoliths of recently-hatched larvae (3.0-5.0 mm SL, 5-21 d) from the two groups were compared to determine if they had originated from a common source population.

This chapter is published in *Frontiers in Marine Science*, **6**: 711.

CHAPTER 5 - Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish

Biophysical models have become a leading approach to predict larval dispersal patterns and subsequent population connectivity in marine systems. In this chapter, a high-resolution hydrodynamic model of South Australia's gulf systems was coupled with a biological model including larval movement and behaviour to simulate dispersal between spawning grounds and nursery areas. Particles were seeded in the model based on the distribution and abundance of eggs collected throughout the recognised spawning area. Larval dispersal was simulated using three increasingly complex behavioural models developed from the biological data collected in Chapters 2 to 4. The predictions of dispersal and population connectivity were compared to the spatial and temporal patterns of otolith chemistry for larvae collected from the spawning grounds (Chapter 4) and nursery areas (Chapters 2 and 3).

This chapter is in review at *Fisheries Oceanography*.

CHAPTER 6 - General Discussion

This chapter provides a synthesis of the key findings from the four preceding data chapters and suggests potential directions for future research. The updated understanding of early life-history processes for King George whiting in South Australia's gulf system are summarised and implications for fishery management are discussed.

Statement of Authorship

Title of Paper	Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Rogers TA, Fowler AJ, Steer MA, Gillanders BM (2019). Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry, *Marine and Freshwater Research*, **70**: 16.

Principal Author

Name of Principal Author (Candidate)	Troy Rogers
Contribution to the Paper	Contributed to the design of the study, conducted the fieldwork, collected the samples, performed the laboratory processing and sample preparation, operated the LA-ICP-MS, collected and analysed the data, applied statistical analyses, wrote the manuscript and acted as corresponding author.
Overall percentage (%)	90%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 14/11/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Anthony Fowler
Contribution to the Paper	Contributed to the design of the study, assisted with sample collection and data interpretation, and revised the manuscript.
Signature	Date 14/11/19

Name of Co-Author	Michael Steer
Contribution to the Paper	Contributed to the design of the study, assisted with data interpretation and revised the manuscript.
Signature	Date 14/11/19

Name of Co-Author	Bronwyn Gillanders
Contribution to the Paper	Contributed to the design of the study, assisted with data interpretation and revised the manuscript.
Signature	Date 17/11/2019

Chapter 2 – Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry

ABSTRACT

Knowledge of the early life-history processes of fish that lead to recruitment is critical for understanding population dynamics. This study explored the early life-history of King George whiting (*Sillaginodes punctatus*) that recruited to an important nursery area in South Australia in 2016 and 2017. The early life-history was reconstructed based on the retrospective analysis of otolith microstructure and chemistry for recently-settled larvae collected fortnightly from July to November. These larvae hatched between March and July, but a three week period in May led to 52-71% of recruitment. Larvae from successive sampling occasions differed in age, size and growth rate, potentially related to seasonal changes in water temperature and food availability. During both years, there were significant changes in otolith elemental chemistry among the groups of larvae that primarily related to changes in concentrations of Sr. There are two hypotheses to account for the differences in otolith chemistry: either a single, primary spawning source and within-season environmental change; or multiple spawning sources. Further investigation with oceanographic models of larval dispersal will help differentiate between these. The retrospective analysis of otoliths has improved the understanding of early life-history for this important species, with implications for fishery management.

This chapter is published as:

Rogers TA, Fowler AJ, Steer MA and Gillanders BM (2019). Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry, *Marine and Freshwater Research*, **70**: 1659-1674.

2.1 INTRODUCTION

Understanding the early life-history processes of fish that contribute to recruitment is critical for interpreting changes in adult populations (Chambers & Trippel 1997, Cowen & Sponaugle 2009). For marine species with spatially distinct spawning and nursery areas, recruitment can be directly related to larval survivorship during dispersal (Leggett & Deblois 1994, Houde 2008, Cowen & Sponaugle 2009). Survival rates can be highly variable as they are influenced by environmental factors including food availability, predator abundance and the prevailing abiotic conditions (Pepin & Myers 1991, Leggett & Deblois 1994, Sponaugle et al. 2006). Even though larvae are small and their behavioural and sensory abilities are poorly understood, larval dispersal is not simply controlled by physical oceanographic processes (Jones et al. 2009, Leis 2010), and biological factors play an important role in larval survival and subsequent recruitment (Houde 1989, Shima & Findlay 2002, Rankin & Sponaugle 2014).

Many fish species are batch spawners that produce offspring repeatedly throughout a protracted spawning season (Brown-Peterson 2011). The larvae that originate from an extensive range of hatch dates can be exposed to different environments throughout their early development (Cargnelli & Gross 1996, Radtke et al. 2001, Cook 2011). Physical and ecological conditions are dynamic and can vary greatly at different spatial and temporal scales. As such, there is the potential for recruits within a single spawning season, and between years, to experience different environments and have considerably different early life-history characteristics (Cargnelli & Gross 1996, Radtke et al. 2001, Cook 2011). For example, as larval growth is highly correlated with temperature, changes in water temperature during the spawning season may affect larval development rates and their subsequent survival (Pepin & Myers 1991, Shima & Findlay 2002, Green & Fisher 2004). Therefore, understanding temporal variation in the biological characteristics of early stage fish may contribute considerably to understanding variation in recruitment.

The biological information stored in calcified structures provides unique opportunities to retrospectively investigate the life-history of fishes (Campana 1999, Campana & Thorrold 2001, Elsdon et al. 2008). Otoliths are paired crystalline structures that function for hearing and orientation and form continuously throughout the lives of bony fishes (Campana & Neilson 1985). The continual accretion of carbonate material at variable rates relative to somatic growth is reflected in the otolith structure as alternating increments that can be used to estimate age (Campana & Neilson 1985). Daily growth increments in the otolith microstructure of early stage fish provide highly-resolved temporal information on age that can be interpreted to inform larval growth, pre-settlement duration and hatch date (Campana & Jones 1992). In addition to microstructure analysis, otolith chemistry is a powerful tool that can be used to discriminate between groups of fish that have occupied different physico-chemical environments throughout their lives (Campana 1999, Elsdon et al. 2008). The material used

for otolith formation is derived from the aquatic environment occupied by the fish. Water chemistry is influenced by extrinsic factors, including temperature and salinity, which vary at different spatial and temporal scales and can influence otolith elemental composition (Campana 1999, Elsdon et al. 2008). The incorporation of elements into the otolith matrix does not simply reflect the concentrations present in the surrounding environment (Izzo et al. 2018), but is regulated by a complex suite of physiological processes that are not yet fully understood (Sturrock et al. 2012, Sturrock et al. 2014). Nevertheless, otolith elemental composition relates to the aquatic environments experienced by a fish (Sturrock et al. 2012), that when interpreted concurrently with age information, describe a chronological record of environmental history (Campana & Thorrold 2001, Elsdon et al. 2008). In the context of early life-history, otolith chemistry has been particularly useful for understanding habitat use (Dorval et al. 2005, Hogan et al. 2017), establishing connectivity between life stages (Gillanders 2002a, Hamer et al. 2003), and delineating natal sources (Swearer et al. 1999, Thorrold et al. 2001).

King George whiting (*Sillaginodes punctatus*; Perciformes) is a demersal finfish species endemic to temperate coastal waters of southern Australia, and is one of the most important fishery species of this region (Kailola et al. 1993, Fowler & Jones 2008). South Australia (SA) is in the centre of this distribution, and supports its highest abundances and most significant fishery (Steer et al. 2018). However, catches and estimated biomass of King George whiting in SA have declined over recent years. In particular, catches from Gulf St. Vincent, one of the important fishery regions of SA, have declined to record lows. Despite a large body of research over the past 30 years to improve the understanding of King George whiting life-history (Jenkins & May 1994, Fowler & Short 1996, Fowler et al. 1999, Fowler et al. 2000b, Jenkins et al. 2000, Jenkins et al. 2016), there remains considerable uncertainty about the spawning sources, population connectivity and early life-history processes that ultimately culminate in recruitment.

The understanding of life-history for King George whiting was that adult fish spawn between March and May in the offshore waters of Investigator Strait and southern Spencer Gulf (Fig. 2.1) (Fowler et al. 1999, Fowler et al. 2000a). This is the only area where adult fish with hydrated oocytes, fertilised eggs and developing larvae have been found in south-eastern Australia. Even though previous research suggests that there are other spawning areas, their locations remain unknown (Fowler et al. 2000b, Jenkins et al. 2000, Jenkins et al. 2016). The developing larvae are subject to a long advection phase of three to five months before they settle to protected bays within the gulfs (Jenkins & May 1994, Fowler & Short 1996, Hamer & Jenkins 1997). Juveniles develop within the nursery for 12-18 months before moving into adjacent deeper water, and eventually migrate southwards as young adults to replenish the offshore spawning population (Fowler et al. 2000a, Fowler et al. 2002). In Gulf St. Vincent, there is one specific nursery area that is particularly significant for the regional population. Barker Inlet is the largest recognised nursery area in Gulf St. Vincent and has a multi-decadal history of annual recruitment

(Fig. 2.1) (Fowler & Short 1996, Fowler & Jones 2008). However, the source of larvae to Barker Inlet and the demographic processes they have experienced prior to settlement remain poorly understood.

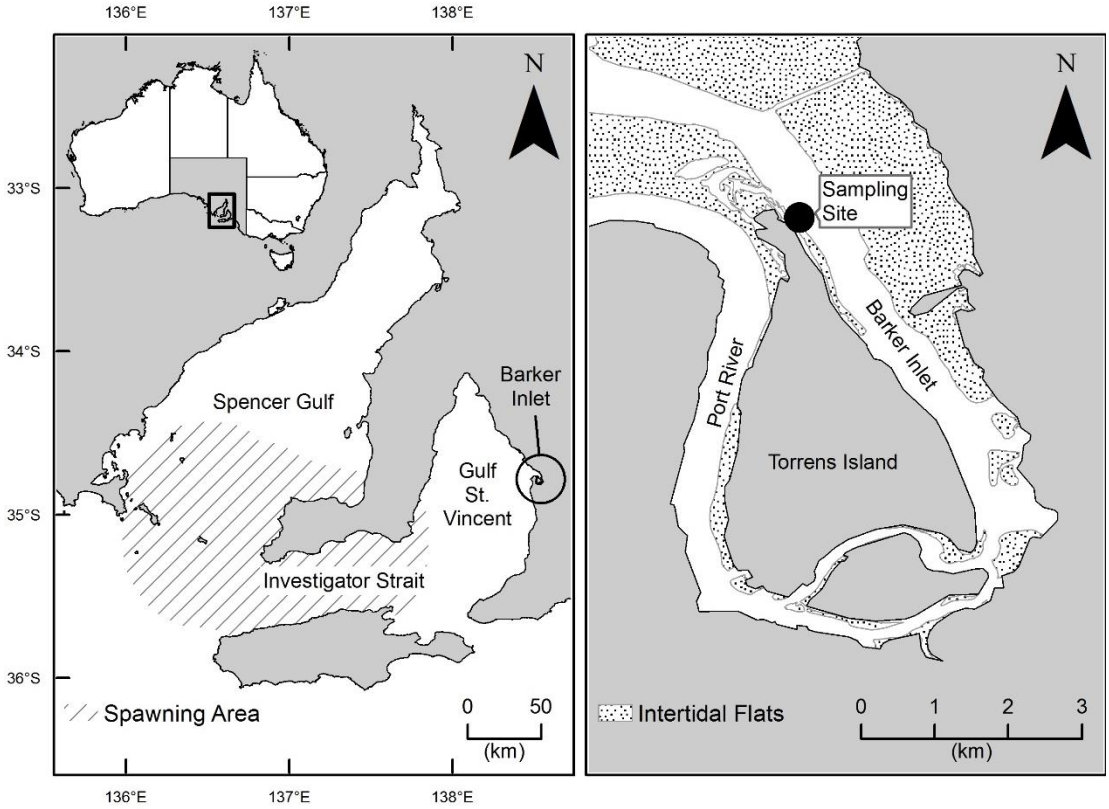

Figure 2.1. Left – Map of South Australia's gulf systems showing the location of Barker Inlet along the eastern coast of Gulf St. Vincent. The dashed area indicates the recognised spawning area (inset – map of Australia showing the location of this region along the southern coastline). Right – the Barker Inlet estuary showing the location of the sampling site.

In order to better inform fishery management, this study aimed to investigate the early life-history of King George whiting that recruited to Barker Inlet. This was achieved through the retrospective interpretation of otolith microstructure and elemental chemistry for new recruits collected throughout two complete settlement seasons. The specific objectives addressed were: (1) to identify when spawning occurred that resulted in settlement, particularly for the peak settlement period; (2) to examine temporal variation in the early life-history characteristics of recruits that settled throughout the season; and (3) to interpret the otolith chemistry of recruits collected at different times through the settlement season in terms of potential spawning sources and larval advection pathways.

2.2 MATERIALS AND METHODS

2.2.1 SAMPLE COLLECTION

Recently-settled King George whiting were collected from Barker Inlet, a semi-enclosed, protected system in Gulf St. Vincent, South Australia (Fig. 2.1). Sampling was done at one site at the northern end of Torrens Island that supported a shallow, subtidal seagrass bed of *Zostera* spp. In each of 2016 and 2017, samples were collected on nine occasions at fortnightly intervals throughout the austral winter-spring settlement period (July to November) (Table 2.1). The day of sampling was determined by the lowest tide for each fortnightly cycle. Sampling was done with a small beach seine net (5 m mouth width, 7 m semi-circular perimeter, 2 m drop, and mesh size of 1 mm^2) that was hauled over 40 m by two people through shallow water (0.5 m). King George whiting were removed from the net, stored in resealable plastic bags on ice immediately post-capture and frozen for later analysis. The total sample area for each seine was 200 m^2. On each occasion six seines were sampled giving a total area sampled of 1,200 m^2.

Table 2.1. Summary of the sample size (*n*) and mean (SD) standard length (SL, mm) of recently-settled King George whiting larvae used for otolith analyses that were collected on nine sampling occasions at Barker Inlet, South Australia, in 2016 and 2017.

2016			2017		
Date	*n*	SL (mm)	Date	*n*	SL (mm)
7-Jul	18	19.7 (0.9)	11-Jul	3	20.3 (0.5)
21-Jul	15	19.8 (0.8)	26-Jul	20	19.0 (0.4)
6-Aug	17	20.3 (0.9)	12-Aug	20	19.6 (0.8)
21-Aug	18	19.8 (0.6)	26-Aug	20	20.0 (0.7)
5-Sept	19	19.3 (0.4)	8-Sept	20	19.2 (0.3)
22-Sept	20	19.1 (0.6)	26-Sept	20	18.6 (0.5)
7-Oct	20	18.2 (0.6)	11-Oct	20	17.0 (0.4)
31-Oct	20	18.5 (0.6)	26-Oct	21	18.5 (1.0)
17-Nov	16	21.0 (1.0)	8-Nov	19	22.8 (1.5)

2.2.2 SAMPLE PROCESSING

King George whiting collected on each occasion generally contained a mix of recently-settled larvae and developing juveniles. No distinctive settlement mark was routinely visible in the otoliths of these fish. This prevented the exact day of settlement from being identified, which was consistent with previous studies of this species in South Australia (Fowler & Short 1996, Fowler et al. 2000b). Settlement for King George whiting has been shown to be more related to size than age (Fowler & Short

1996). Therefore, to best investigate the early life-history characteristics of the newest recruits at different times throughout the settlement season, we considered the smallest fish on each sample occasion to have most recently undergone settlement. As such, each fish was measured for standard length (SL) to the nearest 0.1 mm using digital callipers, and the 20 smallest fish from each sample occasion were used for otolith analyses. Their sagittal otoliths were removed under a dissecting microscope (Olympus SZX7; Tokyo, Japan) using stainless steel needles. Otoliths were rinsed in three drops of ultrapure water, adhering tissue removed, allowed to dry under a laminar flow hood, and stored individually in microcentrifuge tubes.

We followed the widely accepted definitions of early life-history stages for demersal and benthic fishes described by Neira et al. (1998) and Leis and Carson-Ewart (2004). The approximate size range for each stage followed those for King George whiting described by Bruce (1995) and Hamer and Jenkins (1997). Briefly, these were: (1) 'larva' – development stage between hatching and attainment of full meristic complements (fins and scales). Size range: 2.0-15.0 mm SL; (2) 'settlement-stage larva' – development stage during which a larva transitions from the pelagic to benthic environment (settlement), often associated with a morphologic transition from larva to juvenile. Size range: 15.0-20.5 mm SL; and (3) 'juvenile' – development stage from attainment of full meristic complements to sexual maturity. Size range: > 20.5 mm SL.

2.2.3 OTOLITH MICROSTRUCTURE

For each fish, one otolith was randomly chosen for microstructure interpretation whilst the other was used for trace element chemistry analysis. The former was mounted proximal surface upward on a glass microscope slide using thermoplastic glue (Crystalbond™ 509; ProSciTech, QLD, AUS), then ground and polished through the sagittal plane to the level of the primordium using three grades (9 µm, 3 µm, and 1 µm) of aluminium oxide lapping film (AusOptic®). For interpretation, a live digital image of each polished otolith section was viewed on a computer screen using an image analysis system, which consisted of an Olympus DP73 video camera mounted on an Olympus BX51 compound microscope and used Olympus Stream software (v. 1.9.1; Tokyo, Japan). Otolith sections were viewed through a 100× objective using immersion oil. The daily periodicity of increment formation for King George whiting otoliths has previously been validated based on reared larvae of known age (B.D. Bruce and D.A. Short, unpublished). Daily increments were counted from the primordium to the posterior margin (longest axis) (Fig. 2.2), with two successive counts made for each otolith. When these counts differed by less than 5%, their mean was considered the estimated age. If they differed by more than 5%, additional counts were done until an acceptable estimate of the number of increments was achieved. If this was not achieved, the otolith was rejected (n = 9). The date of hatch for each fish was calculated by subtracting the estimated age from the date of capture. Average growth rate (mm d^{-1}) was calculated as:

$$\frac{L_c - L_o}{a} \qquad \text{Eq. (2.1)}$$

where L_c is length at capture, L_o is length at hatch (2.1 mm SL; Bruce 1995), and a is age (d).

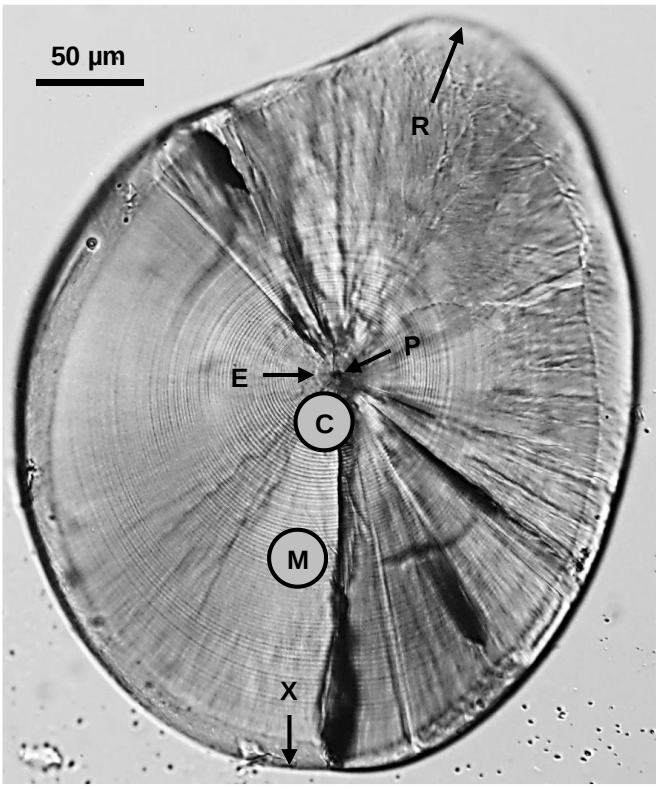

Figure 2.2. Polished sagittal otolith of a recently-settled King George whiting larva viewed at the proximal surface through a 40× objective showing the locations of 30 µm LA-ICP-MS spot ablations: C – 'core', M – 'mid' (R – rostrum, P – primordium, E – exogenous feeding check, X – posterior margin). The fish was 103 days old.

2.2.4 TRACE ELEMENT CHEMISTRY

The method used to prepare otolith sections for trace element chemistry allowed them to be individually polished and relocated to a new slide without altering the orientation of the polished section. Each otolith (n = 326) was embedded proximal surface upward in thermoplastic glue (CrystalBond™ 509; ProSciTech, QLD, AUS) on top of an epoxy resin disc (5 mm diameter x 1 mm high). The thermoplastic glue was spiked with indium (^{115}In) at ~200 ppm to aid discrimination between otolith material and glue during analysis (Reis-Santos et al. 2012). Otoliths were polished through the sagittal plane to the level of the primordium using three grades of aluminium oxide lapping film (9 µm, 3 µm, and 1 µm), rinsed with ultrapure water and allowed to dry. Then, 24 randomly selected polished sections comprising 2-3 otoliths from each of the nine sample occasions within each year were fixed to a new 'analysis' slide

using thermoplastic glue. This resulted in 14 'analysis' slides that were each triple rinsed with ultrapure water, air dried under a laminar flow hood and stored individually in sealed plastic bags.

Otoliths were analysed for trace element chemistry by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The system consisted of a New Wave Research 213 nm high performance (Nd:YAG) ultraviolet probe laser ablation system (Fremont, California, USA) coupled to an Agilent 7900 quadrupole ICP-MS (Santa Clara, California, USA) located at Adelaide Microscopy (University of Adelaide, Australia). Two 'analysis slides' were placed in the sealed chamber at one time and viewed remotely via an image analysis system. Each otolith was sampled at two places using a 30 µm diameter 'spot' ablation (Fig. 2.2). These were: (1) 'core' - posterior to the exogenous feeding check incorporating the first 20 days or so of planktonic larval life, representing the 'natal origin'; and (2) 'mid' - 100-130 µm from the primordium toward the posterior margin, representing a period of the 'larval advection', ca. 60-70 days post hatch.

Each otolith spot was sampled at a pulse rate of 5 Hz and a beam density at the sample of ~11 J cm^{-2}. Otoliths were pre-ablated using the described settings for three seconds to eliminate possible surface contamination. Ablation occurred in a ^{4}He flushed chamber that was mixed with ^{40}Ar for injection into the plasma. The elemental isotopes sampled were ^{7}Li, ^{25}Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb, as well as ^{43}Ca that was used as the internal standard, and ^{115}In used as an indicator to discriminate between otolith material and thermoplastic glue. The concentration of ^{43}Ca in the otolith was assumed to be constant at 38.8% by weight (Yoshinaga et al. 2000). Element concentrations were calibrated against the National Institute of Standards (NIST) 612 glass reference pallet (Lahaye et al. 1997). Trace element measurements of the blank sample gases were recorded for 30 s prior to each sample ablation of 40 s, with a concentration of each mass recorded every 0.30 s. Data reduction, including background subtractions, minimum limits of detection (LOD), and mass count data conversion to concentrations (ppm), was done using Iolite software (v. 2.5; Paton et al. 2011). Elemental data were then converted to molar concentrations and standardised to calcium (element:Ca, µmol mol^{-1}).

Internal precision and accuracy were assessed by analysing the NIST 612 as an unknown sample against the actual concentrations, whilst external precision was assessed by measurements of MACS-3 (United States Geological Survey) calcium carbonate reference material. The NIST 612 and MACS-3 standards were analysed twice at the beginning and end of each sampling session, and after every 12 ablations to correct for short-term instrumental drift. Average recovery (%) for the NIST 612 as an unknown ranged from 100.0 to 100.2% for all elements. Average relative standard deviations (RSD) (%) for NIST 612 were: ^{7}Li 1.7, ^{25}Mg 0.8, ^{55}Mn 1.0, ^{65}Cu 1.7, ^{66}Zn 2.0, ^{88}Sr 0.6, ^{138}Ba 0.7, ^{208}Pb 1.9. External precision (RSD) (%) assessed by measurements of the MACS-3 reference material were: ^{7}Li 2.6, ^{25}Mg 2.3, ^{55}Mn 2.4, ^{65}Cu 5.3, ^{66}Zn 12.8, ^{88}Sr 1.7, ^{138}Ba 3.2, ^{208}Pb 12.8. Average LOD (µmol mol^{-1}) based on three times

the standard deviation of the blank gases adjusted for ablation yield (Lahaye et al. 1997) were: ^{7}Li 0.60, ^{25}Mg 0.45, ^{55}Mn 0.26, ^{65}Cu 0.04, ^{66}Zn 0.19, ^{88}Sr 0.01, ^{138}Ba 0.01, ^{208}Pb 0.01.

2.2.5 DATA ANALYSIS

2.2.5.1 Microstructure

Sizes, ages and average growth rates of the 20 smallest larvae from each sample occasion were compared between sample occasions and years by two-way analysis of variance (ANOVA), with both factors fixed. Normality was assessed by visual examination of histograms, Q-Q plots and the Shapiro-Wilk goodness of fit statistic, and equality of group variances was evaluated using Levene's test. Parametric assumptions were violated for age and length, and therefore the data were transformed to natural logarithms. When significant differences were found, Tukey *post hoc* comparisons were done to identify differences among means.

2.2.5.2 Trace element chemistry

Each element considered, aside from ^{65}Cu, ^{66}Zn and ^{208}Pb, consistently exceeded the detection limits of the ICP-MS. The concentrations of the remaining 5 elemental isotopes, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{88}Sr and ^{138}Ba, conformed to parametric assumptions following fourth root transformation and were considered for analyses. Individual element:Ca ratios were compared between sample occasions and years by two-way ANOVA, and significant differences identified by Tukey *post hoc* comparisons. For each analysis, sample occasion and year were fixed factors within the full factorial model.

Multi-elemental chemistry was compared between sample occasions and years by two-factor multivariate analysis of variance (MANOVA). Pillai's Trace statistic was used as it is considered the most robust to any deviations from multivariate normality. Equivalence of covariance matrices was tested by Box's M. Discriminant function analysis (DFA) determined whether fish could be allocated into groups based on the multi-elemental signals of their otoliths and whether such groups conformed to the different sample occasions. Step-wise DFA determined the most important elements to discriminate between groups. The entry of predictors into the analysis was determined using Wilks' Lambda (Λ) with $p_{(entry)} = 0.05$ and $p_{(exit)} = 0.10$. Leave-one-out, jack-knifed cross-validation calculated whether individual fish could be classified back to their time of capture based on the elemental signals of the remaining samples. Multi-elemental data were visualised through canonical discriminant function plots showing 95% confidence ellipses around group centroids. Statistical analyses were done using SPSS Statistics (v. 26.0; IBM Corp., NY, USA) and figures produced using SigmaPlot (v. 14.0). The sample size for 11-Jul-2017 was small ($n = 4$) and therefore excluded from analyses.

2.3 RESULTS

2.3.1 TEMPORAL ANALYSIS OF FISH SIZE

2.3.1.1 Length frequency distribution

A total of 4,096 settlement-stage larvae and juveniles were captured throughout the study (2,964 and 1,132 in 2016 and 2017, respectively). The temporal trend in catch rate was consistent across years, with the lowest numbers collected in July and August and the highest in late September to November (Fig. 2.3). Length frequency distributions of fish collected at different times were similar between years. Samples from July and August were characterised by fish 19-23 mm SL, and the length range systematically increased from early September to November. An influx of 18-22 mm SL fish in late September and early October represented the highest frequency of any size. Only three fish < 20 mm SL were collected in November across both years.

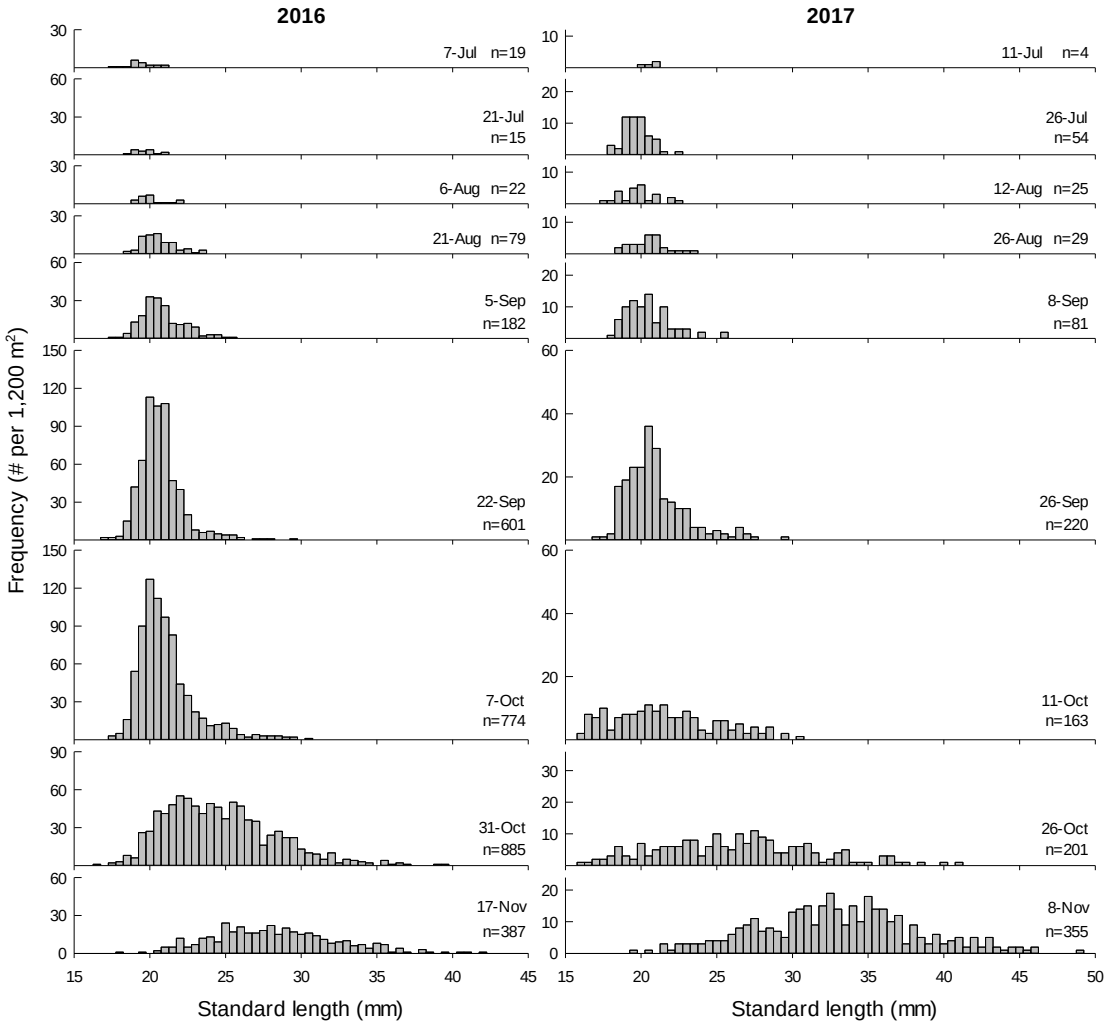

Figure 2.3. Length frequency distributions of settlement-stage larvae and juvenile King George whiting collected on each sample occasion in 2016 (left) and 2017 (right). Data show the total number of fish collected for all (6) transects. Note the different y-axis scale for each year.

2.3.1.2 Settlement pattern

The total number of fish captured and the length frequency distributions for the different sampling occasions do not represent the temporal settlement pattern, as they included juvenile fish that had previously settled. To investigate the temporal settlement pattern, only fish < 20.5 mm SL were considered to represent the most recent recruits (Hamer & Jenkins 1997). The settlement pattern was similar between years (Fig. 2.4). The numbers of new recruits were lowest in July and August, then increased exponentially during early September and peaked in early October. Fish that settled in late September and early October were the smallest and represented 70.7% and 51.7% of total recruits for 2016 and 2017, respectively. The number of new recruits decreased rapidly by late October, and there were almost none by mid-November.

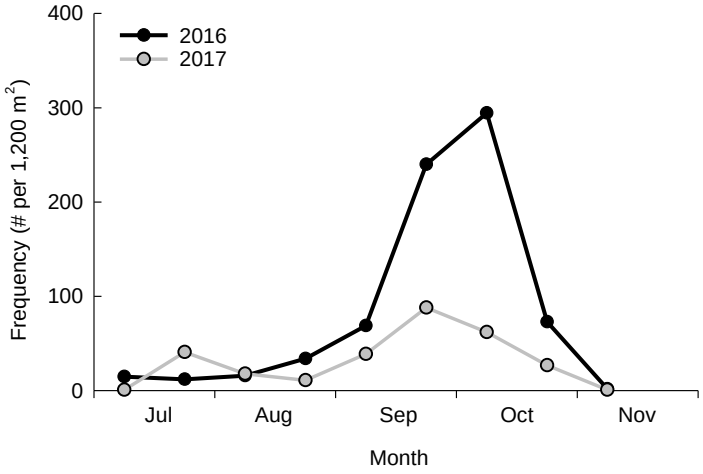

Figure 2.4. Number of settlement-stage larvae (< 20.5 mm SL) collected on each sample occasion in 2016 (black) and 2017 (grey). Data show the total number of fish collected for all six transects.

2.3.2 OTOLITH MICROSTRUCTURE

2.3.2.1 Age and growth

Of the 4,096 fish collected, 326 were aged (n = 163 for each year). Age estimates ranged from 93 to 184 d for 2016 and from 92 to 179 d for 2017 (Fig. 2.5a). Estimated ages were significantly different among sampling occasions and between years, whilst a significant interaction indicated that the pattern of variation among sampling occasions differed between years (Table 2.2). For 2016, mean age increased successively from 108 to 132 d between July and early September, then remained at 128 d until early October, before increasing considerably to 151 and 164 d in late October and November, respectively. In 2017, mean age followed a similar trend, although fish were consistently 5-15 d younger at the same time of year (Supplementary Material S2.1).

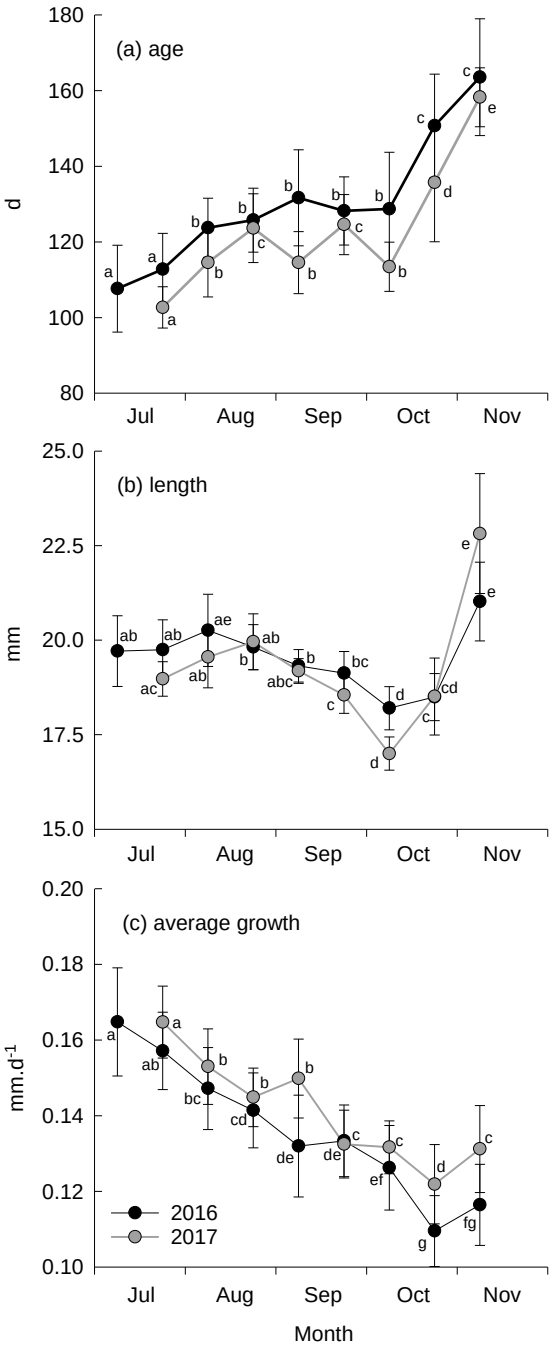

Figure 2.5. Mean (a) age, (b) standard length, and (c) average growth rates of the most recently-settled larvae throughout the settlement seasons in 2016 (black) and 2017 (grey). Error bars are ±1 SD. Letters identify significant differences between sample occasions within each year (means with the same letter are not significantly different).

Table 2.2. Results from two-way ANOVAs for the effects of year and sample occasion on estimated age, length and average growth rate of recently-settled King George whiting larvae collected between July and November from Barker Inlet, South Australia, in 2016 and 2017. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

		Age		Length		Ave. Growth	
	df	MS	*F*	MS	*F*	MS	*F*
Year	1	0.44	66.53***	0.01	6.64*	0.01	48.12***
Occasion	8	0.56	83.59***	0.13	85.16***	0.01	84.36***
Year × Occasion	7	0.02	3.09**	0.02	11.65***	< 0.001	3.47**
Error	306	0.01		0.01		< 0.001	

The patterns of variation in size of the newest recruits differed between years (Table 2.2). Mean length ranged from 16.9 to 22.1 mm SL in 2016 and from 16.1 to 25.3 mm SL in 2017 (Fig. 2.5b). Size was similar in July and August then decreased during September to a minimum in early October. Mean length increased in late October and the largest fish were collected in November. Average growth rate systematically decreased throughout the settlement seasons in both years. It was highest in early July and lowest in late October, and declined from 0.16 to 0.11 mm d^{-1} in 2016 and 0.18 to 0.12 mm d^{-1} in 2017 (Fig. 2.5c).

2.3.2.2 Hatch date

Calculated hatch dates ranged from 26 February to 6 July for 2016, and from 21 March to 13 July for 2017 (Fig. 2.6). As such, the duration of spawning that resulted in recruitment was 131 d for 2016 and 114 d for 2017. Fish sampled at a similar time of the settlement season had hatched earlier in 2016. Mean hatch dates were generally later for larvae collected from July to early October, but then remained similar for those collected in late October and November. There were large differences in mean hatch dates between consecutive sample occasions for early and late September in 2016 (21 d), and for late August to early September in 2017 (22 d). The largest number of new recruits were collected during late September and early October and their mean hatch dates were from 17-May to 31-May for 2016, and from 24-May to 20-Jun for 2017.

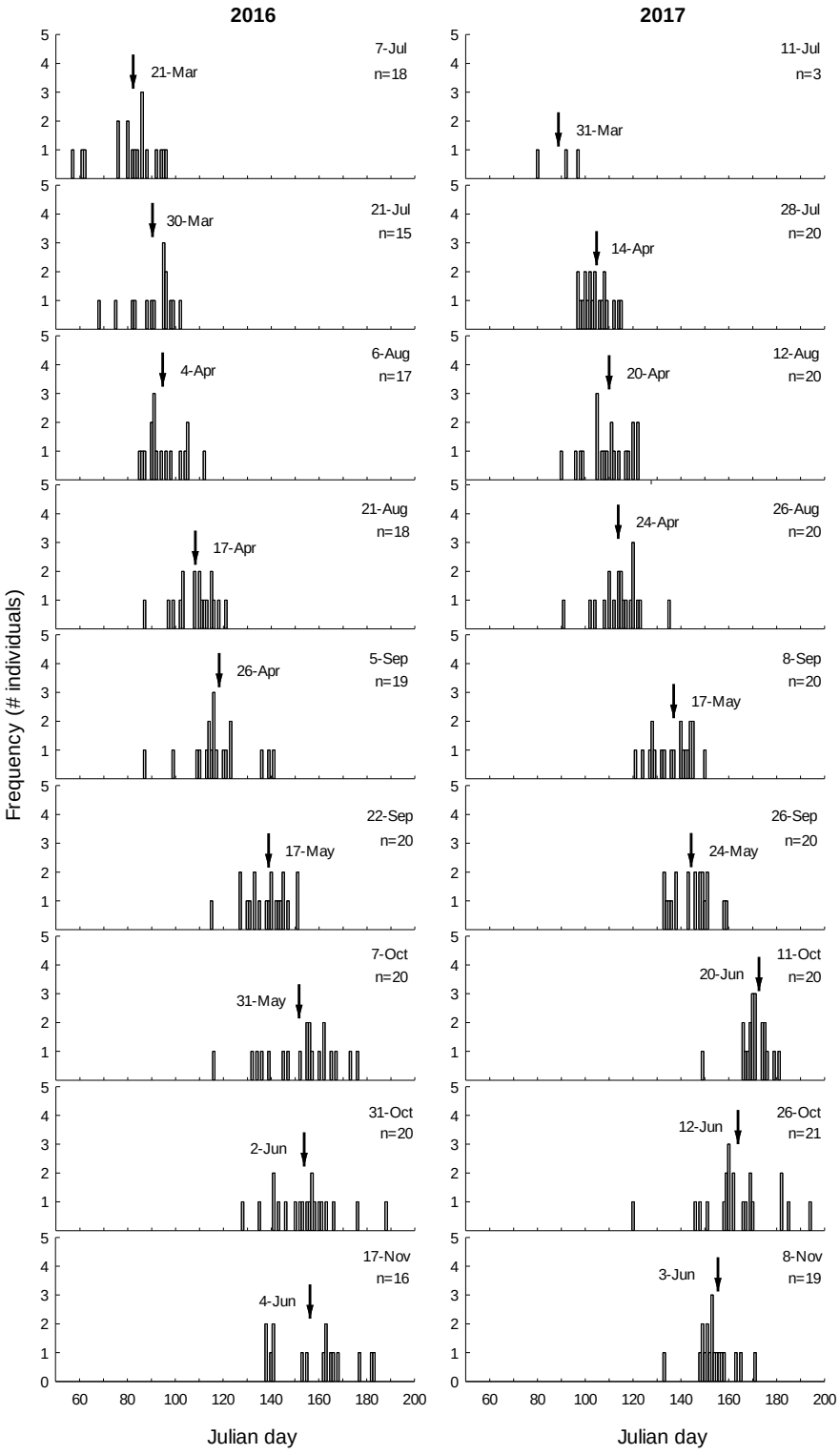

Figure 2.6. Frequency histograms showing the number of aged King George whiting larvae from each sample occasion that hatched on the nominated Julian day in 2016 (left) and 2017 (right). Arrows identify mean hatch date.

2.3.3 OTOLITH CHEMISTRY

2.3.3.1 Individual elements - natal origin

For the otolith core, trace element chemistry varied among sample occasions and between years (Table 2.3). For Li, differences were among sample occasions, but not between years. Li increased during July to a maximum in late August, then declined to a low in late October and November (Fig. 2.7A). Significant differences were between early and late August for 2016, and between late August and late October for 2017. Mg differed among occasions and between years. Mean Mg was higher in July and August for 2016 than 2017, but similar between years from late September to November. Differences for Mn among occasions, but not between years. For 2016, Mn peaked in late July, then decreased to a minimum by late August and remained low until November. In 2017, there were no differences among occasions. For Sr, there were significant differences among occasions and between years, and the pattern of variation between years. Mean Sr increased between fish sampled in July and August and those sampled later, and was consistently higher for 2017. Ba was consistently higher for 2017 than 2016 (Supplementary Material S2.2).

2.3.3.2 Individual elements – larval advection

Elemental differences related to the larval advection life stage varied between elements and years (Table 2.3). The within-season trend for Li was markedly similar between years. Li was highest in late July, then progressively decreased to a minimum in November (Fig. 2.7B). Differences were between late July and November for both years. Significant interactions between occasion and year were detected for Mg, Mn, Sr and Ba indicating that in each case the pattern of variation among sample occasions differed between years. Concentrations of Mg between July and early September were higher for 2016 than 2017. Within-season differences were between August and late September for 2016, and August and November for 2017. For Mn, there was considerable variation within and between years. Mean Mn was consistently higher for 2016 than 2017, particularly during July and early August. Differences were between July to August and September to October for each year. Sr was lowest in early July, increased to a maximum near the start of October, and then remained relatively stable through to November. Mean Sr differed between July and August, and late September to November. There were differences in Ba among occasions and between years. Mean Ba differed between the maximum concentration in late July and the minimum in September (2016) and October (2017) (Supplementary Material S2.3).

Table 2.3. Summary of results of two-way ANOVAs for the effects of year and sample occasion on individual elements related to the otolith core and mid region of recently-settled King George whiting larvae collected at Barker Inlet. Year and occasion were fixed factors within the full factorial design. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

		Li		Mg		Mn		Sr		Ba	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(a) core											
Year	1	0.01	2.90	4.25	14.36***	0.07	3.36	0.94	33.39***	0.27	31.93***
Occasion	7	0.01	5.83***	0.65	2.18*	0.04	2.11*	0.42	15.04***	0.01	0.93
Year × Occasion	7	0.01	0.32	0.55	1.86	0.03	1.66	0.07	2.51*	0.01	0.66
Error	317	0.01		0.29		0.02		0.03		0.01	
(b) mid											
Year	1	0.01	0.02	15.98	55.38***	0.43	84.21***	0.62	50.33***	0.01	6.72*
Occasion	7	0.03	19.04***	2.07	7.18***	0.12	23.90***	0.34	28.14***	0.01	6.68***
Year × Occasion	7	0.01	0.59	1.68	5.83***	0.02	4.62***	0.06	4.61***	0.01	2.97**
Error	324	0.01		0.29		0.01		0.01		0.01	

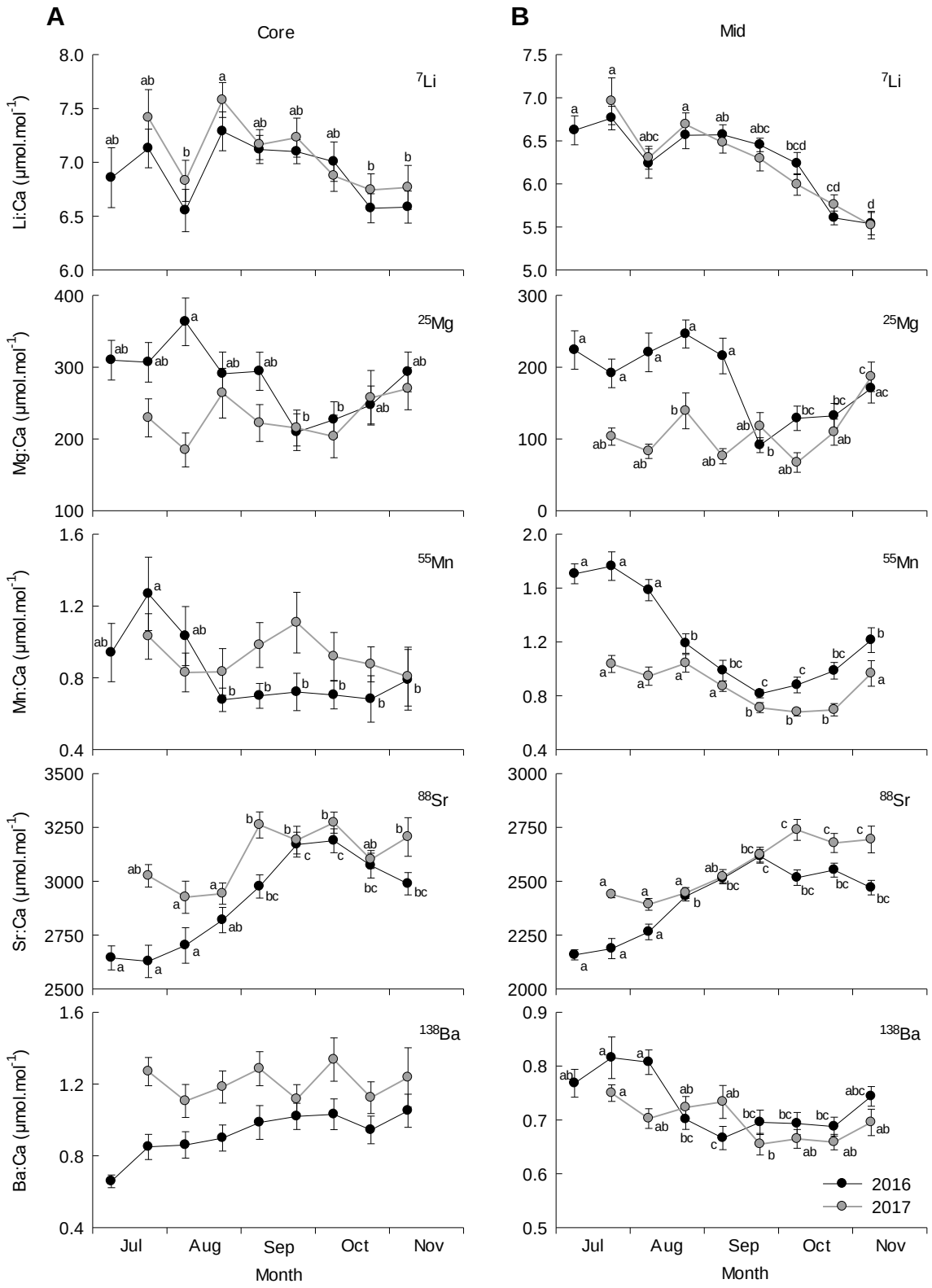

Figure 2.7. Mean element:Ca ratios (µmol mol^{-1}) for the otolith (A) core and (B) mid region of recently-settled King George whiting larvae collected from July to November at Barker Inlet in 2016 (black) and 2017 (grey). Error bars are ±1 SE. Note the different scales on y-axes. No data are shown for early July 2017 as the sample size was small (n = 4). Letters identify significant differences between means (P < 0.05).

2.3.3.3 Multi-element - natal origin

MANOVA identified significant differences in the multi-elemental chemistry related to the natal origin among sample occasions and between years (Table 2.4). For 2016, within-season differences were explained by four discriminant functions ($\Lambda = 0.377$, df = 32, $P < 0.001$), with the first two describing 95% of total variance. Concentrations of Sr and Mn were primarily responsible for group separation along the first axis (81% of total variance), and Li along the second (14%). Sr and Mn broadly separated the data into two clusters along the first axis: larvae sampled in July and August, and those sampled from late September to November (Fig. 2.8a). Variation within these groups was driven by Li, although overlapping 95% confidence ellipses suggested that multi-elemental chemistry was similar. Classification of individuals back to their respective sample occasions was low at 25%, which indicated that although temporal differences were significant, there was considerable variability among samples (Supplementary Material S2.4). Mis-allocation was highest among successive occasions. The temporal distribution of sample occasions was similar for 2017, although group separation was lower. Within-season differences were explained by two discriminant functions ($\Lambda = 0.689$, df = 40, $P < 0.001$), with Sr predominantly responsible for separation along the first axis (79%) and Li along the second (21%) (Fig. 2.8b). Sr drove separation between fish sampled in July and August from those sampled in September to November. High overlap in confidence ellipses between some samples collected months apart indicated that otolith chemistry was similar between them. Classification was marginally lower than 2016 at 24% (Supplementary Material S2.4).

2.3.3.4 Multi-element - larval advection

Multi-elemental chemistry related to the larval advection differed among occasions and between years (Table 2.4). These differences were larger than those identified for the natal origin. For 2016, within-season differences were explained by five discriminant functions ($\Lambda = 0.157$, df = 40, $P < 0.001$), the first two describing 91% of total variance. Concentrations of Sr and Mn were responsible for separation along the first axis (72%), and Li along the second (19%). Fish from July and early August had the lowest Sr and highest Mn, and were clearly separated along the first axis (Fig. 2.8c). Late August to early October samples grouped together, although there was minimal overlap between confidence ellipses, whilst late October and November were separated from all others. Classification success was 33% and mis-classification was highest to adjacent occasions (Supplementary Material S2.5). Within-season differences for 2017 were explained by four discriminant functions ($\Lambda = 0.328$, df = 28, $P < 0.001$), the first two describing 93% of total variation. Like 2016, Sr and Mn drove separation along the first axis (77%) and Li on the second (16%). Fish from late July to early September were separated from those sampled between late September and November (Fig. 2.8d). Overlapping ellipses amongst the July to early September samples indicated similarity in multi-elemental signatures. Fish from late September to November grouped together along the first axis, but separated along the second. Classification success was low at 35% (Supplementary Material S2.5).

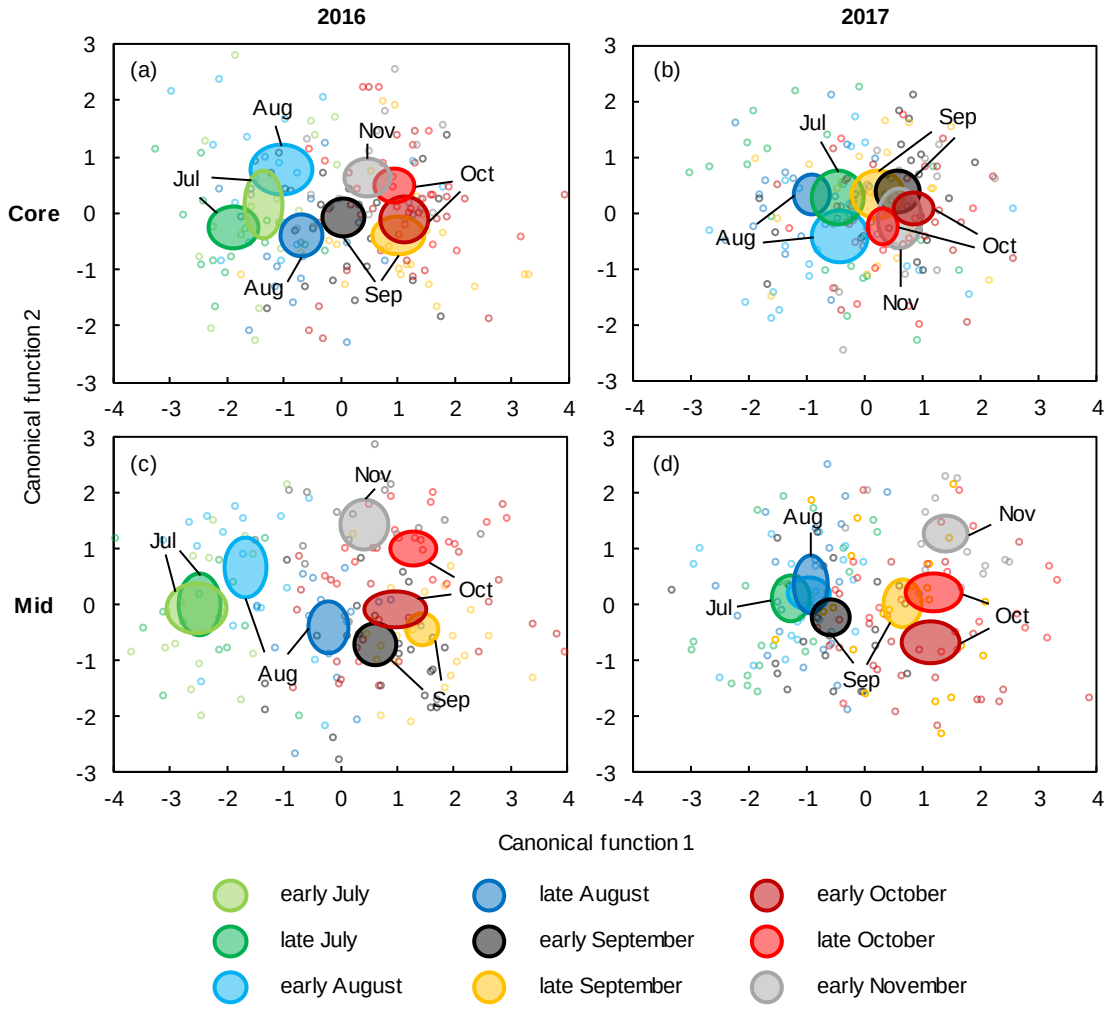

Figure 2.8. Canonical variate plots for the multi-elemental chemistry of the otolith core (top) and mid region (bottom) for King George whiting recruits collected between July and November at Barker Inlet in 2016 (left) and 2017 (right). Ellipses show 95% confidence around group centroids. No data are shown for early July 2017 as the sample size was small (n = 4).

Table 2.4. Summary of results of two-way MANOVAs for the effects of year and sample occasion on multi-elemental chemistry related to the otolith core and mid region of King George whiting recruits. Year and occasion were fixed factors. *P < 0.05; **P < 0.01; ***P < 0.001.

Source	Pillai's trace	df	df_{error}	F
(a) core				
Year	0.174	5	313	13.22***
Occasion	0.556	35	1585	5.66***
Year × Occasion	0.155	35	1585	1.45***
(b) mid				
Year	0.308	5	320	28.46***
Occasion	0.899	35	1620	10.15***
Year × Occasion	0.358	35	1620	3.59***

2.3.3.5 Multi-element comparison between start and end of season

The distribution of sample occasions based on multi-elemental chemistry related to the natal origin and larval advection stages were broadly separated into two groups – those fish sampled 'early' in the season (July to mid-September) and those sampled 'late' (mid-September to November). MANOVAs comparing multi-elemental signatures between the two groups identified significant differences for both life stages (Table 2.5). Irrespective of life stage or year, Sr was foremost responsible for group separation, although separation improved when Mn was influential. For the otolith core, within-season differences were larger for 2016 than 2017 (Fig. 2.9a-b). Classification success was 76% for 2016 and 65% for 2017 (Table 2.6). Comparatively, between-group differences for the larval advection were larger. Fish that settled early or late in the settlement season were divided into two groups based on their otolith chemistry (Fig. 2.9c-d). Within-season differences were greater for 2017 than 2016, supported by classification success of 83% and 79% respectively.

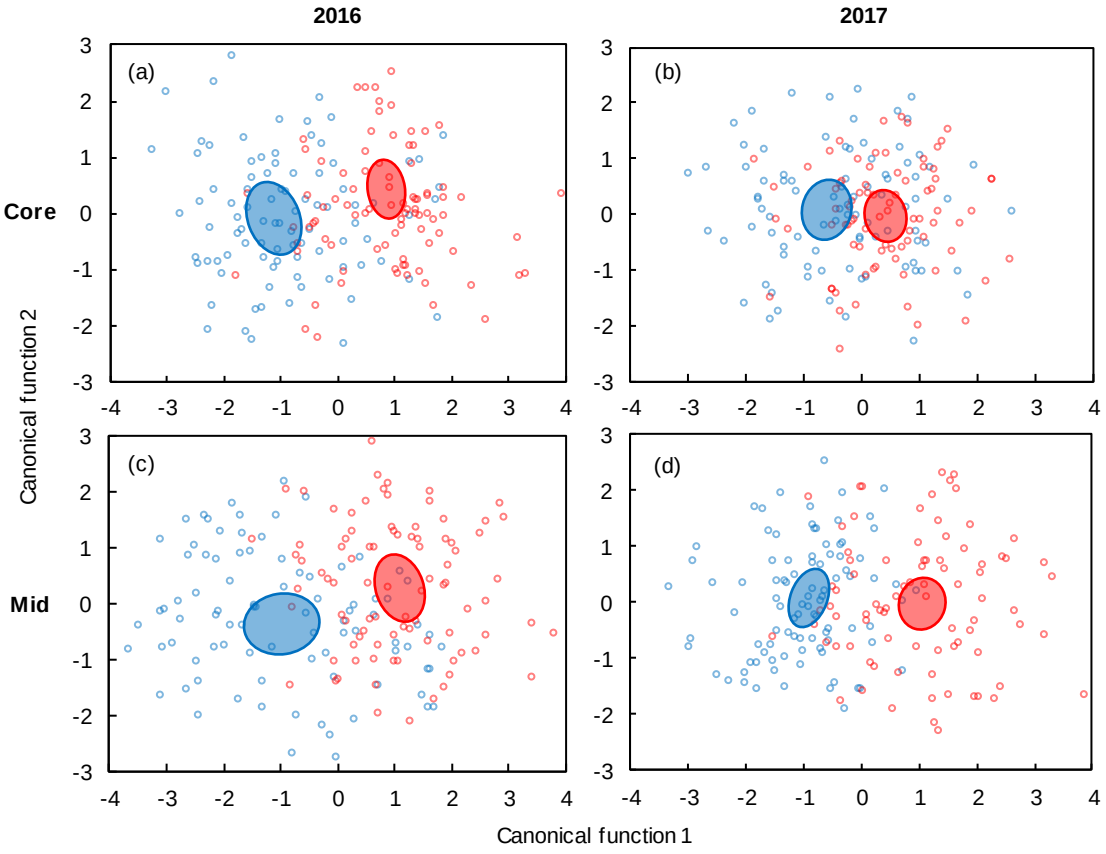

Figure 2.9. Canonical variate plots for the multi-elemental chemistry of the otolith core (top) and mid region (bottom) for recently-settled King George whiting larvae collected early (July to early September; blue) and late (mid-September to November; red) in the settlement season at Barker Inlet in 2016 (left) and 2017 (right). Ellipses show 95% confidence around group centroids.

Table 2.5. Summary of two-way MANOVAs for the effects of year and time of season (early or late) on multi-elemental otolith chemistry related to the otolith core and mid region. Year and time were fixed factors within the full factorial design. *P < 0.05; **P < 0.01; ***P < 0.001.

Source	Pillai's trace	df	df_{error}	F
(a) core				
Year	0.162	5	325	12.59***
Time	0.234	5	325	19.86***
Year × Time	0.050	5	325	3.42***
(b) mid				
Year	0.239	5	322	20.84***
Time	0.409	5	332	45.98***
Year × Time	0.086	5	322	6.27***

Table 2.6. Cross-validated classification success for King George whiting recruits that settled early (July to early September) or late (mid-September to November) in the settlement season based on multi-elemental otolith chemistry. Bold values are correctly classified.

	Year	Time	Early	Late
Core	2016	Early	**76**	24
		Late	24	**76**
		Overall	**76**	
	2017	Early	**65**	35
		Late	35	**65**
		Overall	**65**	
Mid	2016	Early	**78**	22
		Late	21	**79**
		Overall	**79**	
	2017	Early	**87**	13
		Late	23	**77**
		Overall	**83**	

2.4 DISCUSSION

This study investigated the early life-history of recently-settled King George whiting larvae that recruited to an important nursery area over two complete settlement seasons, based on the retrospective analysis of their otoliths. We identified significant temporal variation in early life-history characteristics of larvae within and between years. Otolith chemistry related to the physico-chemical environments occupied during the natal origin and larval advection life-history stages demonstrated considerable temporal variation at multiple scales. When individual elements were combined, they described a significant within-season change in multi-elemental chemistry which related to larvae that hatched at different times.

2.4.1 DEFINING THE SPAWNING SEASON

Throughout the four month settlement season, 52-71% of new recruits were collected in late September and early October, which identified this as the time of peak recruitment. These larvae were spawned between mid-May and early June. As such, spawning during this short, three to four week period made the most significant contribution to annual recruitment. Several other batch spawning species with protracted spawning seasons have also demonstrated a short reproductive window responsible for the majority of recruitment (Cargnelli & Gross 1996, Rankin & Sponaugle 2014, Beveren 2016). However, in this study, the bulk of recruitment corresponded to spawning toward the end of the reproductive season when recruits were smallest and relatively old, rather than at the beginning when they were larger and younger. This seems counterintuitive, as the older and slower growing larvae are likely to have experienced higher mortality during an extended critical period, and subsequently have lower survivorship (Hjort 1914, May 1974, Houde 2008). There are several possibilities to explain this. One is that changes in the plankton community resulted in lower predation on King George whiting eggs and larvae that subsequently improved survivorship (Black et al. 2016, Jenkins & Black 2019). Another option is that spawning success improved, either through higher gamete production or increased fertilisation rates, which culminated in higher recruitment (Leggett & Deblois 1994, Chambers & Trippel 1997). A third possibility is that the larval dispersal pathways changed throughout the spawning season and became more favourable for settlement to Barker Inlet later on. It is likely that larval dispersal would be considerably affected by seasonal changes in larval duration and prevailing oceanographic conditions (Fowler et al. 2000b, Jenkins et al. 2000).

There was some similarity between the settlement pattern to Barker Inlet in 2017 and that for 1993, i.e. 24 years earlier (Fowler & Short 1996). In that year, settlement was characterised by two distinct cohorts, the first that settled in June and July, and the second in late September and October. For 2017, it appears that a second, more abundant, cohort appeared in late September and October. The settlement

pattern for 2016 aligned with the second phase of recruitment, corresponding with the timing of settlement for King George whiting in other regions of its distribution (Jenkins & May 1994, Hyndes et al. 1996, Hamer & Jenkins 1997, Fowler et al. 2000b). Higher settlement during late September and October is likely associated with either improved larval survivorship, a change in larval dispersal pathways, or higher reproductive output later in the season (Leggett & Deblois 1994). It would be unlikely for survivorship to have improved, based on the longer pre-settlement durations and slower growth rates of the late season recruits (Pepin & Myers 1991, Shima & Findlay 2002). Furthermore, the timing and duration of spawning that resulted in recruitment to Barker Inlet does not completely align with the recognised spawning season for Investigator Strait. Estimated hatch dates indicated that spawning occurred from March until July, although adult reproductive activity is highest between March and May (Fowler et al. 1999). This period of spawning corresponds to the first cohort of recruits from July to mid-September, but does not account for the high abundance of new settlers in late September and October that were responsible for the majority of recruitment.

2.4.2 TEMPORAL VARIATION IN EARLY LIFE-HISTORY

Larval King George whiting that hatched at different times throughout the long autumn-winter spawning season displayed differences in early life-history characteristics in response to changes in environmental conditions (Pepin & Myers 1991, Cargnelli & Gross 1996, Radtke et al. 2001). Estimates of size-at-age decreased successively throughout the settlement season, with those larvae sampled at the time of peak recruitment being smaller and having experienced a longer pre-settlement duration than those that settled earlier. The difference in pre-settlement duration between those that settled in July and October was 24 days, representing a 20% increase. Also, over the same period, average growth rate declined by 23-26%. As such, larvae that settled later in the season not only had longer larval durations, but developed at considerably slower rates. The larval phase is when fish are most vulnerable, and therefore any prolongation of pre-settlement duration is likely to be reflected in survivorship and subsequent recruitment (May 1974, Houde 1989, Houde 2008). However, here settlement was highest later in the season when recruits were smaller and had experienced longer larval durations. Larval growth is strongly correlated with temperature, such that even relatively small changes in water temperature can affect growth and development (Houde 1989, Green & Fisher 2004). The systematic decline in average growth rate throughout the season is consistent with the progressively decreasing water temperatures during the autumn-winter period (Fowler & Short 1996). Furthermore, decreasing water temperature may also influence primary productivity and plankton density, which has implications for larval development. It is possible that the decline in average growth rate may also relate to changes in prey availability, as well as the direct effect of water temperature on growth (Leggett & Deblois 1994, Meekan et al. 2003, Black et al. 2016). It also needs to be recognised that this study was focused at a single site, and therefore the results may not be representative of recruitment for this species

at a broader scale. As such, differences in temporal recruitment patterns to other nursery areas could have implications for management recommendations.

2.4.3 TRACE ELEMENT CHEMISTRY

Fish that settled early in the season had significantly lower Sr than those that settled later, which broadly separated the samples into two groups. Strontium is one of the most widely used elements in otolith chemistry analysis to reconstruct environmental histories and differentiate between groups of fish that have occupied different environments (Walther & Thorrold 2006, Izzo et al. 2018). Laboratory experiments and field studies have demonstrated positive relationships between the Sr concentration in otoliths and ambient concentrations in the aquatic environment (Bath 2000, Elsdon & Gillanders 2003b, Izzo et al. 2018). This is likely to be associated with the ability of Sr ions to directly substitute for Ca ions at the accreting surface of the otolith (Doubleday et al. 2013). However, in this study it is difficult to disentangle the physico-chemical influences responsible for the changes in otolith chemistry. Regardless, the observed differences either directly relate to physical environmental conditions, or are mediated by their effects on the physiology of the fish (Sturrock et al. 2012, Sturrock et al. 2014).

Even though Sr was primarily responsible for within-season differences, changes in Li, Mg, Mn and Ba also contributed to group separation and significant multi-elemental differences between sample occasions. The distribution of sample occasions in multivariate space was similar between years, with successive occasions generally showing the greatest similarity in elemental composition. However, there was considerable variation in otolith chemistry within and between sample occasions. For both years, within-season differences in elemental composition were larger for the larval advection life stage compared to the natal origin. It is difficult to determine whether this related to greater environmental heterogeneity during larval dispersal compared to the spawning source, or if ontogenetic influences during early development compromised environmental signals (Ruttenberg et al. 2005, Chittaro et al. 2006, Walther et al. 2010). Several studies have identified changes in otolith chemistry associated with the primordium of otoliths for marine and freshwater fish, which may mask environmental influences and affect the ability to delineate natal origins (Brophy 2004, Ruttenberg et al. 2005, Macdonald et al. 2008). However, we specifically sampled otoliths outside the primordium to reduce such influence.

Daily age information from otolith microstructure provided a highly-resolved temporal scale to assist the interpretation of otolith chemistry (Campana 1999, Campana & Thorrold 2001). Although we identified variation in multi-element chemistry among successive sample occasions, the largest differences were between the two broad groups of recruits that were sampled at the beginning and end of the settlement season. Daily increment counts determined that recruits collected from July to early September hatched from March until the end of April, whilst those collected from mid-September to

November hatched from mid-May into June. The differences in otolith chemistry between these two groups of recruits corresponded to a three week difference in mean hatch date at the beginning of May. Only a handful of fish for each year overlapped in hatch date between these groups, the transition of which was between early and late September in 2016, and August to September in 2017. The corresponding changes in otolith chemistry suggest that recruits collected at different stages of the settlement season were hatched into and developed in different physico-chemical environments.

2.4.4 ECOLOGICAL INTERPRETATION AND FISHERY IMPLICATIONS

The recruits that settled to Barker Inlet between July and early September were spawned in March and April, whilst those that settled in mid-September and October were spawned from mid-May into June. Therefore, different groups of developing larvae were moved towards Barker Inlet between March and October. The two groups of recruits had significantly different early life-history characteristics. Larvae that settled early were larger, faster growing, had shorter pre-settlement durations and had significantly different otolith chemistry compared to those that settled in mid-September and October. However, the latter contributed most to annual recruitment. There are two hypotheses to account for these within-season changes in early life-history characteristics and otolith chemistry. The first is that all recruits to Barker Inlet throughout the long settlement season originated from a primary spawning source and followed a similar larval advection pathway. Here, the differences in otolith chemistry would reflect a temporal change in the physico-chemical environment experienced by the larvae between the time of hatch and throughout the period of larval development. The second possibility is that the different multi-elemental signals represent different spawning sources with different physico-chemical conditions, and that these sources contributed recruits to Barker Inlet at different stages of the settlement season. In this scenario, the geographic source that produced the larvae that settled from mid-September to November made the greatest contribution to annual recruitment. Furthermore, inter-annual variation in reproductive success and larval survival for each spawning source would be reflected in recruitment to Barker Inlet, and help explain the inter-annual variation in settlement (Fowler & Short 1996).

Two approaches are being used to differentiate between these hypotheses. To understand the spatial distribution of spawning that lead to recruitment, we will use a high-resolution biophysical model to simulate larval dispersal and identify potential spawning sources for larvae that recruited throughout the settlement season. Secondly, plankton surveys throughout the recognised spawning area will be undertaken to improve the spatial understanding of spawning activity. The spatial distribution of larvae from these surveys could be used to investigate the hypothesis of multiple spawning grounds contributing to recruitment. The two approaches will provide complementary spatial information to be interpreted along with the information on otolith microstructure and chemistry.

Understanding the spatial and temporal variation in early life-history characteristics will help to develop the most appropriate fishery management strategies. Most recruitment to Barker Inlet occurred in late September and early October, corresponding to spawning between mid-May and June. These recruits had significantly different early life-history characteristics and otolith chemistry compared to those that settled earlier. Since 2017, a seasonal closure has been imposed throughout an extensive area in Investigator Strait and southern Spencer Gulf for the month of May to protect aggregations of spawning whiting (Steer et al. 2018). Nevertheless, there may be temporal and spatial issues associated with this closure. Based on the timing of peak settlement and the retrospective hatch dates of these recruits, the closure does not completely encompass the period of spawning responsible for the majority of recruitment to Barker Inlet. The discrepancy in the timing of spawning that resulted in peak recruitment to Barker Inlet and the current seasonal spawning closure has potential implications for management. Resolving the underlying cause of the significant change in otolith chemistry throughout the reproductive season should produce better spatial information regarding where King George whiting originate. This could lead to refinement of the current seasonal spawning closure. Combining otolith microstructure and trace element chemistry has improved our understanding of early life-history for this important fishery species.

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Name of Principal Author (Candidate)	Troy Rogers
Contribution to the Paper	Contributed to the design of the study, conducted the fieldwork, collected the samples, performed the laboratory processing and sample preparation, operated the LA-ICP-MS, collected and analysed the data, applied statistical analyses, wrote the manuscript and acted as corresponding author.
Overall percentage (%)	90%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 14/11/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Contributed to the design of the study, assisted with data interpretation and revised the manuscript.
Signature	Date 17/11/2019

Chapter 3 - Spatial connectivity during the early life-history of a temperate marine fish inferred from otolith microstructure and geochemistry

ABSTRACT

Connectivity during the ontogenetic development of fishes identifies the spatial scale over which a population functions, which is the appropriate scale for conservation and management. For many marine species, spawning grounds and nursery areas are spatially segregated and larval dispersal is an obligate process that connects life-history stages. This study investigated the spatial scale of early life-history for one such species, the King George whiting (*Sillaginodes punctatus*; Perciformes), through the retrospective analysis of otolith microstructure and elemental chemistry of recently-settled larvae. The aim was to determine whether the South Australian population constitutes a single panmictic stock, or if it comprises multiple sub-populations. Sizes (15.1-25.1 mm SL), ages (85-183 d) and hatch dates (24-Apr to 1-Aug) of larvae varied considerably between nursery areas at different spatial scales. Regional differences in multi-elemental otolith signatures indicated that multiple spawning grounds contribute to recruitment, and larvae that settled in each region dispersed through different water masses. Within each region, there were differences in hatch dates and otolith chemistry indicative of finer-scale relationships between particular spawning grounds and nursery areas, consistent with local oceanographic circulation patterns. Although multi-elemental signatures were year-specific, concentrations of Ba and Mn were largely responsible for spatial differences and assigned larvae to regional groups with 52-66% accuracy. The results suggest the State-wide stock is replenished by three putative source populations, and provide an example of how otolith chemistry can discriminate among geographically-close, yet-ecologically separated groups of fish in coastal marine ecosystems.

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3.1 INTRODUCTION

A common feature in the life-history of marine fishes is coastal spawning followed by larval ingress to nursery areas, which is strongly influenced by physical oceanographic processes (Norcross & Shaw 1984, Beck et al. 2001, Teodosio et al. 2016). For such species, nursery areas provide critical habitats for larvae to settle and then develop into juveniles, before they move to adjacent coastal populations during their ontogenetic development (Cowen et al. 2000, Beck et al. 2001, Cowen & Sponaugle 2009). Larval transport provides the potential for extensive dispersal and the opportunity for mixing between geographically segregated populations (Cowen & Sponaugle 2009, Leis et al. 2011). As such, understanding connectivity during ontogenetic development is necessary to determine the spatial scale over which fish populations operate, which informs population dynamics, stock structure, and ultimately underpins effective conservation and management strategies.

The extent of mixing between larvae from different spawning grounds during dispersal determines whether fish populations are essentially self-recruiting stocks, or if they form part of a larger meta-population where recruits originate from multiple sources (Bailey 1997). Historically it was assumed that marine fish populations were open systems and that recruitment was largely independent of local reproduction (Hjort 1914, Caley et al. 1996, Cowen et al. 2000). However there has been a growing body of evidence that connectivity between habitats and life stages is far more complex than originally considered, and that life-history processes can occur over much finer spatial scales (Swearer et al. 1999, Thorrold et al. 2001, Jones et al. 2005). Empirically quantifying larval movement is logistically challenging because of the inherently small size and difficulty in marking larvae. However, the refinement of analytical techniques has facilitated the use of biological structures as ‘natural tags’. Of those considered (reviewed by Gillanders 2009), analysis of the incremental structure and chemistry of otoliths has become a leading approach for assessing connectivity in fish populations (Campana 1999, Elsdon et al. 2008, Reis-Santos et al. 2013).

Fish otoliths, or ‘ear stones’, are paired crystalline structures that assist with balance and orientation which precipitate throughout the lives of teleosts (Campana & Neilson 1985). They are metabolically inert and form bi-partite structures on a daily periodicity, which can be used to estimate age (Pannella 1971). When interpreted correctly, the incremental microstructure of otoliths can provide highly-resolved temporal information on early life-history characteristics including larval growth, pre-settlement duration and hatch date (Campana & Neilson 1985, Campana & Jones 1992). Such information can then be used to discriminate between fish that hatched and developed in different environments (Campana & Jones 1992, Clausen et al. 2007, Watai et al. 2018). The calcium carbonate used for increment formation is derived from the surrounding aquatic environment, which contains elements in minor and trace quantities that can be incorporated into the otolith at the accreting surface

(Campana 1999, Elsdon et al. 2008). Ambient concentrations of these elements are influenced by extrinsic factors which vary at different spatial and temporal scales. However, elemental concentrations incorporated into otoliths do not directly reflect ambient concentrations (Izzo et al. 2018), but are regulated by physiological processes and molecular interactions along the incorporation pathway (Sturrock et al. 2014, Izzo et al. 2016, Thomas et al. 2017). Nevertheless, otolith elemental composition does relate to the physico-chemical environment experienced by the fish, which can be coupled with age information to describe a chronological record of environmental history (Campana 1999, Elsdon et al. 2008). The retrospective analysis of otolith chemistry has been used to reconstruct movement patterns (Elsdon & Gillanders 2003a, Hamer et al. 2006), assess stock structure (Ferguson et al. 2011, Tanner et al. 2016), discriminate natal or nursery origins (Thorrold et al. 2001, Walther et al. 2008, Tanner et al. 2012), and evaluate the contribution of nursery areas to adult populations (Gillanders 2005, Reis-Santos et al. 2013).

King George whiting (*Sillaginodes punctatus*; Perciformes) is a demersal marine finfish species that is endemic to temperate coastal waters of southern Australia where it supports important fisheries (Kailola et al. 1993). In South Australia, which has historically provided the highest State-based catches, commercial catches and estimated biomass have declined to record lows in recent years (Steer et al. 2018). There are considerable differences in population characteristics and biomass between regions in South Australia that may relate to recruitment variability (Steer et al. 2018). Despite extensive research into the life-history of this species (Fowler & Short 1996, Fowler et al. 1999, Fowler et al. 2000b, Jenkins et al. 2000, Jenkins 2005, Jenkins et al. 2016), the relationships between spawning grounds and nursery areas, and the spatial scale over which the early life-history operates, remain poorly understood. Nursery areas can be up to several hundred kilometres from the nearest spawning ground, which means that larval dispersal is an obligate process that connects life-history stages (Fowler et al. 2000b, Jenkins et al. 2000, Jenkins 2005). Adults spawn in coastal waters near low-profile reef systems during the austral autumn and early winter. The only known spawning areas are throughout Investigator Strait and southern Spencer Gulf (Fowler et al. 1999, Fowler et al. 2000a) (Fig. 3.1), although it is recognised that spawning occurs elsewhere. The developing larvae experience an extended dispersal phase of 3-5 months, before they settle in protected bays during winter and spring (Jenkins & May 1994, Fowler & Short 1996, Rogers et al. 2019a; Chapter 2). Juveniles develop and reside in these nursery areas for up to two years, and eventually move to adjacent coastal populations as adults after 3-4 years. Adults form a mixed size and age population in the spawning area where they remain resident for the rest of their lives (Fowler et al. 2000a, Fowler & Jones 2008). The distribution of King George whiting around southern Australia is divisible into three broad stocks based on genetic divergence: the Western Australian Stock; the South Australia and Victoria Stock; and the Tasmanian Stock (Jenkins et al. 2016). Genetic homogeneity among South Australian and Victorian populations indicates that there is some movement of fish between these geographic regions (Kent et al. 2018), although each State-wide

population is considered a separate biological stock (Jenkins et al. 2016). However, within South Australia there are considerable geographic differences in population characteristics and biomass (Steer et al. 2018), suggesting that finer-scale stock structure may exist.

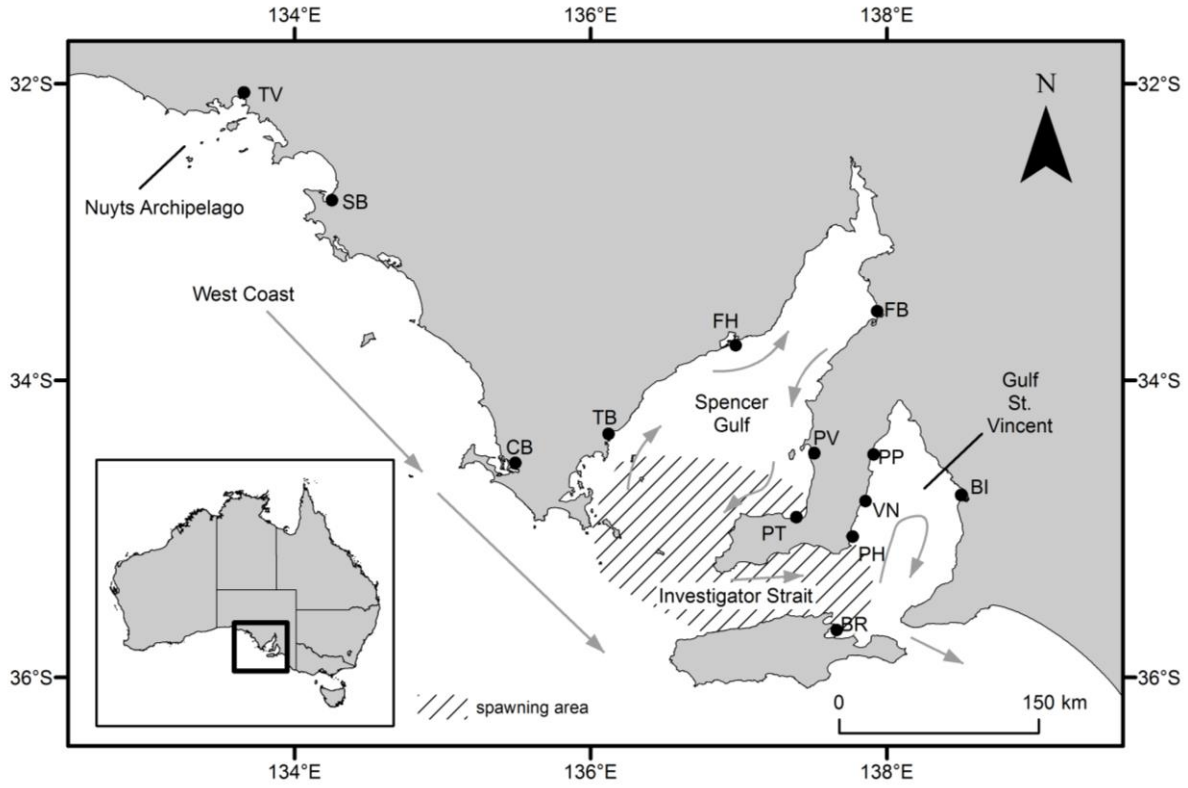

Figure 3.1. Map of South Australia showing the sites and regions sampled for recently-settled King George whiting larvae in 2016 and 2017. The shaded zone identifies the known spawning area of Southern Spencer Gulf and Investigator Strait. Site names are in Table 3.1. Arrows show the general circulation of water in the study area. Inset – map of Australia identifying the study area along the southern coastline.

There are two hypotheses regarding the spatial scale over which the early life-history operates and its influence on population biology. The first hypothesis is that larvae which recruit to South Australian nursery areas originate from a single spawning source, be it the known spawning area in Investigator Strait and southern Spencer Gulf, or elsewhere. This is synonymous with the proposed model of replenishment of nursery areas in Victoria (Jenkins et al. 2000, Jenkins 2005), and suggests the South Australian population would constitute a single, panmictic stock. The second hypothesis is that the larvae that recruit to nursery areas in different regions originate from different spawning grounds. This is the model proposed by Fowler et al. (2000b) based on physical oceanographic modelling, which suggested larvae are sourced from different spawning grounds and then disperse relatively short distances (50-200 km). In this case, the stock would be comprised of multiple small, self-recruiting populations. This proposition remains untested empirically. This study aimed to differentiate between these hypotheses and to identify the spatial scale over which the early life-history operates. We

compared the otolith microstructure and trace element chemistry of recently-settled larvae from two successive annual cohorts at the regional and site level. Spatial differences in otolith chemistry would support the hypothesis of localised population processes, whereas no differences suggest larvae originated from a single, intermixed source.

3.2 MATERIALS AND METHODS

3.2.1 STUDY AREA

The South Australian coastline is geographically extensive and supports a diversity of marine ecosystems. Recently-settled King George whiting larvae were sampled from three regions: the West Coast (WC) of Eyre Peninsula; Spencer Gulf (SG); and Gulf St. Vincent (GSV; Fig. 3.1). The West Coast of Eyre Peninsula is an open, high energy coastline characterised by rocky cliffs interspersed with protected bays, adjacent to the eastern Great Australian Bight. When larvae are dispersing during the austral winter, an eastward coastal current is driven by seasonal winds, thermohaline gradients and the residual Leeuwin Current (Middleton & Bye 2007). Onshore Ekman transport leads to downwelling, which pushes cold, nutrient poor water onto the shelf and into coastal areas. In contrast, the physical environmental characteristics of Spencer Gulf and Gulf St. Vincent are largely influenced by their complex topography, shallow bathymetry and local meteorology, rather than through shelf-gulf exchange (Bye & Kampf 2008). Spencer Gulf and Gulf St. Vincent are semi-enclosed seas that display a positive salinity gradient from the mouth to head, which classifies them as inverse estuaries (Nunes & Lennon 1986). This is because evaporation exceeds precipitation and there is no permanent freshwater input (Petrusevics 1993). Both gulfs demonstrate seasonal reversals in temperature gradients. Temperature fluctuation is highest (12-24 °C) toward the heads due to their shallow bathymetry and irregular flushing, and moderate (15-19 °C) near the mouth and Investigator Strait because of increased depth and seasonal shelf exchange (Petrusevics 1993, Middleton & Bye 2007). Local wind forcing and density-driven thermohaline circulation are largely responsible for the general clockwise movement of water within the gulfs (Bye 1976, Bye & Kampf 2008).

The described geographic differences in environmental characteristics underpin the use of otolith-based techniques to investigate ontogenetic connectivity and stock structure of King George whiting. In 2016, recently-settled larvae were sampled at four sites in Spencer Gulf and five sites in Gulf St. Vincent. In 2017, the same sites were sampled, as well as three sites on the West Coast and another site in Spencer Gulf due to additional funding (Fig. 3.1; Table 3.1). The sites are semi-protected embayments which support shallow beds of *Zostera* spp. seagrass and have previously been identified as nursery areas for King George whiting (Fowler & Jones 2008).

Table 3.1. Mean (SD) standard length (SL, mm) and sample size (*n*) of recently-settled King George whiting larvae collected from each region and site in 2016 and 2017.

		2016		2017	
Region (Site)		SL (mm)	*n*	SL (mm)	*n*
West Coast	**WC**	**-**	**-**	**20.8 (1.1)**	**48**
Thevenard	TV	-	-	20.2 (0.9)	19
Streaky Bay	SB	-	-	21.2 (1.1)	14
Coffin Bay	CB	-	-	21.1 (1.0)	15
Spencer Gulf	**SG**	**20.4 (2.2)**	**60**	**20.8 (1.9)**	**98**
Tumby Bay	TB	-	-	19.5 (1.7)	19
Franklin Harbour	FH	18.4 (0.3)	20	18.6 (0.8)	20
Fishermans Bay	FB	22.6 (1.5)	14	22.0 (1.4)	20
Port Victoria	PV	21.3 (2.6)	9	21.9 (0.6)	20
Point Turton	PT	20.6 (1.4)	17	22.2 (1.3)	19
Gulf St. Vincent	**GSV**	**18.3 (1.1)**	**100**	**18.0 (1.4)**	**91**
Point Hickey	PH	16.7 (0.6)	20	16.5 (0.3)	19
Port Vincent	VN	18.5 (0.6)	20	18.1 (1.3)	18
Pine Point	PP	18.4 (0.8)	21	18.4 (0.9)	20
Barker Inlet	BI	18.2 (0.6)	20	17.0 (0.4)	20
Brownlow	BR	19.7 (0.6)	19	19.9 (0.8)	14

3.2.2 SAMPLE COLLECTION AND PROCESSING

Each site was sampled once during October, which is the time of peak settlement (Fowler & Short 1996, Fowler et al. 2000b, Rogers et al. 2019a; Chapter 2). All sites across this broad geographic range (ca. 1000 km; Fig. 3.1) were sampled over a six day period in each year to limit the influence of temporal confounding. Sampling was done using a small beach seine net (mouth width 5 m, semicircular perimeter 7 m, drop 2 m, mesh 1 mm^2) hauled by two people through shallow water (0.5-1.0 m). Sampling continued until > 25 larvae were collected. King George whiting were sorted from the net, stored in resealable plastic bags on ice and frozen for later analysis. The samples from each site generally consisted of a mix of recently-settled larvae and developing juveniles. The definitions and descriptions of early life-history stages for King George whiting used in this study were summarised by Rogers et al. (2019a; Chapter 2).

All King George whiting were measured for standard length (SL) to the nearest 0.1 mm, and the twenty smallest larvae from each sample were used for otolith analyses. Their sagittal otoliths were removed under a dissecting microscope (Olympus SZX7; Tokyo, Japan) using stainless steel needles, triple

rinsed in ultrapure water to remove adhering tissue, air dried and stored in individual microcentrifuge tubes. No settlement mark was evident in the otoliths of these larvae, preventing the determination of a settlement day. This was consistent with previous studies of this species in South Australia (Fowler & Short 1996, Fowler et al. 2000b, Rogers et al. 2019a; Chapter 2). Settlement of King George whiting is more related to size than age, and therefore we considered the smallest fish to have most recently undergone settlement (Fowler & Short 1996, Rogers et al. 2019a; Chapter 2).

3.2.3 OTOLITH MICROSTRUCTURE

One otolith from each fish (n = 435) was arbitrarily selected for microstructure examination, and the other was used for trace element chemistry analysis. The former was mounted sulcus face upwards on a microscope slide using thermoplastic glue (CrystalBond 509; ProSciTech, QLD, AUS), then hand polished through the sagittal plane to the primordium using three grades (9 µm, 3 µm and 1 µm) of aluminium oxide lapping film (AusOptic; NSW, AUS). An image analysis system was used to aid interpretation of the otolith microstructure (Olympus BX51 compound microscope, Olympus DP73 video camera, Olympus Stream v. 1.9.1; Tokyo, Japan). Otolith sections were viewed through a 100× objective using immersion oil. Daily periodicity of increment formation for King George whiting has been validated from known age larvae (B.D. Bruce and D.A. Short, unpub.). Increments were counted from the primordium to the posterior margin, and repeated counts were made for each otolith. When these counts differed by < 5%, their mean was considered the estimated age. If not, counts were repeated until an acceptable estimate of the number of increments was achieved. If not, or if the otolith was damaged during processing, it was rejected (n = 38). Hatch date was calculated by subtracting the estimated age from the known date of capture. Average growth rate (mm d^{-1}) was calculated as:

$$\frac{L_c - L_h}{a} \qquad \text{Eq. (3.1)}$$

where L_c is the length at capture, L_h is the length at hatch (2.1 mm SL; Bruce, 1995) and a is age (d).

3.2.4 TRACE ELEMENT CHEMISTRY

Elemental chemistry preparation and analytical procedures followed Rogers et al. (2019a; Chapter 2). The remaining otolith from each fish was embedded sulcus face upwards in thermoplastic glue (CrystalBond 509) on a small epoxy resin disc (Struers). Thermoplastic glue and epoxy resin were spiked with indium (^{115}In) at ~200 ppm to aid discrimination during analysis (Reis-Santos et al. 2012). Otoliths were polished to the primordium as previously described, rinsed in ultrapure water and air dried. Then, 24 otolith sections randomly selected from different sites were transferred to a new slide for analysis. Each analysis slide (n = 17) was triple rinsed with ultrapure water, air dried and stored in a sealed plastic bag.

Otolith sections were analysed for trace element chemistry by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The system consisted of a New Wave Research (Fremont, CA, USA) 213 nm high performance (Nd:YAG) ultraviolet probe laser ablation system coupled to an Agilent (Santa Clara, CA, USA) 7900 quadrupole ICP-MS located at Adelaide Microscopy (Adelaide, SA, Australia). Each otolith was sampled at two places: (1) 'core' – posterior to the exogenous feeding check incorporating the first 20 or so days of larval life, representing the 'natal origin'; and (2) 'mid' - 100-130 µm from the primordium toward the posterior margin, representing a period of larval dispersal, ~60-70 d post hatch (Fig. 3.2). Ablation sites were located by comparing pre-prepared high resolution photographs of the samples with the video image of the ablation chamber (Ferguson et al. 2011).

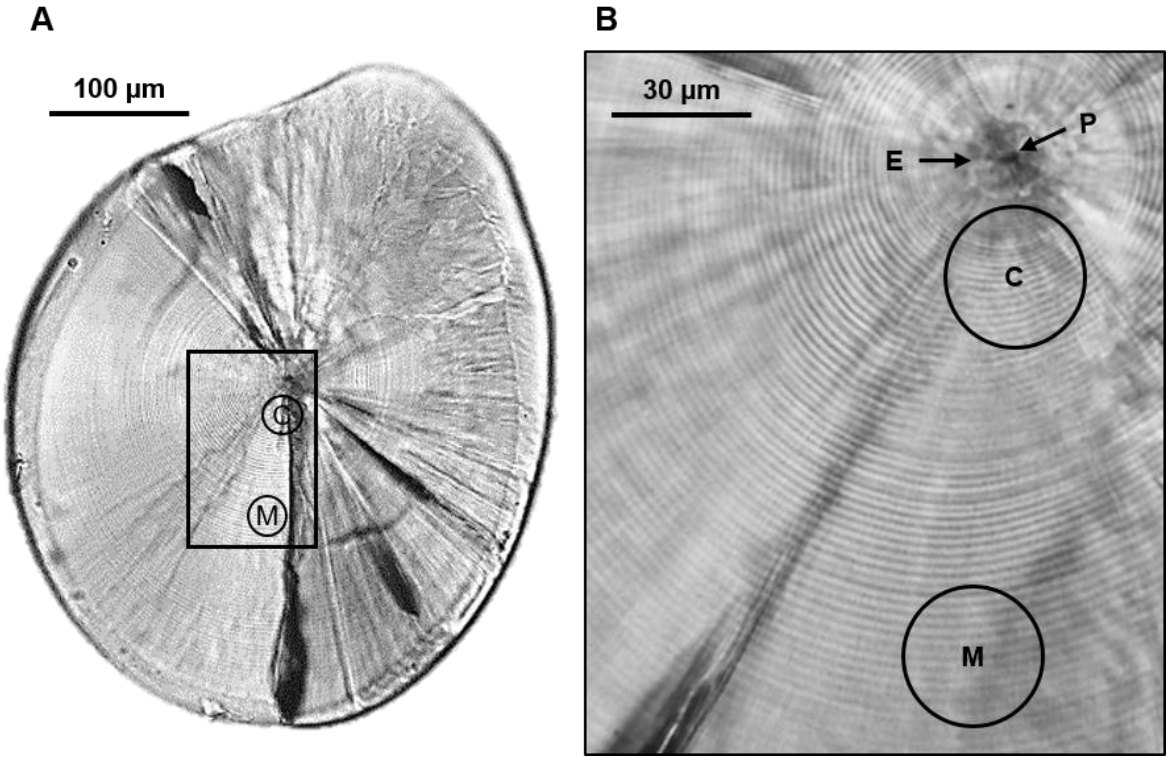

Figure 3.2. Polished sagittal otolith from a recently-settled King George whiting larva viewed at the proximal surface (sulcus face upward). (A) Otolith viewed through a 20× objective; (B) highlighted section from A viewed through a 60× objective. Both images show the locations of 30 µm LA-ICP-MS spot ablations: C – core; M – mid-section (P – primordium; E – exogenous feeding check).

Each otolith was sampled using a 30 µm diameter laser 'spot' at a pulse rate of 5 Hz and a beam density at the sample of ~11 J cm^{-2}. Otoliths were pre-ablated for three seconds to remove possible surface contamination. Ablation occurred in a ^{4}He flushed chamber that was mixed with ^{40}Ar for injection into the plasma. Five elements were quantified (^{7}Li, ^{25}Mg, ^{55}Mn, ^{88}Sr and ^{138}Ba), as well as ^{43}Ca that was measured for use as the internal standard. ^{115}In was measured to aid discrimination between otolith material and thermoplastic glue during analysis (Reis-Santos et al. 2012). The concentration of otolith

^{43}Ca was assumed to be constant at 38.8% (Yoshinaga et al. 2000). Data were calibrated against the National Institute of Standards (NIST) 612 glass reference (Lahaye et al. 1997). Background measurements were recorded for 30 s and a concentration of each element was recorded every 0.17 s. Data reduction was done offline in the Igor Pro workspace using Iolite software (v. 2.5; Paton et al. 2011). Data were converted to molar concentrations and standardised to calcium (element:Ca, µmol mol^{-1}). All analyses used the element:Ca data.

Internal precision and accuracy were assessed by analysing the NIST 612 as an unknown sample against the known concentrations, and external precision was assessed by measurements of MACS-3 (United States Geological Survey; Virginia, United States) calcium carbonate reference material. NIST 612 and MACS-3 were analysed twice at the beginning and end of each session, and after every 12 ablations to correct for short-term instrument drift (n = 104). Mean recovery for the NIST 612 was 100.0-100.2% for each element. Mean relative standard deviation (RSD) for NIST 612 was: 1.8% (Li), 1.0% (Mg), 0.8% (Mn), 0.7% (Sr), and 0.8% (Ba). External precision (RSD) assessed by measurements of the MACS-3 reference material was: 6.2% (Li), 1.9% (Mg), 1.6% (Mn), 1.5% (Sr), and 2.1% (Ba). Mean MDL (µmol mol^{-1}) based on three times the standard deviation of the blank gases adjusted for ablation yield (Lahaye et al. 1997) were: 0.66 (Li), 0.51 (Mg), 0.26 (Mn), 0.01 (Sr), and 0.01 (Ba).

3.2.5 DATA ANALYSES

3.2.5.1 Early life-history characteristics

The data for the sizes, ages and average growth rates of larvae violated the parametric assumptions of normality (Shapiro-Wilk goodness of fit statistic) and homoscedasticity (Levene's test). Therefore, these characteristics were compared between years, regions and sites by three-factor permutational analysis of variance (PERMANOVA). Year and region were fixed factors, and site was nested in region. Resemblance matrices were calculated from Euclidean distance dissimilarity, and analysed by unrestricted permutation with 9,999 repeats. When significant differences were found, *post-hoc* pair-wise comparisons were used to identify the source of differences among group means.

3.2.5.2 Trace element chemistry

Otolith data for each element (^{7}Li, ^{25}Mg, ^{55}Mn, ^{88}Sr and ^{138}Ba) consistently exceeded (98.9-100%) the detection limits of the ICP-MS, and so were considered for statistical analyses. Elemental ratios of Li, Mg, Sr and Ba satisfied parametric assumptions following fourth-root transformation. However, Mn:Ca violated assumptions regardless of transformation type. Therefore, to apply a consistent statistical approach to all elements and enable them to be combined for multivariate analyses, non-parametric tests were used. Elemental data were compared between years, regions and sites using a three-factor PERMANOVA design for each element individually and all elements combined (Anderson 2001). Year

and region were fixed factors, and site was nested in region. Element:Ca data were normalised prior to constructing resemblance matrices based on Euclidean distance dissimilarity, and analysed using unrestricted permutation with 9,999 random repeats. Euclidean distance was used because it is the most appropriate distance measure for continuous variables where data are not on comparable scales or ranges. *Post-hoc* pair-wise comparisons identified the source of differences between means. Multivariate data were reduced to two-dimensions and visualised using non-metric multidimensional scaling (nMDS) and canonical analysis of principal coordinates (CAP). CAP is a constrained method of ordination for discriminating between *a priori* groups (Anderson & Willis 2003) and was considered an appropriate approach to assess spatial and temporal variation in multi-elemental data because: (1) constrained ordination has been traditionally applied to otolith chemistry studies which aim to identify variation in the natal signatures of juvenile or adult fish (e.g. Gillanders & Kingsford 1996, Thorrold et al. 2001, Jenkins et al. 2016); (2) constrained ordination is considered a practical approach for assigning fish to possible sources (White & Ruttenberg 2007); and (3) the complex multi-factor design of the study could confound visually identified groupings from an unconstrained ordination. To aid interpretation of the canonical plots, vector diagrams were included to show the influence of elements to sample positioning in multivariate space. The relative length and direction of each vector correspond to its discriminating ability. Jack-knifed cross validation was used to classify fish to groups for each year and region based on the multi-elemental signatures of the remaining samples. Statistical analyses were done using PRIMER (v. 7.0.13; Auckland, NZ) and figures produced using SigmaPlot (v. 14.0; Systat Software Inc, San Jose, CA, USA).

3.3 RESULTS

3.3.1 EARLY LIFE-HISTORY CHARACTERISTICS

There were significant spatial differences in the sizes and ages of recently-settled larvae (Table 3.2). In both years, larvae from Spencer Gulf were significantly larger and older than those from Gulf St. Vincent (Fig. 3.3). There were no differences in mean size or age between Spencer Gulf and the West Coast. Average growth rates did not differ between regions, but were significantly different between years. Larvae grew 10.1% faster in 2017 than 2016 (mean growth 0.142 and 0.129 mm d^{-1}, respectively). Mean size and age of larvae differed significantly among sites, the pattern of which varied between years (Table 3.2). In general, size and age increased from west to east among sites within each region (Fig. S3.1). Larvae ranged in size from 15.1-25.4 mm SL and 15.7-23.9 mm SL in 2016 and 2017, respectively. Age ranged from 92-183 d in 2016 and 85-160 d in 2017.

Table 3.2. Summary of results from three-factor PERMANOVAs for the effects of year, region and site on the length, age and average growth rate of recently-settled King George whiting larvae. *P < 0.05; **P < 0.01; ***P < 0.001.

		Length		Age		Ave. Growth	
	df	MS	*F*	MS	*F*	MS	*F*
Year	1	0.04	0.03	28.41	33.46***	62.85	78.46***
Region	2	61.30	6.00*	46.03	5.39*	6.38	2.30
Site (Region)	10	13.17	49.79***	10.97	28.42***	3.41	5.52***
Year × Region	1	1.43	1.30	0.01	0.01	1.11	1.38
Year × Site (Re)	7	1.15	4.34***	0.87	2.26*	0.81	1.31
Residuals	358	0.26		0.39		0.62	

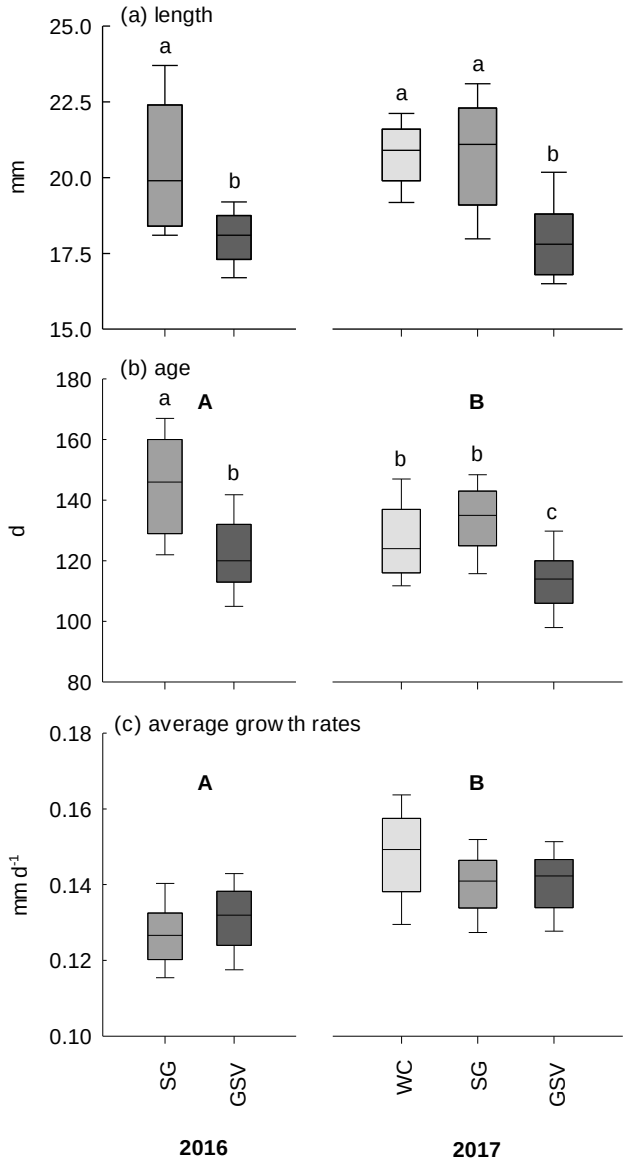

Figure 3.3. Regional comparisons of standard length (mm), age (d) and average growth rates (mm d^{-1}) for recently-settled King George whiting larvae sampled in 2016 and 2017. Box and whisker plots show median, box 25-75%, error bars 10 and 90%. Letters identify significant differences ($P < 0.05$; capitals = between years, lower case = between regions). WC – West Coast; SG – Spencer Gulf; GSV – Gulf St. Vincent.

Hatch dates ranged from 24 April to 1 August in 2016 and from 11 May to 29 July in 2017 (Fig. 3.4). The mean and range of hatch dates differed between regions. Larvae from Spencer Gulf hatched 13-17 d earlier than those from Gulf St. Vincent. The range of hatch dates was considerably larger for Gulf St. Vincent than Spencer Gulf in 2016 (99 and 57 d, respectively), but similar in 2017 (73 and 72 d, respectively). Larvae hatched 6-10 d later in 2017 than 2016. Hatch dates were very similar between Spencer Gulf and the West Coast. There was considerable variation in hatch dates among sites, particularly in Gulf St. Vincent. Larvae at the eastern sites (Pt. Hickey, Pt. Vincent and Pine Point) hatched several weeks later those elsewhere (Fig. S3.2).

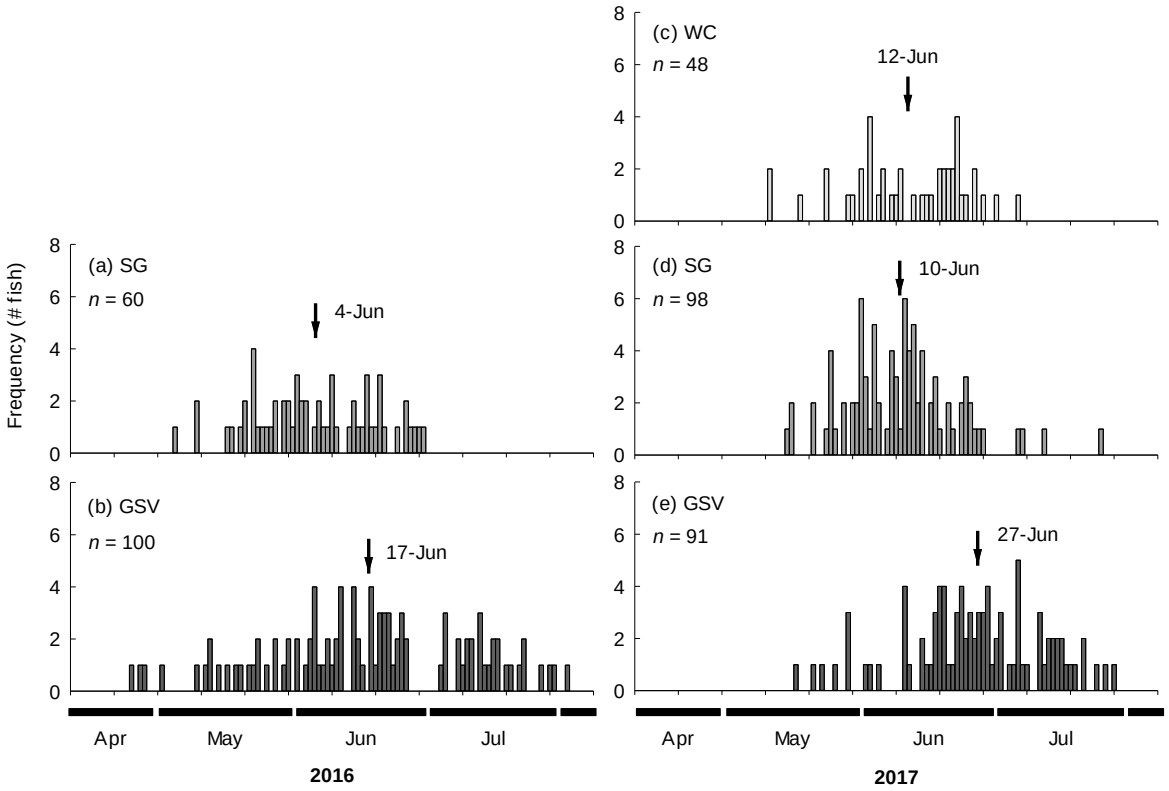

Figure 3.4. Frequency histograms showing the number of aged King George whiting larvae from each region that hatched on the nominated Julian day in 2016 and 2017. Arrows identify mean hatch day. WC – West Coast; SG – Spencer Gulf; GSV – Gulf St. Vincent.

3.3.2 OTOLITH TRACE ELEMENT CHEMISTRY

3.3.2.1 Individual elements

Spatial and temporal differences in trace element chemistry varied among individual elements (Table 3.3). For the otolith core, there were regional differences for Sr and Ba. In 2016, only Sr differed significantly and was higher for Gulf St. Vincent than Spencer Gulf (Fig. 3.5*a*). Ba was consistently higher for Gulf St. Vincent, although only significant in 2017. There were differences between years for Li, Mn and Sr, which were most pronounced in Mn and Sr (Fig. 3.5*a*). At a finer spatial scale, elemental ratios of Li, Mg and Ba varied significantly among sites within regions (Table 3.3; Fig. S3.3*a*). There were significant differences among sites between years for Li, Mg and Ba. Concentrations of Li and Ba were higher in 2016 than 2017.

For the mid-section of the otolith, only Mn differed between regions (Table 3.3). In 2016, Mn was significantly higher for Spencer Gulf than Gulf St. Vincent (Fig. 3.5*b*). However in 2017, there was no difference between Spencer Gulf and Gulf St. Vincent, but the West Coast was significantly higher. Significant differences between years occurred for Sr and Mn. Concentrations of Sr were higher in 2017, consistent with the patterns seen in the otolith core. There were no differences between regions or years for Li, Mg and Ba, with Ba almost constant at ~0.690 µmol mol^{-1}. PERMANOVAs detected differences (P<0.001) among sites within regions for all elements (Table 3.3). Site-level differences were largest for Li and Mg, although these elements did not differ between regions or years (Fig. S3.3*b*).

Table 3.3. Summary of three-factor PERMANOVAs for the effects of year, region and site on individual and combined element:Ca ratios for the otolith (a) core and (b) mid-section of recently-settled King George whiting larvae. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

		Li		Mg		Mn		Sr		Ba		All elements	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(a) core													
Year	1	12.20	6.39*	1.70	0.31	51.27	39.30***	25.77	34.85***	2.38	1.13	93.32	8.08**
Region	2	10.54	3.58	0.62	0.18	2.34	1.98	3.46	6.15*	18.29	8.68**	35.26	3.44*
Site (Region)	10	3.54	4.44***	4.18	5.01***	1.29	1.60	0.46	0.49	2.46	3.01**	11.93	2.85***
Year × Region	1	0.11	0.06	1.12	0.20	0.62	0.47	1.14	1.55	1.94	0.92	4.93	0.43
Year × Site (Re)	7	1.97	2.47*	5.73	6.87***	1.33	1.66	0.73	0.78	2.18	2.66*	11.94	2.85***
Residuals	358	0.80		0.83		0.80		0.94		0.82		4.19	
(b) mid													
Year	1	9.71	4.17	0.23	0.09	4.91	5.89*	42.06	27.85**	2.69	0.99	59.60	5.97**
Region	2	11.79	3.28	2.37	0.45	39.46	12.32**	4.05	1.45	1.73	0.71	59.41	3.43**
Site (Region)	10	4.36	5.64***	6.51	8.06***	3.87	5.24***	3.33	4.27***	2.84	3.06***	20.91	5.19***
Year × Region	1	1.85	0.79	1.52	0.59	1.04	1.25	6.62	4.38	0.13	0.05	11.16	1.12
Year × Site (Re)	7	2.41	3.12**	2.70	3.34**	0.84	1.14	1.54	1.99	2.80	3.01**	10.30	2.56***
Residuals	358	0.77		0.81		0.74		0.78		0.92		4.03	

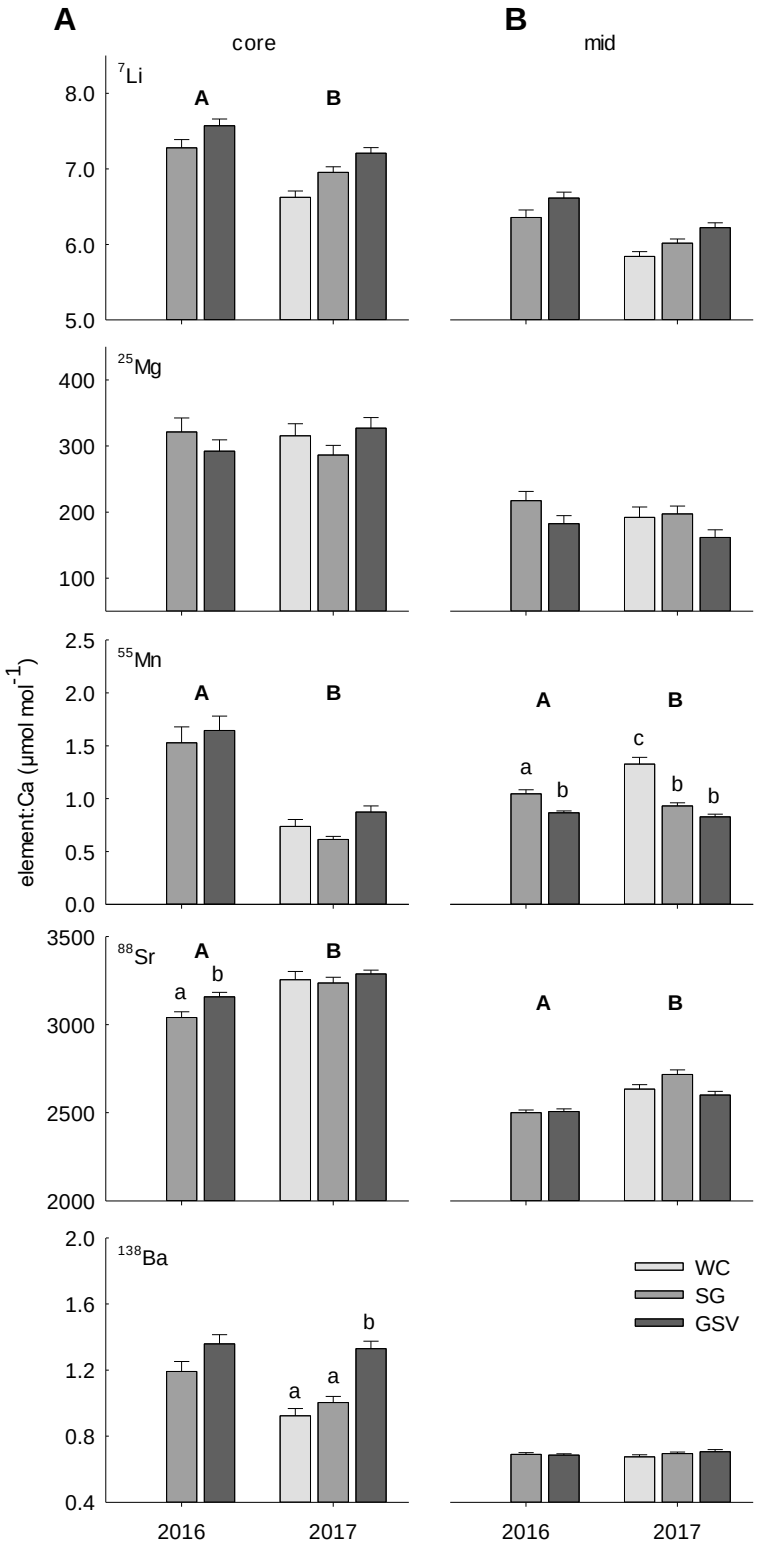

Figure 3.5. Regional comparisons of mean element:Ca ratios (µmol mol^{-1}) for the otolith (A) core and (B) mid-section of recently-settled King George whiting larvae in 2016 and 2017. Letters identify significant differences ($P < 0.05$; capitals = between years; lower case = between regions). Error bars are +1 SE. WC – West Coast; SG – Spencer Gulf; GSV – Gulf St. Vincent.

3.3.2.2 Multi-element comparisons

When individual elements were combined into a single matrix, PERMANOVAs detected significant differences in multi-element chemistry between regions, years and sites (Table 3.3). For the otolith core, regional differences between Spencer Gulf and Gulf St. Vincent in 2016 were primarily driven by Sr, although Ba was also influential (Fig. 3.6*a*). In 2017, clear separation between the West Coast, Spencer Gulf and Gulf St. Vincent was largely driven by differences in Ba and Li. Multi-elemental signatures differed considerably between years, primarily associated with inter-annual differences in Sr and Mn. Classification of larvae back to their year and region of capture based on multi-element signatures was low at 37%, but improved to 52-60% when years were considered individually (Table S3.1). At a finer spatial scale, multi-element signatures varied considerably among sites within regions, particularly for Gulf St. Vincent. Despite this, otolith chemistry was generally more similar among sites within regions, than between regions (Fig. 3.7). Site-level classification success was highest for Pt. Turton (38-39%), Pt. Hickey (42-53%) and Barker Inlet (35-38 %) in each year (Table S3.2).

Regional differences in multi-elemental signatures for the mid-section of the otolith were largely influenced by Mn. Spencer Gulf and Gulf St. Vincent were clearly separated in 2016, which related to considerably higher Mn for Spencer Gulf (Fig. 3.6*b*). In 2017, multi-elemental signatures were significantly different between the West Coast, Spencer Gulf and Gulf St. Vincent. Although Mn was influential, Sr and Li drove regional differences. Similar to the otolith core, differences between years were driven by concentrations of Sr and Mn. Larvae were allocated to their year and region of capture with 42% accuracy, which improved to 62-66% when years were considered separately (Table S3.1). There were distinct differences in multi-elemental otolith chemistry among sites within each region (Fig. 3.7*b*), which related to differences in Mn, Sr and Li. Variation was largest for sites in Gulf St. Vincent, with Barker Inlet clearly separated and supporting the highest site-level classification success of 63% (Table S3.3).

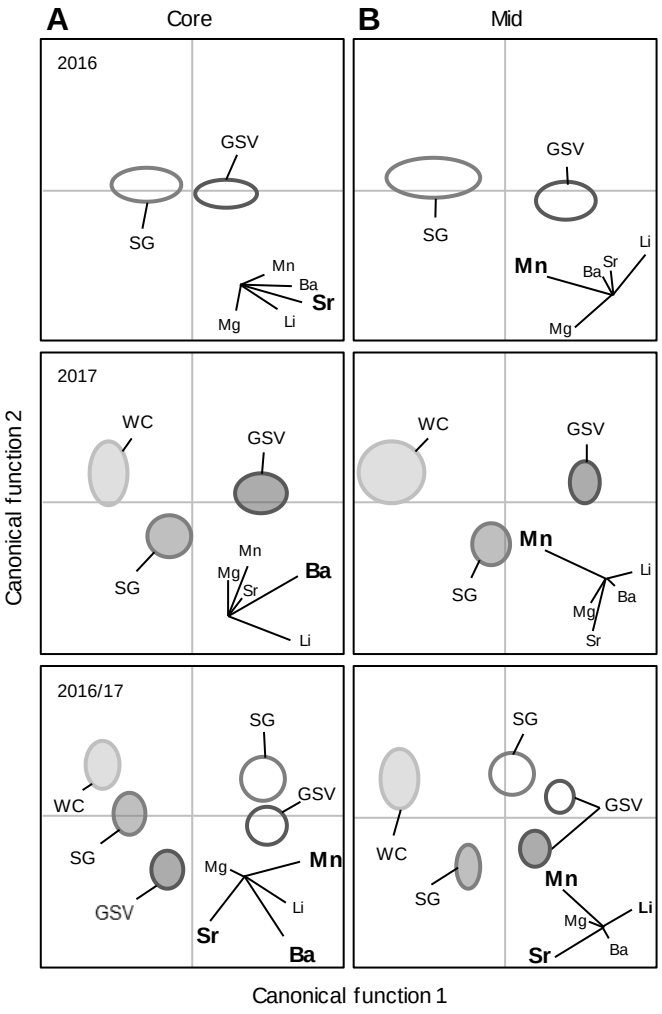

Figure 3.6. Canonical variate plots for the regional multi-elemental chemistry of the otolith (A) core and (B) mid-section of recently-settled King George whiting larvae in 2016 (open) and 2017 (shaded). Ellipses represent 95% confidence around group centroids. Vector diagrams show the direction and weight of individual elements to sample distribution. Elements in bold contribute most to group differences. WC – West Coast; SG – Spencer Gulf; GSV – Gulf St. Vincent.

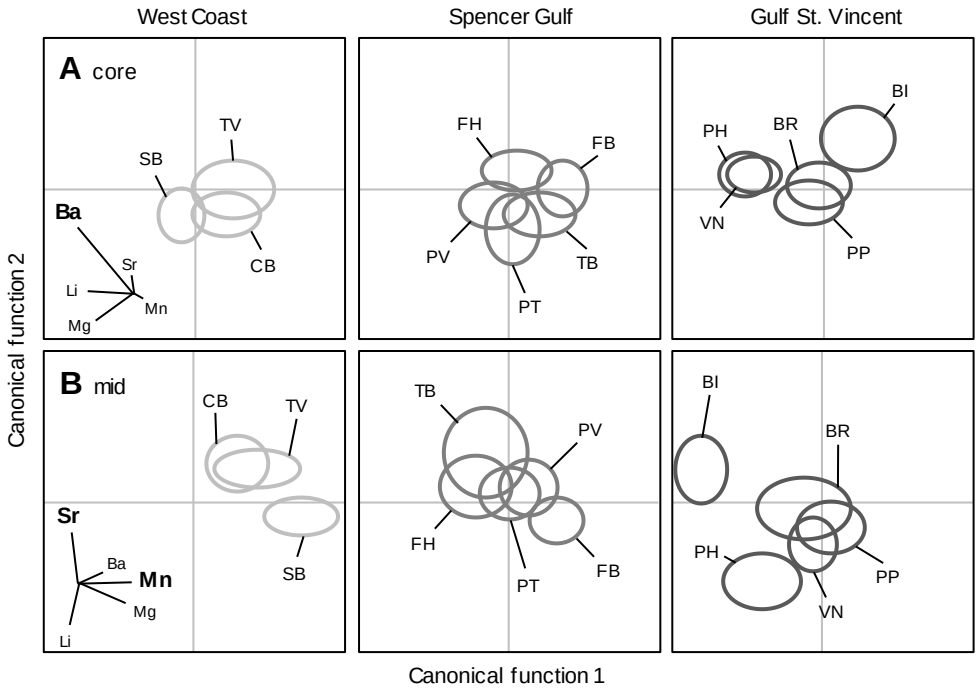

Figure 3.7. Canonical variate plots for the multi-elemental chemistry of the otolith (A) core and (B) mid-section of recently-settled King George whiting larvae sampled in 2017, grouped by site. Ellipses represent 95% confidence around group centroids. Site names in Table 3.1. Figures were separated by region for clarity. Vector diagrams show the direction and weight of individual elements to sample distribution. Elements in bold contribute most to group differences.

3.4 DISCUSSION

This study investigated the spatial scale over which the early life-history for King George whiting operates in South Australia, in order to infer spatial connectivity during ontogenetic development and inform stock structure. We aimed to differentiate between two hypotheses concerning the natal origins and dispersal pathways of larvae. The first hypothesis was that all larvae which settle to nursery areas in South Australia originate from a common spawning source (Kent et al. 2018). As such, the population would constitute a single, panmictic stock. Alternatively, larvae may originate from multiple spawning grounds in closer proximity to regional nursery areas (Fowler et al. 2000b). In the latter case, the State-wide population would comprise numerous smaller, self-recruiting populations.

3.4.1 REGIONAL COMPARISON

Recently-settled larvae from the West Coast and Spencer Gulf had very similar early life-history characteristics, with no differences in sizes, ages or hatch dates. However, they differed from larvae that settled in Gulf St. Vincent which were smaller, younger and had hatched two to three weeks later. Average growth rates did not differ between regions. King George whiting is a multiple batch spawner which spawns between March and July (Fowler et al. 1999). The regional differences in hatch dates suggest that throughout the protracted spawning season, either a single spawning area produces progeny and larvae disperse along different pathways, or multiple spawning grounds produce larvae at different times. Larval transport is predominantly influenced by physical oceanographic processes and larval behaviour (Norcross & Shaw 1984, Leis et al. 2011, Teodosio et al. 2016), and therefore larvae with longer pre-settlement durations have the potential to disperse over longer distances. The longer larval durations of recruits to the West Coast and Spencer Gulf may relate to increased distances between spawning grounds and nursery areas relative to Gulf St. Vincent, or could reflect different physical oceanographic processes between regions. For example, larvae could potentially be entrained in eddies which prevent settlement or extend the larval duration.

In this study, spatial differences were the primary interest for the otolith chemistry analyses as they relate to the physico-chemical environments experienced by fish at different life-history stages (Campana 1999, Gillanders 2005, Elsdon et al. 2008). We identified significant differences in multi-elemental otolith signatures between regions for the natal origin and larval dispersal life stages. Differences relating to the natal portion of the otolith suggest larvae which settled to nursery areas in each of the West Coast, Spencer Gulf and Gulf St. Vincent hatched into different physico-chemical environments. Recruits to the West Coast and Spencer Gulf hatched at almost the same time but displayed different multi-elemental signatures in their otoliths, providing strong evidence that they originated from spatially distinct spawning grounds. The otolith chemistry of recruits to Gulf St.

Vincent differed from those to the West Coast and Spencer Gulf. However, they hatched two to three weeks later. Otolith chemistry relating to larval dispersal also differed between regions, indicating that not only did recruits to the West Coast, Spencer Gulf and Gulf St. Vincent hatch into different environments, but they also dispersed through different water masses prior to settlement.

3.4.2 SITE-LEVEL COMPARISON

At a finer spatial scale, there was considerable variation amongst sites in the sizes and ages of recruits. For the nursery areas within each region, the sizes and ages of larvae increased from west to east. This is consistent with larvae having originated from spawning grounds in the west and dispersed east-ward. Differences in sizes and larval durations of recruits within regions reflect the greater distances between a single regional spawning ground and progressively distant nursery areas. This was particularly apparent for sites within Gulf St. Vincent. Larvae sampled at Pt. Hickey, the closest site to the spawning area in Investigator Strait, were the smallest and youngest. Larvae systematically increased in size and age up the west coast of Gulf St. Vincent from Pt. Hickey to Pt. Vincent and Pine Point. Larval dispersal of King George whiting is strongly influenced by the prevailing south-westerly winds during the austral winter (Fowler et al. 2000b, Jenkins et al. 2000, Jenkins 2005, Middleton & Bye 2007). This hypothesis of a single regional spawning ground that seeds multiple nursery areas is consistent with the general clockwise movement of currents in Gulf St. Vincent (Bye 1976, Bye & Kampf 2008). A similar pattern of increasing larval durations at nursery areas from west to east is apparent in Victoria, for which there is a long-held hypothesis that recruitment depends on seasonal wind forcing that disperses larvae from a western spawning ground, possibly in South Australia, to the Victorian nursery areas (Jenkins et al. 2000, Jenkins 2005, Jenkins et al. 2016).

Multi-elemental otolith signatures were more similar among sites within regions than between regions, providing support for the regional-scale differences in otolith chemistry. Despite this, there were larvae at sites within the same region that had similar hatch dates but different otolith chemistries, indicating they had occupied spatially discrete water masses (Campana 1999, Elsdon et al. 2008). For the West Coast, otolith chemistry for the natal origin was similar among nursery areas, but there were two different larval advection signatures. As such, larvae potentially originate from a common origin but disperse along two different pathways. The exact locations of spawning grounds on the West Coast are unknown. Larval duration of recruits at nursery areas increased eastward, which is consistent with larval dispersal entrained in the seasonal eastward circulation of the South Australian inshore coastal current (Middleton & Bye 2007). This suggests that the most likely location of a common spawning ground is in the adjacent coastal waters nearest the most western nursery area, Thevenard. The offshore islands of the Nuyts Archipelago meet these geographic requirements, and support the appropriate low-profile

reef and moderate wave energy characteristics of the recognised spawning area in Investigator Strait and southern Spencer Gulf (Fowler et al. 1999, Fowler et al. 2000a).

There was greater variation in otolith chemistry among nursery areas within Spencer Gulf. Larvae from Franklin Harbour (western side) and Pt. Turton (east) had similar hatch dates, but different multi-elemental signatures for each life-history stage, indicating that they were spawned and developed in spatially segregated water masses. Larval advection signatures were most similar among nursery areas along the two sides of the gulf, suggesting that there were two different dispersal pathways. Therefore, it is more likely that within Spencer Gulf there are at least two different spawning grounds and dispersal pathways that lead to recruitment (Fig. 3.8). There were no differences in hatch dates or otolith chemistry between recruits from Pt. Turton and Pt. Victoria, suggesting that these larvae hatched and developed in the same water mass. Larval transport along the western coast of Spencer Gulf conforms with the general clockwise circulation within the gulf (Nunes & Lennon 1986), and appears the most likely dispersal trajectory for recruits to northern nursery areas.

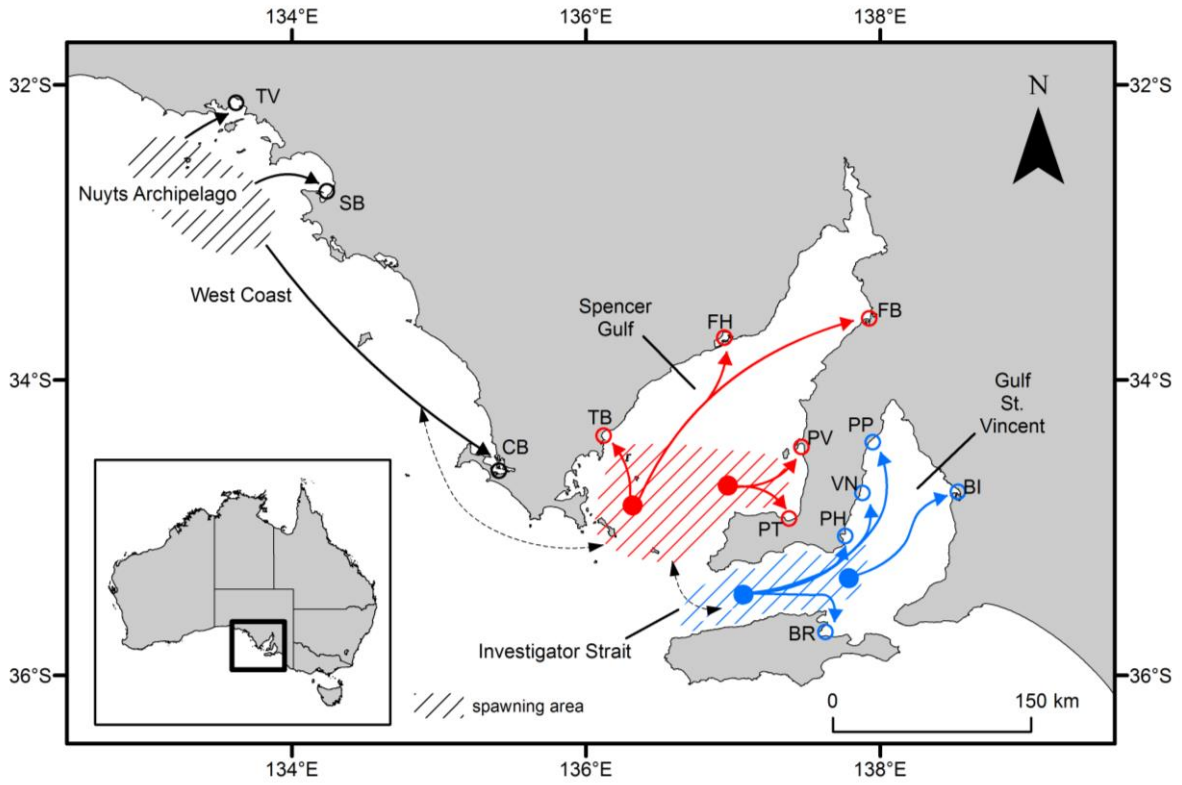

Figure 3.8. Theoretical spatial scale of early life-history for King George whiting in South Australia based on the retrospective analysis of otolith microstructure and multi-elemental chemistry of recently-settled larvae. Shaded areas represent hypothesised regional spawning grounds; solid circles represent theoretical spawning 'hot spots'; open circles represent recognised nursery areas; solid arrows show potential dispersal pathways to nursery areas; dotted arrows represent mixing between adjacent populations. Site names are in Table 3.1.

The largest variation in otolith chemistry among sites was in Gulf St. Vincent. Multi-elemental signatures were most different for Barker Inlet compared to other Gulf St. Vincent nursery areas, even those with similar hatch dates, indicative of at least two different spawning sources and dispersal pathways. Similarities in otolith chemistry among the other nursery areas corresponded with comparable hatch dates. Despite having different natal signatures, similar otolith chemistry related to the larval advection stage for Pt. Hickey, Pt. Vincent and Pine Point suggested larvae dispersed through a similar water mass. It is possible for physical environmental conditions at one location to change considerably over time, which could manifest as different otolith elemental signatures (Gillanders 2002b, Reis-Santos et al. 2012, Rogers et al. 2019a; Chapter 2). As such, the differences in natal otolith chemistry between larvae from adjacent nursery areas with different hatch dates may relate to temporal variation in physico-chemical conditions at a single spawning ground, rather than the contribution of multiple sources. Given the progressive increase in larval durations at nursery areas from west to east, consistency with physical oceanographic circulation (Bye & Kampf 2008), and previously described temporal change in natal otolith chemistry for recruits in this region (Rogers et al. 2019a; Chapter 2), it seems likely that larvae which settled on the western coast of Gulf St. Vincent originated from a common spawning ground (Fig. 3.8).

3.4.3 SPATIAL SCALE OF EARLY LIFE-HISTORY

Overall, the empirical data suggest that larvae that recruited to the West Coast, Spencer Gulf and Gulf St. Vincent originated from different spawning areas and dispersed through different water masses, indicating the early life-history of King George whiting in South Australia operates over a regional scale. As such, our results support the hypothesis that the State-wide stock comprises multiple small, self-recruiting populations (Fig. 3.8; Fowler et al., 2000b). In which case, it is most likely that the known spawning area of Investigator Strait and southern Spencer Gulf constitutes two separate spawning grounds that provide larvae to Gulf St. Vincent and Spencer Gulf, respectively. Within Spencer Gulf and Gulf St. Vincent, we identified differences in hatch dates, larval durations and otolith chemistry among sites, suggesting that early life-history processes may occur at an even finer spatial scale. Multi-elemental signatures were most similar among nursery areas along either side of each gulf, i.e. those along the western coast and those along the east. Consequently, we hypothesise that within each regional spawning ground there are localised ‘hot spots’ of egg production that replenish particular nursery areas (Fig. 3.8). Despite each putative regional population being largely independent from an ecological perspective (Bailey 1997), there must be some degree of mixing between populations to maintain genetic homogeneity (Kent et al. 2018). Analysis of tag-recapture records for adult fish has demonstrated that inter-regional adult movement is uncommon (Fowler et al. 2002). However, given the proximity of regional spawning grounds, only small-scale movement would be required for exchange between populations.

3.4.4 ECOLOGICAL INTERPRETATION OF OTOLITH GEOCHEMISTRY

The elements most influential to spatial discrimination differed among life-history stages, but remained largely consistent between years. Spatial differences for the natal origin were predominantly driven by Ba, which increased between regions from west to east. Barium has demonstrated a positive correlation between ambient water and otolith concentrations, and varying relationships with temperature and salinity (Bath 2000, Walther & Thorrold 2006, Izzo et al. 2018). Salinity negatively affects Ba uptake and is considered the primary extrinsic driver of otolith incorporation (Elsdon & Gillanders 2005, Izzo et al. 2018). Manganese was the only element that differed between regions for the larval dispersal stage. Unlike Ba, there are no recognised relationships between otolith Mn uptake and temperature or salinity, and there is likely to be considerable physiological regulation to otolith incorporation (Elsdon & Gillanders 2003b, Limburg et al. 2015, Turner & Limburg 2015). Manganese is present in aqueous form as a redox product following oxidation, and therefore shares a relationship with oxygen (Slomp et al. 1997, Limburg et al. 2015). As such, regional differences in otolith Mn could relate to spatial variation in dissolved oxygen. However, without an understanding of the ambient concentrations of Ba and Mn, it cannot be determined whether the differences in otolith concentrations are driven by extrinsic factors or simply reflect environmental availability.

It is also important to recognise the potential for temporal variation to confound spatial differences, which can adversely affect our ability to interpret connectivity patterns and stock structure (Gillanders 2002b, Reis-Santos et al. 2012). Average growth rates did not vary spatially, but were different between years with larvae growing faster in 2017. Larval growth is strongly correlated with ambient water temperature and food availability (Houde 1989, Leggett & Deblois 1994), and therefore differences in growth rates likely relate to inter-annual differences in primary productivity and/or water temperature. Inter-annual differences in multi-elemental chemistry for each life-history stage were predominantly for Sr, which was considerably higher in 2017. Otolith Sr incorporation reflects ambient availability and has shown a generally positive relationship with temperature for marine species (Bath 2000, Walther & Thorrold 2006, Izzo et al. 2018). The higher Sr ratios suggest warmer ambient water temperatures in 2017 and were consistent with faster larval growth rates.

The authors acknowledge that applying a constrained ordination to the multi-elemental data can potentially inflate group assignment compared to unconstrained methods because of *a priori* probabilities. Alternative approaches that do not consider *a priori* groupings and have been used to identify potential source populations include multidimensional scaling (MDS) with cluster analysis (Tanner et al. 2012), and unconditional Bayesian mixture models (Neubauer et al. 2013). However, because of the complexity of the dataset (three factors with 2-12 levels, $n = 397$), it was highly unlikely that the direction of greatest total variation in the multivariate data cloud was the same as the greatest

variation among groups. Constrained ordinations have traditionally been applied to multi-elemental otolith chemistry studies that aimed to identify differences in the natal signatures of juvenile or adult fish (Gillanders & Kingsford 1996, Thorrold et al. 2001, Jenkins et al. 2016). Discriminant function analysis (DFA) has been the most commonly used technique to investigate this aim and is considered a generally practical approach for assigning fish to possible sources (White & Ruttenberg 2007, Neubauer et al. 2013). We used canonical analysis of principal coordinates (CAP) as a non-parametric alternative of constrained ordination to DFA because the data violated the assumption of multivariate normality required for discriminant analysis.

3.4.5 IMPLICATIONS AND FUTURE DIRECTIONS

South Australian waters are divided into three regional populations for assessment and management purposes: the West Coast of Eyre Peninsula, Spencer Gulf and Gulf St. Vincent (Steer et al. 2018). The results indicate the early life-history processes of King George whiting occur over this regional scale, which conforms to the spatial scale currently used for management. Spatial differences in otolith chemistry suggest the recognised spawning area comprises numerous segregated spawning grounds that replenish particular regional nursery areas. As such, this level of spatial information has the potential to support management of key spawning grounds and nursery areas once they are adequately defined. Given the differences in otolith chemistry relating to the natal origin from settled larvae, a logical approach to discriminate among natal source populations would be to compare the otolith chemistry of larvae prior to dispersal. Although the chemical analysis of otoliths from recently-hatched larvae presents methodological challenges, the technique has previously been applied to address similar ecological questions (Barbee & Swearer 2007, Schaffler et al. 2009, Lazartigues et al. 2017). In addition, the hypothesised connectivity between spawning grounds and nursery areas, based on hatch dates and otolith chemistry, could be tested by simulating larval dispersal using highly-resolved oceanographic models (Fowler et al. 2000b, Jenkins et al. 2000). However, the accuracy of such predictions is constrained by the biological understanding of early life-history. Subsequently, future research investigating the locations of coastal spawning grounds that contribute recruits to nursery areas within each region would considerably enhance the ability to interpret the results of this study, and help elucidate spatial connectivity during early ontogeny.

This study has demonstrated how the incremental structure and elemental analysis of otoliths can be applied to discriminate between geographically close, yet ecologically-separated groups of settled larvae that hatched and developed in different marine environments. The underlying conceptual approach was to compare the otolith chemistry of larvae that hatched at the same time to infer spatial connectivity. One of the fundamental properties of otoliths is their ability to record biological information at a daily time scale (Pannella 1971, Campana & Neilson 1985). However, many early life-

history studies that use otolith chemistry tend to ignore within-season variation in hatch date, despite documented changes in elemental composition at temporal scales ranging from weeks to months (Gillanders 2002b, Reis-Santos et al. 2012, Rogers et al. 2019a; Chapter 2). Consequently, this study emphasises the importance of interpreting otolith chemistry concurrently with microstructure to investigate spatial connectivity during early life-history.

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Statement of Authorship

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Chapter 4 - Discriminating natal source populations of a temperate marine fish using larval otolith chemistry

ABSTRACT

The life cycles of many marine species depend on a dispersive larval stage that connects spatially segregated populations. However, quantifying larval movement among populations remains one of the greatest challenges in marine ecology. Such movement determines whether a population is essentially a self-recruiting stock, or if it forms part of a larger meta-population where recruits originate from multiple sources. Previous research has struggled to differentiate between such stock structure models for King George whiting (*Sillaginodes punctatus*; Perciformes) in southern Australia, largely due to difficulties in identifying the source populations of dispersing larvae. In this study, pelagic larvae were collected throughout the only recognised spawning area in South Australia in 2017 and 2018. First, we identified that the distribution of larvae was broadly divisible into two groups – those in southern Spencer Gulf and those in Investigator Strait. Then, the incremental structure and elemental composition of otoliths of larvae from the two regions were compared to determine if they had originated from a common source population. There were no spatial differences in the sizes (3.0-5.0 mm SL), ages (5-21 d), hatch dates (7-24 April) or average growth rates (0.09-0.21 mm d^{-1}) of larvae. However, multi-elemental (Li, Mg, Mn, Sr and Ba) otolith signatures differed significantly between the two regions, primarily driven by differences in concentrations of Li and Ba. Although otolith signatures were year-specific, larvae were assigned to their region of capture with 70-82% accuracy. Larvae in each region hatched at the same time yet had significantly different otolith chemistry, providing strong evidence that those in southern Spencer Gulf and Investigator Strait originated from spatially segregated water masses. This study has demonstrated the ability of otolith chemistry to discriminate source populations of pelagic larvae in a fully marine environment, which provides a basis to quantify larval movement between fish populations.

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4.1 INTRODUCTION

Many marine species conform to a bi-partite life cycle whereby spawning grounds and nursery areas are spatially segregated, and larval transport is an obligate process that connects life-history stages (Cowen et al. 2000). The dispersal of larvae in marine environments is heavily influenced by physical oceanographic processes, which results in a high probability of mixing between larvae that originate from different source populations (Norcross & Shaw 1984, Cowen & Sponaugle 2009, Leis et al. 2011). Identifying the degree of larval exchange among populations is necessary to understand population dynamics and inform stock structure. However, quantifying larval movement among populations remains a significant challenge. On one hand, a population may be primarily maintained by larval production and dispersal from other populations, and is considered demographically open (Caley et al. 1996). Alternatively, larvae may recruit to the population to which they were born and the population is demographically closed as it is essentially self-recruiting (Swearer et al. 1999, Jones et al. 2005). It is likely that most marine populations are maintained by a combination of both processes – i.e. a proportion of self-recruitment that is supplemented by larval production from other populations (Swearer et al. 2002, Cowen & Sponaugle 2009).

The most significant barrier to empirically quantifying larval movement has been the difficulty in differentiating between larvae from different source populations (Thorrold et al. 2002, Cowen & Sponaugle 2009). This is largely associated with the logistical challenges involved in marking inherently small larvae that experience extremely high mortality rates. In recent years, natural environmental markers, such as the geochemical signatures in calcified structures, have become a leading approach to study the biology of even the smallest aquatic animals (Campana 1999, Thorrold et al. 2007, Tzadik et al. 2017). The otoliths (ear stones) of teleost fishes are paired crystalline structures that form during embryonic development that record environmental information at a highly-resolved temporal scale (Campana 1999, Elsdon et al. 2008). They form increments at a daily periodicity which can be used to estimate age, and in turn, calculate hatch dates and larval growth rates (Campana & Neilson 1985, Campana & Jones 1992). The calcium carbonate generated during daily increment formation is derived from the surrounding aquatic environment, which contains elements in minor and trace quantities that can be incorporated into the otolith at the precipitating surface. Once incorporated, these elements are permanently retained and represent a chronological record of environmental history (Campana 1999, Elsdon et al. 2008). The geochemical signatures in otoliths can be used as a tool to discriminate between fish populations, and have been successfully applied to assess stock structure (Campana et al. 1994, Tanner et al. 2016), reconstruct ontogenetic movement patterns (Elsdon & Gillanders 2003a, Fowler et al. 2005), and evaluate the contributions of source populations to nursery areas (Gillanders & Kingsford 1996, Tanner et al. 2012).

The analysis of otolith chemistry has the potential to discriminate between source populations of larvae by comparing the elemental signatures of their otoliths prior to dispersal (Thorrold et al. 2002, Barbee & Swearer 2007). Most studies of larval otolith chemistry have considered the otoliths of embryonic larvae from substrate-attached egg masses before the larvae have hatched (e.g. Warner et al. 2005, Ruttenberg & Warner 2006, Barbee & Swearer 2007, Standish et al. 2008). In such cases, the entire otolith relates to embryonic development at the single location where the eggs were collected. However, most commercially important marine fish species are broadcast spawners that produce large numbers of pelagic eggs and larvae (Sadovy 2001, Murua & Saborido-Rey 2003). Relatively few studies have considered pelagic larvae (but see Ludsin et al. 2006, Lazartigues et al. 2014, Lazartigues et al. 2017), and even fewer have considered pelagic larvae in marine environments (but see Brophy et al. 2003, Schaffler et al. 2009). This likely relates to a number of factors that include: the logistical challenges involved in collecting recently-hatched larvae from open marine ecosystems; the limited amount of calcified material deposited at the natal origin because of the potential for dispersal immediately after spawning; and the analytical difficulties in detecting differences in elemental signatures where environmental gradients are potentially less pronounced (Barbee & Swearer 2007, Standish et al. 2008).

King George whiting (*Sillaginodes punctatus*; Perciformes) is a demersal marine finfish species that is endemic to temperate coastal waters of southern Australia, where it supports important commercial and recreational fisheries (Kailola et al. 1993, Steer et al. 2018). South Australia is at the centre of this geographic distribution and provides the highest State-based catches (Mobsby 2018). However, in recent years, catches from two of the most productive fishery regions, Spencer Gulf and Gulf St. Vincent, have declined to record lows. In addition, there are differences in regional population characteristics that may be indicative of localised population processes (Steer et al., 2018). Despite extensive research of the life-history of this species (Fowler & Short 1996, Jenkins et al. 1997, Fowler et al. 1999, Fowler et al. 2000b, Jenkins et al. 2000, Jenkins et al. 2016), there remains considerable uncertainty about the source populations that replenish nursery areas in different regions. Across south-eastern Australia, the only recognised spawning area for King George whiting is in southern Spencer Gulf and Investigator Strait, which is the region that connects the two gulfs (Fowler et al. 1999, Fowler et al. 2000a) (Fig. 4.1). Adults are multiple batch spawners that release pelagic eggs repeatedly between March and June (Fowler et al. 1999). The larvae undergo a prolonged dispersal phase of 3-5 months during the austral winter, from which the survivors eventually settle in protected bays between July and November (Fowler & Short 1996, Jenkins et al. 1997, Fowler et al. 2000b, Rogers et al. 2019a; Chapter 2). However, in South Australia, the relationships between the recognised spawning ground and nursery areas are poorly understood.

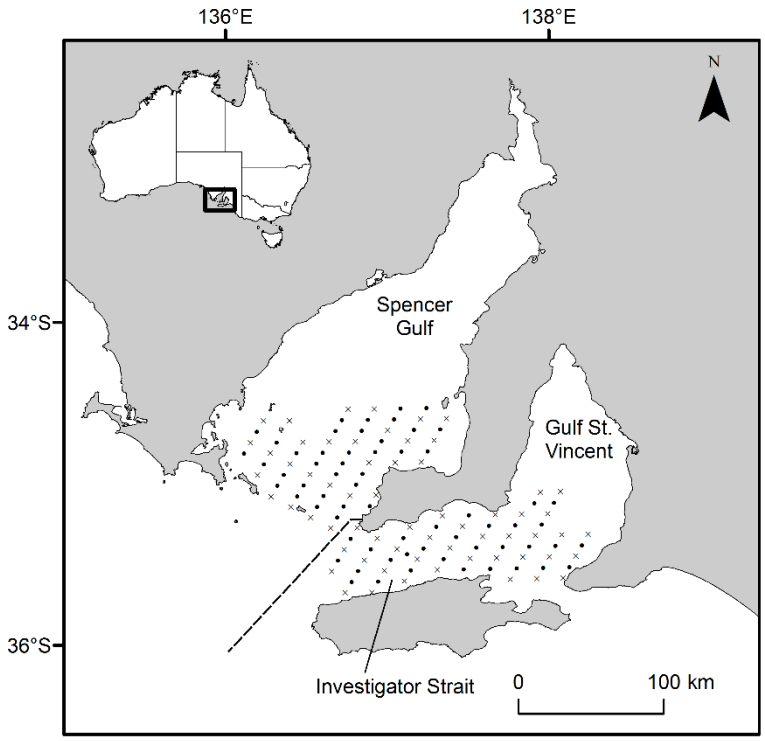

Figure 4.1. Map of South Australia's gulf systems showing the geo-referenced stations where plankton samples were collected in 2017 and 2018. Oblique tows (●) were done at every station (n = 126) and vertical tows with CTD casts (×) at almost every second station (n = 62). The dashed line separates Southern Spencer Gulf and Investigator Strait stations. Inset – map of Australia showing the study area along the southern coastline.

A recent study identified significant differences in the natal otolith chemistry of larvae that settled to nursery areas in Spencer Gulf and Gulf St. Vincent, suggesting that recruits in each gulf originated from different source populations (Rogers et al. 2019b; Chapter 3). Because of the short distances between the nursery areas in these regions and the recognised spawning area, there are two primary hypotheses regarding the sources of larvae to nursery areas in Spencer Gulf and Gulf St. Vincent. The first hypothesis is that the recognised spawning area is comprised of multiple different source populations that replenish nursery areas in each region. Alternatively, larvae that recruit to one region may originate from the recognised spawning area, whilst the larvae that recruit to the other region may originate from a different spawning source elsewhere. The aim of this study was to determine if larval King George whiting in their natal waters of southern Spencer Gulf and Investigator Strait originated from a common source population. The specific objectives were to: (1) identify the distribution and abundance of recently-hatched larvae throughout southern Spencer Gulf and Investigator Strait; and (2) compare the early life-history characteristics and otolith chemistry of larvae to assess the potential for different source populations. Otolith chemistry signatures could then be compared between larvae and recruits to evaluate hypotheses concerning population connectivity and stock structure.

4.2 MATERIALS AND METHODS

4.2.1 STUDY AREA

This study focused on recently-hatched King George whiting larvae in the recognised spawning area throughout southern Spencer Gulf and Investigator Strait, South Australia (Fowler et al. 1999, Fowler et al. 2000a) (Fig. 4.1). This area connects the semi-enclosed seas of Spencer Gulf and Gulf St. Vincent, to the oceanic waters of the eastern Great Australian Bight. The region experiences significant seasonal changes in physical environmental characteristics and oceanographic regimes which largely relate to the formation of frontal systems at the mouths of Spencer Gulf and Investigator Strait (Petrusevics 1993, Middleton & Bye 2007, Petrusevics et al. 2011). Temperature and salinity increase in the gulfs during the austral summer, which leads to the formation of thermohaline fronts that inhibit shelf-gulf exchange. The frontal systems dissipate as gulf temperatures decrease in late autumn (May), and shelf/gulf exchange resumes as lower density shelf water is drawn into southern Spencer Gulf and Investigator Strait.

4.2.2 SAMPLE COLLECTION

Larvae were sampled on two research cruises from 25 April to 1 May 2017 and 24 to 30 April 2018 aboard the RV *Ngerin* at 126 geo-referenced stations arranged in a 4 × 2 nm grid pattern (Fig. 4.1). Plankton samples were collected from a combination of oblique and vertical tows using paired bongo nets of 0.57 m diameter with 500 µm mesh. An oblique tow was done at every station (n = 126), whilst a vertical tow was done at almost every second station (n = 62). Each net was fitted with a flow-meter which was calibrated using factory coefficients to estimate the distance travelled by each net during each tow (General Oceanics 2030; FL, USA). Plankton samples were preserved in 100% ethanol and refrigerated at 4 °C prior to sorting. A SBE 19plus V2 SeaCAT Profiler CTD (SeaBird Scientific, WA, USA) was attached to the bottom of the vertical net frame and recorded temperature (°C) and salinity at 1 m intervals during each vertical net tow. King George whiting eggs are buoyant and the larvae remain near the surface until post-flexion (Bruce 1995, Ham & Hutchinson 2003). Therefore, we considered the mean CTD data from the surface to 5 m depth to best represent the environmental conditions experienced during early ontogeny. Maps were created in ArcGIS (v. 10.6; ESRI, CA, USA).

4.2.3 SAMPLE PROCESSING

Larval fish were sorted from the plankton using a modified Sedgwick-Rafter sorting tray under a dissecting microscope (Olympus SZX7; Tokyo, Japan). Larval King George whiting were identified

following the morphological descriptions by Bruce (1995). The primary diagnostic characteristics were: shallow-bodied, elongate larvae with a small head; a single series of dorsal and ventral melanophores; and a moderate to long uncoiled gut (Bruce 1995) (Fig. 4.2A). To aid identification, larvae were viewed at 20× magnification on a computer screen using an Olympus DP73 video camera attached to the microscope, and used Olympus Stream software (v. 1.9.1; Tokyo, Japan). Morphological identifications were verified using an *in situ* hybridisation (ISH) molecular technique. This technique uses a horseradish peroxidase (HRP) enzyme conjugated oligonucleotide probe that binds specifically to mitochondrial 16S ribosomal RNA of King George whiting and generates a blue colour through oxidisation with a HRP reactive substrate (Oxley et al. 2017) (Fig. 4.3). The ISH probe was applied to a tissue sample from each larva after the head had been removed to prevent potential contamination of the otoliths. The identification of all King George whiting larvae used in otolith analyses (n = 134) was verified using the ISH molecular technique (Table 4.1).

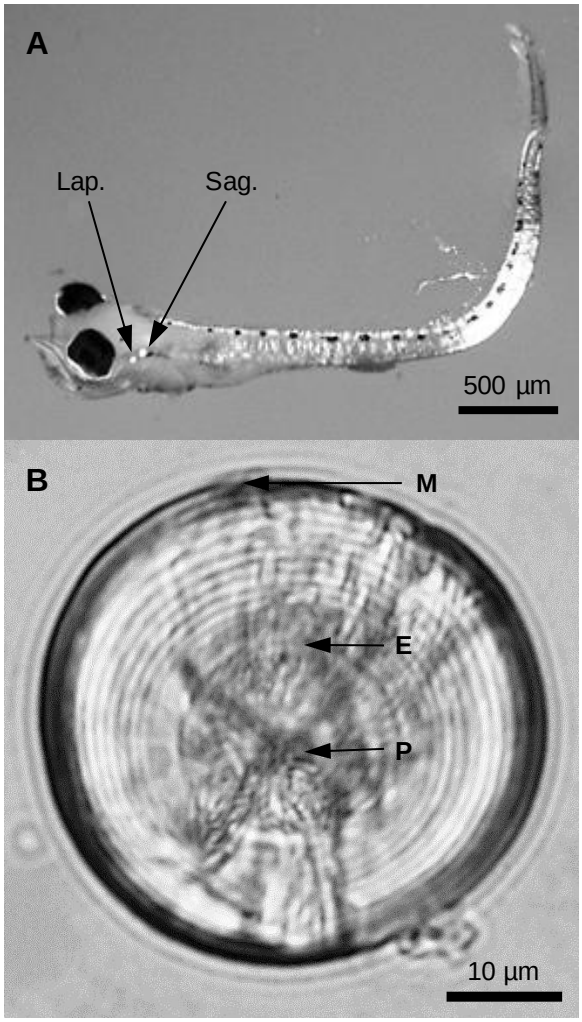

Figure 4.2. (A) A pre-flexion larval King George whiting of 3.7 mm SL viewed at 20× magnification under a dissecting microscope. Polarised light was used to illuminate the otoliths (Lap. – lapillus; Sag. – sagitta). (B) Whole sagittal otolith of a larval King George whiting viewed from the proximal surface (sulcus upward) at 1,000× magnification using immersion oil (P – primordium; E – exogenous feeding check; M – posterior margin). Otolith diameter was 48.9 µm and the larva was 18 d old and 4.7 mm SL.

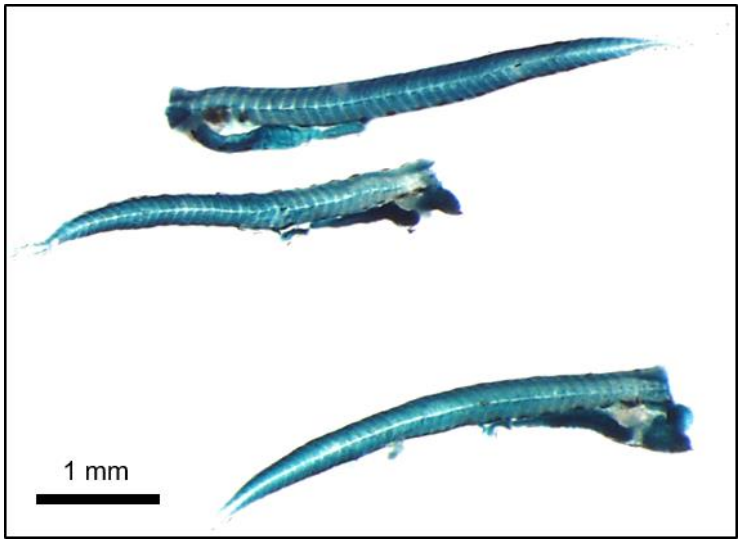

Figure 4.3. Larval King George whiting following confirmation of identification using the *in-situ* hybridisation (ISH) molecular technique. Larvae were viewed at 20× magnification under a dissecting microscope. The head of each larva was removed to prevent potential contamination of the otoliths.

Table 4.1. Summary of sample details used to compare otolith microstructure and elemental chemistry of larval King George whiting from southern Spencer Gulf and Investigator Strait in 2017 and 2018. *n* – number of larvae; stations – no. of stations larvae sourced from. All other values are in the form mean (SD).

Year	Region	*n*	Stations	Fish length (mm)	Otolith diameter (μm)	Age (d)	Ave. growth rate (mm d^{-1})	Temperature (°C)	Salinity (ppt)
2017	Spencer Gulf	19	13	4.0 (0.6)	40.6 (8.7)	13.3 (3.0)	0.140 (0.03)	19.2 (0.7)	37.0 (0.6)
	Investigator Strait	31	11	3.9 (0.6)	41.3 (9.1)	13.7 (3.5)	0.136 (0.03)	18.6 (0.2)	36.2 (0.1)
2018	Spencer Gulf	39	19	3.9 (0.4)	41.9 (7.9)	13.1 (2.6)	0.137 (0.02)	19.5 (0.5)	36.7 (0.6)
	Investigator Strait	42	17	3.8 (0.4)	37.4 (7.0)	11.8 (2.6)	0.140 (0.02)	19.2 (0.5)	36.4 (0.2)

At each station, the density of larvae per volume of water filtered was estimated by the equation:

$$D = \frac{n}{V} \quad \text{Eq. (4.1)}$$

where D is the density of larvae (ind. per m^3), n is the number of larvae in each sample, and V is the volume of water filtered (m^3). V was calculated as the area of the paired nets ($2 \times \pi r^2$) multiplied by the distance travelled according to the flowmeter readings. Each larva was measured for standard length (SL) to the nearest 0.1 mm. Larvae ranged in size from 1.6 to 9.3 mm SL. However, we constrained otolith analyses to larvae of ≤ 5 mm to minimise potential dispersion from their natal environment. In 2018, the abundance of larvae in Investigator Strait was considerably higher than elsewhere, and therefore a random sub-sample of larvae (n = 42) representative of the overall distribution was used for otolith analyses. The otoliths (sagittae and lapilli) were clearly visible in the heads of the larvae under a dissecting microscope at 60× magnification fitted with a polarising filter (Fig. 4.2A). Only the sagittal otoliths were used for analyses, which were extracted using stainless steel dissecting needles. Hereafter, a fine-tipped synthetic paintbrush was used for all otolith transfers to prevent contamination from metallic instruments. Otoliths were rinsed in a drop of ultrapure water, then transferred to a bath of ultrapure 15% H_2O_2 buffered with 0.1 N NaOH for 15 minutes to remove adhering organic material (Warner et al. 2005, Barbee & Swearer 2007, Standish et al. 2011). Otoliths were then transferred through three drops of ultrapure water to remove residual cleaning solution and were then air dried under a laminar flow hood.

4.2.4 OTOLITH MICROSTRUCTURE

The sagittae from King George whiting larvae are hemispherical in shape, with a convex proximal surface (sulcus face) and almost flat distal surface. One otolith from each larva (n = 134) was randomly selected for microstructure analysis, and the other was used for trace element chemistry. The former was viewed whole, orientated sulcus face upwards in immersion oil, at 1,000× magnification under a compound microscope (Olympus BX51; Tokyo, Japan). For interpretation, each otolith was viewed on a computer screen using an image analysis system (Olympus DP73 video camera, Olympus Stream v. 1.9.1; Tokyo, Japan). Otoliths were measured from the anterior to posterior margins to the nearest 0.1 µm. Daily increment formation for otoliths of King George whiting has been validated from reared larvae of known age (B.D. Bruce and D.A. Short, unpub.). Increments were counted from the primordium to the posterior margin (Fig. 4.2B). Two counts were done for each otolith. If these counts differed, additional counts were done until an acceptable estimate of age was achieved. If not, the otolith was rejected (n = 3). After ageing, each otolith was cleaned of oil and mounted in thermoplastic glue (CrystalBond 509; ProSciTech, QLD, AUS) for storage.

Larval growth rates were calculated in two ways. The 'average growth rate' (mm d^{-1}) provided an estimate of mean daily growth rate from hatch to capture, and was calculated as:

$$\frac{L_c - L_o}{a} \quad \text{Eq. (4.2)}$$

where L_c is length at capture, L_o is length at hatch (2.1 mm; Bruce, 1995), and a is age (d). However, this method provides no information on daily variation in growth rate. As such, retrospective length at age and daily growth rates were calculated from otolith increment widths using the 'back-calculation with biological intercept algorithm' (Campana 1990, Campana & Jones 1992). This technique depends on proportional somatic and otolith growth, for which there were strong linear relationships in each year (r^2 = 0.79 and 0.78 in 2017 and 2018, respectively; Fig. S4.1). Daily increments were measured from the primordium to posterior margin to the nearest 0.1 µm, and the size of each larva on successive days was estimated by the equation:

$$L_a = L_c + \left(\frac{(O_a - O_c)(L_c - L_o)}{(O_c - O_o)}\right) \quad \text{Eq. (4.3)}$$

where L_a is the length at age a, L_c is length at capture, L_o is length at hatch (2.1 mm; Bruce, 1995), O_a is the otolith radius at age a, O_c is the otolith radius at capture, and O_o is otolith radius at hatch. Here, O_o was defined as the distance from the closest increment to the primordium, and O_a was calculated from accumulating successive increments (Fowler & Short 1996). The estimates of size at age and daily growth rate were averaged across larvae in each region and year.

4.2.5 TRACE ELEMENT CHEMISTRY

Otolith chemistry preparation and analytical processing were modified from Barbee and Swearer (2007). A gridded microscope slide was coated with a thin layer of thermoplastic glue (CrystalBond 509; ProSciTech, QLD, AUS), which was spiked with indium (^{115}In) at ~200 ppm to aid discrimination during analysis (Reis-Santos et al. 2012). On each slide, up to 20 otoliths were orientated sulcus face upward on individual grid squares on top of the hardened thermoplastic glue. The slide was then heated on a hotplate for 3 seconds at 80 °C to soften the glue, which caused the otoliths to 'sink' into it using their own mass. This meant that each otolith was mounted in glue around its margin, but presented a largely exposed proximal surface for laser ablation (Fig. 4.4A). A total of seven such slides were prepared and stored in resealable plastic bags prior to analysis.

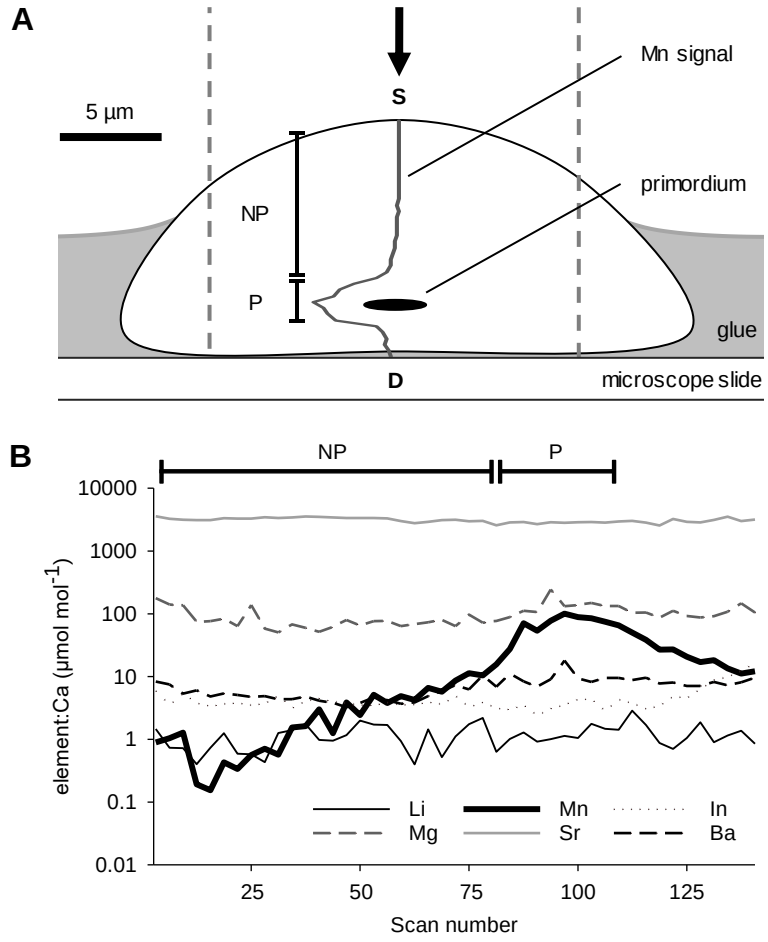

Figure 4.4. (A) Schematic cross-section of a sagittal otolith from a larval King George whiting mounted sulcus face upward on a microscope slide in preparation for elemental analysis. Dashed lines show the area that the LA-ICP-MS ablation path encompassed. The arrow indicates the direction of laser ablation. ^{55}Mn signal is overlaid on the otolith. (B) Example of a time-resolved multi-elemental profile for a larval otolith ablated through the primordium from the proximal (S) to distal (D) surfaces. Note the logarithmic scale. NP – non-primordial area; P – primordial area.

Otoliths were analysed for trace element chemistry by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The system consisted of a Resonetics ASI (ACT, AUS) 193 nm excimer LA system coupled to an Agilent (Santa Clara, CA, USA) 7900 quadrupole ICP-MS, which was located at Adelaide Microscopy (Adelaide, SA, Australia). Up to four slides, each bearing 20 otoliths, were placed in the sealed chamber at one time and viewed remotely using a video camera. Otoliths were ablated using a 19 µm diameter laser 'spot' at a pulse rate of 4 Hz and a beam density at the sample of ~3.5 J cm^{-2}. Each otolith was sampled through the primordium from the proximal to distal surfaces. Due to the small diameter of larval otoliths (21.2-61.7 µm), no pre-ablation pass was done. Instead, the first 2 s of data were cropped to remove data that could have been affected by possible surface contamination. Ablation occurred in a ^{4}He flushed chamber that was mixed with ^{40}Ar for injection into the plasma. The elements quantified for analysis were ^{7}Li, ^{25}Mg, ^{55}Mn, ^{88}Sr and ^{138}Ba, as well as ^{43}Ca

that was used as the internal standard. The concentration of otolith ^{43}Ca was assumed to be constant at 38.8% (Yoshinaga et al. 2000). ^{115}In was also measured to aid discrimination between otolith material and thermoplastic glue during analysis (Reis-Santos et al. 2012), and ^{27}Al was measured as an indicator of metallic contamination (Lazartigues et al. 2016). Calibration was achieved against the National Institute of Standards (NIST) 612 glass standard (Lahaye et al. 1997). Background measurements were recorded for 30 s prior to each sample ablation, with a measurement of each element recorded every 0.17 s. Sample acquisition times ranged from ~15 to 35 s due to the different sizes of otoliths. Data reduction, including background subtractions and calculation of minimum detection limits (MDL), was done offline in the Igor Pro workspace using Iolite software (v. 2.5; Paton et al., 2011). Data were converted to molar concentrations and standardised to calcium (element:Ca, µmol mol^{-1}). All analyses used the element:Ca data.

Internal precision and accuracy were assessed by analysing the NIST 612 as an unknown sample against the known concentrations, and external precision was assessed by measurements of MACS-3 (United States Geological Survey; VA, USA) calcium carbonate reference material. NIST 612 and MACS-3 were analysed twice at the beginning and end of each session, and after every 10 ablations to correct for instrumental drift (n = 18). Mean recovery for the NIST 612 was 99.99-100.05% for each element. Mean relative standard deviation (RSD) for NIST 612 was: 0.6% (Li), 1.1% (Mg), 0.3% (Mn), 0.3% (Sr), and 0.5% (Ba). External precision (RSD) assessed by measurements of MACS-3 was: 3.3% (Li), 3.9% (Mg), 2.0% (Mn), 3.7% (Sr) and 2.7% (Ba). Mean MDL (µmol mol^{-1}) based on three times the standard deviation of the blank gases adjusted for ablation yield (Lahaye et al. 1997) were: 0.39 (Li), 1.63 (Mg), 0.56 (Mn), 0.02 (Sr) and 0.02 (Ba).

4.2.6 DATA ANALYSIS

4.2.6.1 Environmental and biological characteristics

Environmental (temperature and salinity) and biological (length, age, otolith diameter and average growth rate) characteristics were compared between regions and years by two-way analysis of variance (ANOVA). Region and year were fixed factors in the full factorial model. Parametric assumptions of normality (Shapiro-Wilk) and equality of group variances (Levene's Test) were satisfied after square-root transformation. When significant differences were found, Tukey HSD *post-hoc* comparisons were used to determine the source of differences between group means. Individual growth trajectories calculated from daily increment widths were compared between regions and years by repeated-measures analysis of variance (RM-ANOVA). Statistical analyses were done using SPSS Statistics (v. 26.0; IBM Corp., NY, USA).

4.2.6.2 Trace element chemistry

The concentration of Mn was used to separate the elemental data in two parts (Barbee & Swearer 2007). Mn concentrations spiked at the primordium, which were consistent with larval otolith chemistry studies of other species (e.g. Brophy 2004, Barbee & Swearer 2007, Macdonald et al. 2008, Lazartigues et al. 2017). The two areas were: (1) 'primordial area'– mean elemental concentrations of the 25 scans surrounding the peak count in Mn (Fig. 4.4B). This incorporated the primordium and the immediately surrounding otolith material, and relates to the earliest stages of embryonic development and larval growth. It is representative of the 'natal origin' of pelagic larvae (0 to ~5 d post fertilisation). (2) 'Non-primordial area'– mean elemental concentrations from the proximal surface to the primordial area. This corresponds to larval development from the onset of exogenous feeding until the time of capture (~5 to 20 d).

The elemental ratios of Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca exceeded the detection limits of the ICP-MS for all samples (100%). Parametric assumptions were violated for Li:Ca regardless of transformation type. Therefore, to apply a consistent statistical approach to all elements and enable them to be combined for multivariate analyses, non-parametric tests were used. For each area of the otolith, elemental chemistry was compared between regions and years using a two-factor PERMANOVA design for each element individually and all elements combined (Anderson 2001). Region and year were fixed factors in the full factorial model. Element:Ca data were normalised prior to constructing resemblance matrices based on Euclidean distance dissimilarity, and analysed using unrestricted permutation with 9,999 iterations. When significant differences were found, *post-hoc* pair-wise comparisons were used to identify the source of differences between means. Multivariate data were reduced to two-dimensions and visualised using non-metric multidimensional scaling (nMDS) and canonical analysis of principal coordinates (CAP) (Anderson & Willis 2003). Multi-elemental data were compared between spatial groups of larvae in each year, and then pooled together in a single analysis to evaluate variation between years. Vector diagrams in each canonical plot show the influence of individual elements to sample positioning in multivariate space. The relative length and direction of each vector correspond to its discriminatory ability. Leave-one-out cross validation was used to classify larvae to groups for each region and year based on the multi-elemental signals of the remaining samples. The performance of CAP to discriminate between groups was evaluated using Cohen's Kappa (κ) statistic, which is a method of calculating the chance-corrected percentage of agreement between actual and predicted group memberships. Values of κ range from 0 to 1, where 0 indicates that the CAP resulted in no improvement over chance, and 1 indicates perfect agreement (Titus et al. 1984). Statistical analyses were done using PRIMER (v. 7.0.13; Auckland, NZ) and figures were produced using SigmaPlot (v. 14.0; Systat Software Inc, San Jose, CA, USA).

4.3 RESULTS

4.3.1 ENVIRONMENTAL CHARACTERISTICS

In both 2017 and 2018, there were strong temperature and salinity gradients that increased northward in southern Spencer Gulf and eastward in Investigator Strait (Fig. 4.5). In 2017, temperature ranged from 17.8 to 19.9 °C in southern Spencer Gulf and 17.9 to 19.6 °C in Investigator Strait. Despite within-region variation, southern Spencer Gulf was significantly warmer than Investigator Strait (Fig. S4.2). Water temperatures across the study area were 0.5-0.7 °C warmer in 2018. Salinity also showed greater variation within than between regions. The largest variation in salinity was in southern Spencer Gulf in 2017 which increased from 35.6 to 37.5. In 2017, salinity was significantly higher in southern Spencer Gulf than Investigator Strait, but there were no differences in 2018 or between years. Although there were no regional differences for temperature and salinity in 2018, T-S plots separated the sampling stations into 2 clusters in each region (Fig. 4.5C): (1) low temperature (18.6-19.3 °C) and low salinity (35.8-36.5); and (2) high temperature (19.5-20.5 °C) and high salinity (36.5-37.5).

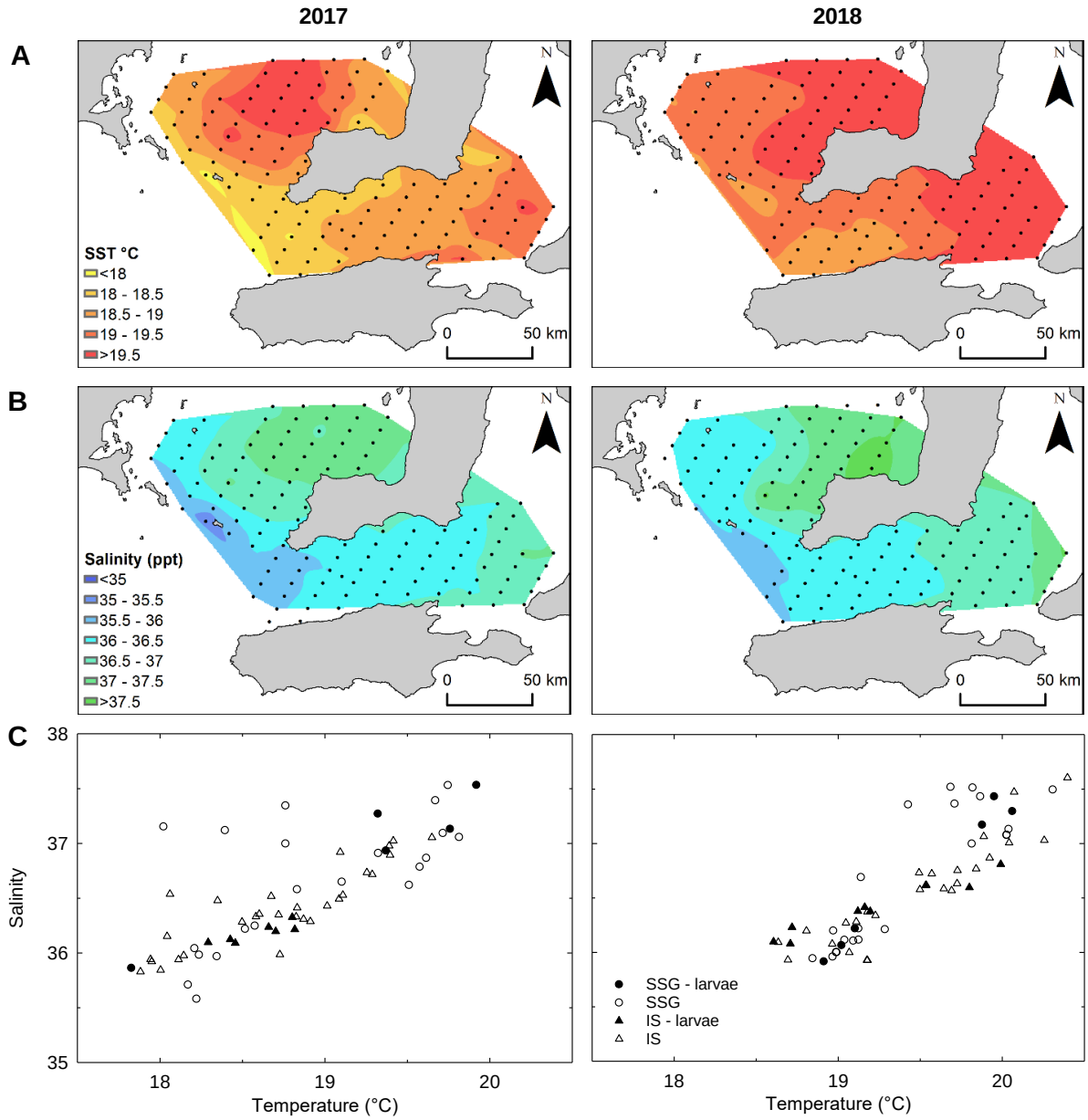

Figure 4.5. (A) Interpolated mean temperature (°C) and (B) salinity to a depth of 5 m from the surface determined from CTD casts (n = 62) in 2017 and 2018. (C) Temperature-Salinity plots for sampling stations where CTD casts were done in each year. Stations where larvae were captured and used in otolith analyses are represented by closed symbols.

4.3.2 LARVAL DISTRIBUTION

In total, 360 larval King George whiting were captured throughout the study (n = 100 in 2017 and n = 260 in 2018). Larvae ranged in size from 1.6 to 7.6 mm SL in 2017, and 1.7 to 9.3 mm SL in 2018. The spatial distribution of larvae was similar in both years. Larvae in Investigator Strait were distributed as a single group at a high density, whereas larvae in southern Spencer Gulf were distributed in small patches at lower densities (Fig. 4.6A). In both years, larval abundance and density was higher in Investigator Strait than southern Spencer Gulf. There was a break in the distribution of larvae between the two regions. Based on the spatial distribution, larvae were separated into two groups: (1) southern Spencer Gulf and (2) Investigator Strait. The larvae considered for otolith analyses from these two groups were ≤ 5.0 mm SL to ensure that there had been minimal dispersion from the place where they had been spawned. The distribution of larvae of this size range was consistent with the overall distribution of larvae of mixed sizes (Fig. 4.6B). The highest density of larvae ≤ 5.0 mm SL was in Investigator Strait in both years.

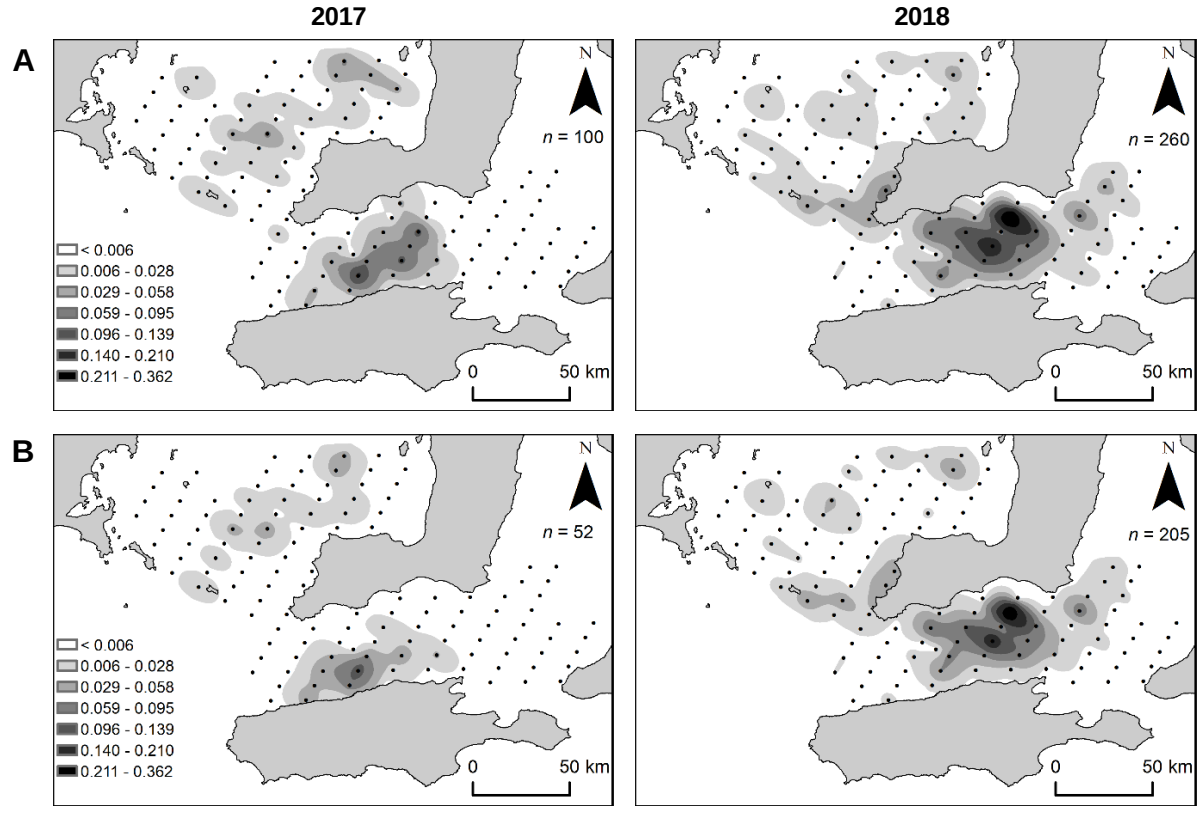

Figure 4.6. Spatial distribution and density (larvae m^{-3}) of King George whiting larvae collected from southern Spencer Gulf and Investigator Strait, South Australia, in 2017 and 2018. (A) All larvae captured; (B) larvae ≤5 mm SL. For clarity, maps show larval density from oblique tows which accounted for 89.7% of larvae captured (n = 323 of 360).

4.3.3 EARLY LIFE-HISTORY CHARACTERISTICS

The larvae used for otolith analyses ranged in size from 3.0 to 5.0 mm SL and in age from 5 to 21 d (Fig. 4.8). There were no differences in the sizes and ages of larvae between regions or years (Table S4.1). Otolith diameter for these larvae ranged from 21.2 to 61.7 µm and were strongly related to standard length and age (r^2 = 0.75 and r^2 = 0.78, respectively). Otoliths of larvae ≤ 3.0 mm SL were not large enough (≤ 20 µm diameter) for elemental analysis with LA-ICP-MS. In 2017 larvae hatched from 8 to 24 April, and in 2018 from 7 to 23 April. Mean hatch dates did not differ between regions or years. There was considerable variation in average growth rates which ranged from 0.09 to 0.21 mm d^{-1}. However, there were no differences between regions or years. Furthermore, there were no differences in average growth rates between larvae from the low and high temperature groups in 2018 (*t*-test; t = 0.44, d.f. = 24, P = 0.667). Mean daily growth rates calculated from increment measurements showed a significant ontogenetic shift, although there were no differences between regions (P = 0.236) or years (P = 0.975) (Table S4.2). The range was from 0.17 to 0.20 mm d^{-1} for age 0-4 d, then declined considerably on day 5 from 0.11 to 0.13 mm d^{-1} and then remained consistent from age 5 to 15 d (Fig. 4.7).

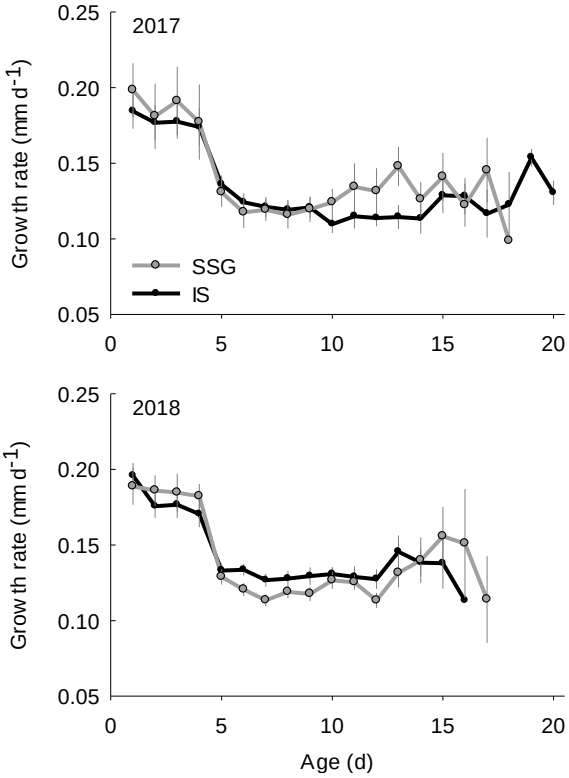

Figure 4.7. Comparison of mean daily growth rates (mm d^{-1}) for King George whiting larvae from southern Spencer Gulf (SSG; light) and Investigator Strait (IS; dark) in 2017 and 2018. Error bars are ± 1 SE.

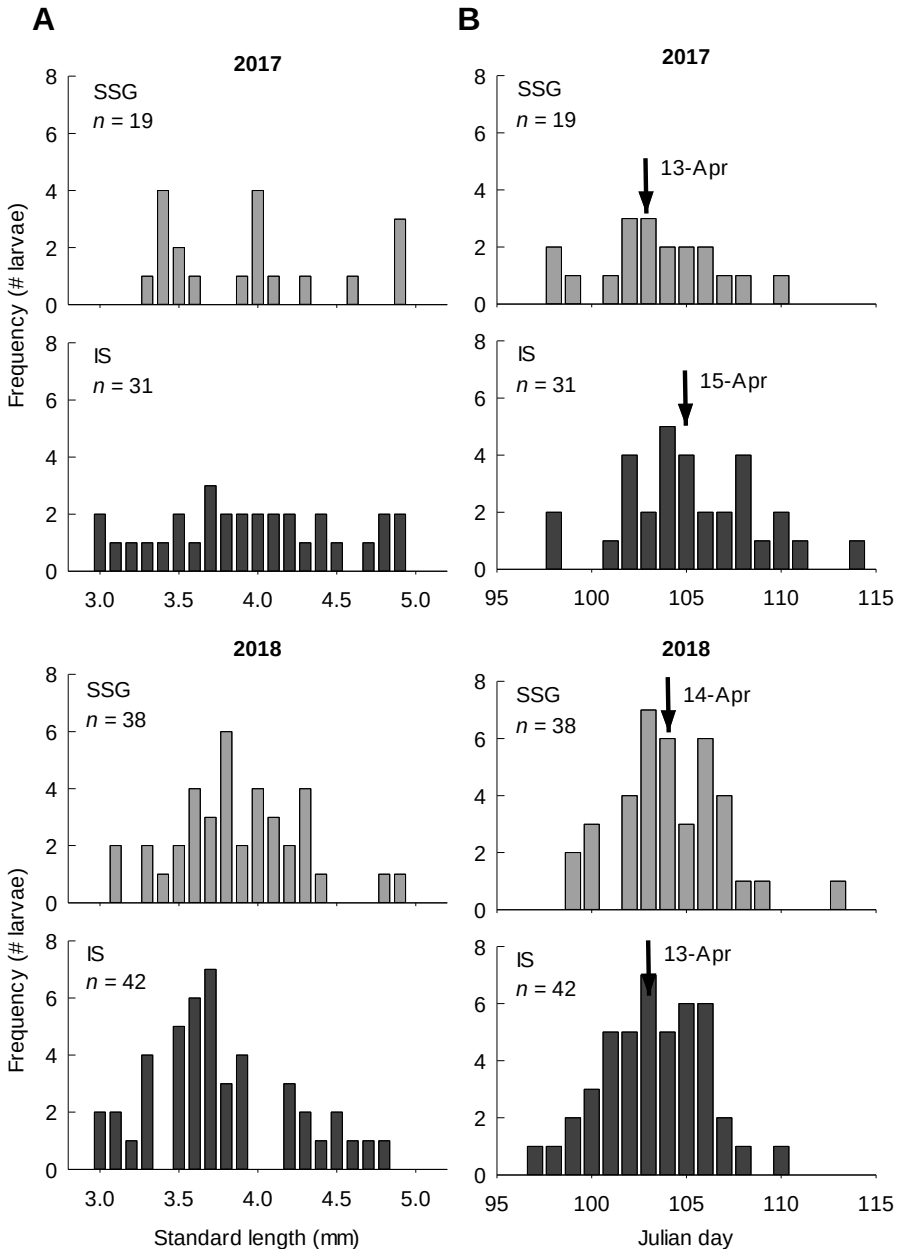

Figure 4.8. (A) Length frequency distributions of King George whiting larvae from southern Spencer Gulf (SSG) and Investigator Strait (IS) in 2017 and 2018. Size was constrained to larvae of ≤ 5 mm SL. (B) Frequency histograms showing the number of aged larvae that hatched on the nominated Julian day in 2017 and 2018. Arrows identify the mean hatch day.

4.3.4 TRACE ELEMENT CHEMISTRY

4.3.4.1 Individual elements

There was considerable variation in the elemental concentrations in otoliths that resulted in numerous spatial and temporal differences (Fig. 4.9; Table 4.2). For the primordial area, concentrations of Li and Ba differed between regions in 2017, being considerably higher for Investigator Strait than southern Spencer Gulf (Fig. 4.9A). Li concentrations were 4-fold higher for Investigator Strait in 2017, but in 2018, there were no differences. Instead, regional differences were observed for Mg and Ba which were both higher for Investigator Strait. Ba was consistently higher for Investigator Strait than southern Spencer Gulf in each year. The concentrations of each element differed significantly between years. Concentrations of Li, Mn and Ba were higher in 2017, whilst Mg and Sr were higher in 2018. Concentrations of Mn were 10-20 fold higher for the primordial area compared to the remainder of the otolith.

For the non-primordial area, Li was the only element that differed between regions (Table 4.2). Concentrations of Li were higher for Investigator Strait than southern Spencer Gulf in each year, with the magnitude of difference considerably greater in 2017 (Fig. 4.9B). There were significant between-year differences for the concentrations of Li, Mg and Ba, which were consistent with the primordial area of the otolith. Concentrations of Sr were higher in Investigator Strait in 2017, but higher in southern Spencer Gulf in 2018.

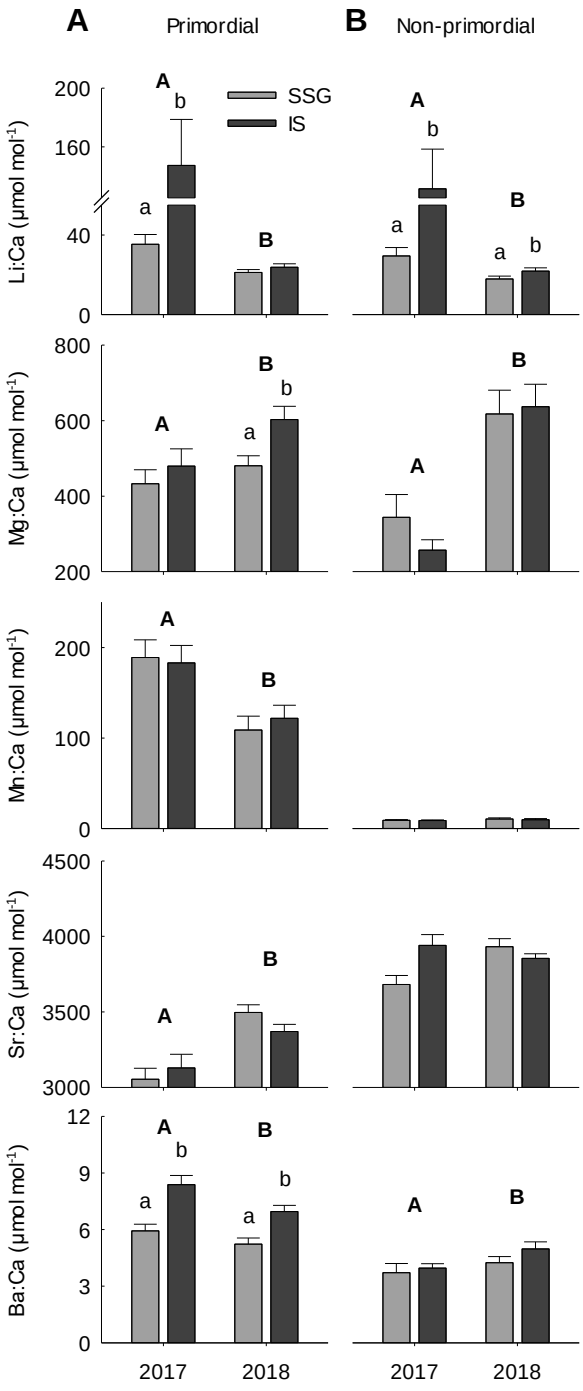

Figure 4.9. Regional comparisons of mean element:Ca ratios (µmol mol^{-1}) for the (a) primordial area and (b) non-primordial area of larval King George whiting otoliths in 2017 and 2018. Letters identify significant differences ($P < 0.05$; capitals = between years; lower case = between regions). Error bars are + 1 SE. SSG – southern Spencer Gulf (light); IS – Investigator Strait (dark).

Table 4.2. Summary of two-factor PERMANOVAs for the effect of year and region on individual and combined element:Ca ratios for the (a) primordial area and (b) non-primordial area of larval King George whiting otoliths. Year and region were fixed factors. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

		Li		Mg		Mn		Sr		Ba		All elements	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(a) primordial													
Year	1	22.35	35.08***	6.70	7.32**	12.48	13.65***	23.05	28.45***	5.76	7.43**	70.34	17.36***
Region	1	10.87	17.06***	3.64	3.97*	0.01	0.01	0.15	0.19	19.88	25.65***	34.55	8.53***
Year × Region	1	8.14	12.78***	0.99	1.08	0.36	0.40	1.61	1.99	0.08	0.11	11.18	2.76*
Residuals	127	0.64		0.92		0.91		0.81		0.78		4.05	
(b) non-primordial													
Year	1	22.20	35.52***	30.48	41.48***	<0.01	<0.01	2.39	2.56	4.66	4.85*	59.73	13.95***
Region	1	13.03	20.85***	0.23	0.32	<0.01	<0.01	2.62	2.79	2.87	2.99	18.76	4.38**
Year × Region	1	7.57	12.11**	0.47	0.64	<0.01	<0.01	8.25	8.81**	0.01	0.01	16.31	3.81**
Residuals	127	0.62		0.73		1.03		0.94		0.96		4.28	

4.3.4.2 Multi-elemental signatures

When individual elements were combined into a single matrix, significant differences were evident between regions in each year for the primordial area (Table 4.2). Regional differences were considerably greater in 2017 due to higher concentrations of Li for Investigator Strait. Regional differences in 2017 were driven by Li and Ba, and in 2018 by Ba (Fig. 4.10A). Inter-annual differences in Li, Mn and Sr were responsible for significant differences in regional multi-elemental signatures between years. Overall classification of larvae back to their region of capture was 82% and 70% in 2017 and 2018, respectively, and ranged from 66 to 94% for individual regions (Table 4.3).

For the non-primordial area, multi-elemental signatures differed between regions in 2017 but not 2018. Regional differences in 2017 were exclusively driven by higher concentrations of Li for Investigator Strait. Inter-annual variation in concentrations of Li, Mg and Sr were responsible for differences in regional multi-elemental signatures between years (Fig. 4.10B). Overall classification success was 80% in 2017, but was considerably lower in 2018 at 53% (Table 4.3). When both regions and years were combined in a single analysis, elemental signatures were most similar among regions within the same year (Table S4.2).

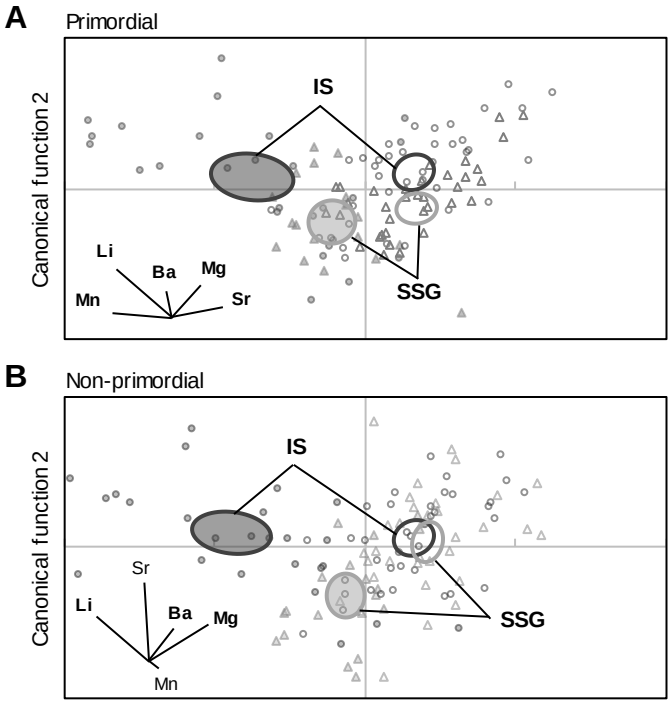

Figure 4.10. Canonical variate plots of the multi-elemental chemistry for the (a) primordial area and (b) non-primordial area of larval King George whiting otoliths in 2017 (shaded) and 2018 (open). Ellipses show 95% confidence around group means. Vector diagrams show the direction and weight of individual elements to sample distribution. Elements in bold contribute most to group differences. SSG – southern Spencer Gulf (▲; light); IS – Investigator Strait (●; dark).

Table 4.3. Classification success for the spatial comparison of multi-elemental chemistry for the (a) primordial and (b) non-primordial areas of larval King George whiting otoliths in 2017 and 2018. Data represent the percentage (%) of larvae from the region of capture (row) allocated to each region (column). Bold values are correctly assigned. SSG – southern Spencer Gulf; IS – Investigator Strait.

		SSG	IS
(a) primordial			
2017	SSG	**94**	6
	IS	26	**74**
	Overall	**82**	
	κ	0.65	
2018	SSG	**66**	34
	IS	26	**74**
	Overall	**70**	
	κ	0.39	
(b) non-primordial			
2017	SSG	**89**	11
	IS	26	**74**
	Overall	**80**	
	κ	0.60	
2018	SSG	**60**	40
	IS	53	**47**
	Overall	**53**	
	κ	0.07	

4.4 DISCUSSION

The aim of this study was to determine whether larval King George whiting in their natal waters of southern Spencer Gulf and Investigator Strait originated from a common source population. Firstly, we described the distribution and abundance of larvae throughout the only recognized spawning area in southern Australia. We then used the incremental structure and multi-elemental signatures recorded in their otoliths to assess the potential for different source populations within the large spawning area.

4.4.1 ENVIRONMENTAL CHARACTERISTICS

The incremental structure and elemental composition of calcified structures is heavily influenced by the aquatic environment, and as such, geographic differences in environmental characteristics underpin the use of otolith-based techniques to discriminate between fish populations (Campana 1999, Elsdon et al. 2008). Physical and chemical gradients in open marine environments are generally less pronounced compared to estuarine and freshwater systems (Standish et al. 2008). Even so, only small changes in environmental characteristics may be sufficient to be manifested as detectable differences in otolith elemental concentrations. For example, Atlantic croaker larvae collected from three water masses in the Mid-Atlantic Bight based on temperature and salinity showed significantly different otolith chemistry, suggesting that multiple source populations contributed to recruitment (Schaffler et al. 2009). In the present study, significant differences in temperature and salinity were evident between southern Spencer Gulf and Investigator Strait in 2017 that corresponded to distinct otolith signatures for larvae in each region. In 2018, environmental differences between regions were less prominent, which was reflected in greater similarity among regional otolith signatures. However, unlike Schaffler et al. (2009), we were unable to confidently discriminate between water masses based solely on environmental characteristics. Temperature and salinity demonstrated a similar gradient within each region in both years that increased away from the mouth into the two gulfs. These environmental gradients are characteristic of the seasonal thermohaline frontal systems that form at the entrance of Spencer Gulf and Investigator Strait (Bruce & Short 1990, Petrusevics 1993, Petrusevics et al. 2011).

4.4.2 DISTRIBUTION AND ABUNDANCE OF LARVAE

The spatial distribution and density of larvae differed considerably between southern Spencer Gulf and Investigator Strait. Larval abundance and density was higher for Investigator Strait in both 2017 and 2018, with larvae concentrated in the middle of the strait. In contrast, larvae in southern Spencer Gulf were distributed as a mosaic of small patches of low densities. For the latter region, in 2018, the highest densities of larvae were collected at the frontal zone across the mouth of the gulf. The patterns of larval

distribution and areas of highest abundance in this study were consistent with those observed throughout the same study area in 1999 (Fowler 2000). There was a discontinuity in the distribution of larvae between the two regions that corresponded to the environmental gradients at the entrances to Spencer Gulf and Investigator Strait. These observations provide further support to the findings of previous studies which have suggested that the frontal systems act as environmental barriers which inhibit water exchange, and subsequent plankton transport, during the austral summer and autumn (Bruce & Short 1990, Petrusevics 1993, Fowler 2000, Petrusevics et al. 2011). Because the seasonality of the frontal systems coincides with the peak spawning period for King George whiting (Fowler et al. 1999, Fowler et al. 2000a), it is possible that eggs and larvae in Spencer Gulf and Investigator Strait are separated until exchange between the gulfs and the waters of the continental shelf resumes after the fronts have dissipated.

4.4.3 EARLY LIFE-HISTORY CHARACTERISTICS

Otolith microstructure analysis is a useful tool for investigating the early life-history characteristics of developing larvae, which can be used to discriminate between fish that have occupied different environments (Campana & Neilson 1985, Campana & Jones 1992, Watai et al. 2018). The discriminatory power of this technique largely depends on variation in somatic growth rates that manifest as differences in otolith increment widths. However, despite spatial differences in temperature, we found no differences in the sizes, ages, hatch dates or average growth rates of larvae between regions or years. Furthermore, there were no spatial differences in daily growth trajectories from the otolith increment widths. The only difference evident was an ontogenetic shift in the daily growth rates of all larvae at 5 days post hatch, that would be associated with the transition from endogenous to exogenous feeding (Bruce 1995). Because of the similar environmental gradients in each region, it is possible that spatial differences were masked when samples were combined into regional groups. However, that appears unlikely because of the weak relationships between early life-history (e.g. average growth rate) and environmental characteristics (e.g. temperature). Another possibility is that the spatial variation in temperature (~2 °C) and salinity (~2 ppt) may have been insufficient to affect somatic growth, and therefore was not manifested in the otolith structure. Regardless, we were unable to discriminate between groups of larvae based on the characteristics derived from otolith microstructure analysis.

The primary interest of the analyses of otolith chemistry was to compare the multi-elemental signatures of larvae to determine if they had originated from a common source population. We identified significant differences in otolith chemistry between larvae from southern Spencer Gulf and Investigator Strait in each year, although the magnitude of difference was considerably greater in 2017. The differences suggest larvae from the two regions were hatched into, and subsequently developed in, water masses with different physical and/or chemical conditions that influenced otolith chemistry (Campana

1999, Elsdon et al. 2008). There was considerable inter-annual variation in regional multi-elemental signatures, with the magnitude of difference between years greater than regional differences within years. Such inter-annual variation has implications for characterising the geochemical signatures of larval populations, and also for studies that consider the natal signatures of otoliths from juvenile or adult fish (Gillanders 2002b, Reis-Santos et al. 2012).

In addition to differences among regions and years, there was considerable variation in elemental concentrations within otoliths. Spatial and temporal variation was considerably higher in the primordial area compared to the non-primordial area. The largest difference was a significant increase in Mn concentration at the primordium. Such elevated concentrations are unlikely to reflect environmental availability, but rather relate to physiological processes during embryonic development or crystallisation of the otolith nucleus (Brophy 2004, Ruttenberg et al. 2005). Barbee and Swearer (2007) separated larval otolith chemistry data in the same way, but found equally strong differences among populations for the primordial and non-primordial areas. The early life-history of their species of interest involves substrate-attached egg masses, and therefore all the otolith material related to embryonic development at a single location. In contrast, the pelagic eggs and larvae of broadcast spawning species are subject to dispersal immediately after being released (Norcross & Shaw 1984, Cowen & Sponaugle 2009). The potential for transport is even greater for marine species that reproduce in exposed coastal or open waters. Because larvae of such species may only remain near their spawning ground for a short period of time, such as a few days, the amount of otolith material that corresponds to the natal environment is likely to be very limited (Barbee & Swearer 2007, Standish et al. 2008). As such, for pelagic marine larvae the elemental composition of the otolith material that immediately surrounds the primordium may best reflect the natal environment. However, recent research suggests that otolith formation during embryonic development is considerably influenced by maternally derived chemical signatures, which could potentially mask environmentally-driven variation (Hegg et al. 2018, Loeppky et al. 2018). Consequently, it is necessary to separate the elemental data accordingly to account for such maternal effects.

4.4.4 ECOLOGICAL INTERPRETATION OF ELEMENTAL SIGNATURES

Larvae from southern Spencer Gulf and Investigator Strait hatched at the same time and developed at a similar rate, but had significantly different multi-elemental signatures relating to their natal origin. The differences in otolith chemistry indicate that larvae in the two regions occupied spatially segregated water masses, and suggest that each region supports its own spawning population (Campana 1999, Elsdon et al. 2008). Evidence of two distinct spawning populations within the recognised spawning area has considerable implications for understanding the ontogenetic connectivity and stock structure of King George whiting. Spatial differences in multi-elemental signatures were primarily driven by

concentrations of Ba and Li. Specifically, in both years, the otoliths of larvae from Investigator Strait showed significantly higher Ba concentrations compared to those from Spencer Gulf. Otolith Ba incorporation in marine fishes generally reflects ambient availability and shows a negative relationship with salinity (Elsdon & Gillanders 2005, Walther & Thorrold 2006, Izzo et al. 2018). A similar trend in otolith Ba concentration was identified for the natal signatures of settled King George whiting recruits, with those in Gulf St. Vincent having considerably higher Ba concentrations than Spencer Gulf (Rogers et al. 2019b; Chapter 3). As such, the most parsimonious hypothesis is that each putative source population replenishes nursery areas in the adjacent gulf region, and as such, each gulf supports a largely closed population. That is, for Spencer Gulf, larvae are spawned in the south and disperse northward to the nursery areas, whilst larvae spawned in Investigator Strait disperse eastward and replenish nursery areas in Gulf St. Vincent. This scenario is consistent with the meta-population structure of King George whiting in South Australia hypothesised by Fowler et al. (2000b) and Rogers et al. (2019b; Chapter 3). Because the two spawning grounds are separated by 50-100 km, only small-scale adult movement is required for exchange between populations to maintain genetic homogeneity (Kent et al. 2018).

Otolith Li concentrations for 10 of 25 larvae collected from Investigator Strait in 2017 were drastically higher than for larvae from elsewhere in either year. The mean Li concentration in the otoliths of these larvae was 287.4 µmol mol^{-1} (compared to 32.9 µmol mol^{-1} for the other larvae in the sample), which exceeded any published otolith Li concentrations (<50 µmol mol^{-1}). These larvae were collected at three consecutive sampling stations within two hours of each other, and all larvae from these stations demonstrated exceedingly high Li values. We believe that sample contamination is unlikely because larvae were randomised at each stage of processing, were processed in a clean laboratory, and there were no systematic differences in the concentrations of the other elements measured from the same otoliths. One possible explanation is that the pronounced Li concentrations reflect a localised environmental phenomena that was directly manifested in otolith chemistry (Elsdon et al. 2008, Izzo et al. 2018). Lithium is only present in the salt fraction of endolymphatic fluid and is therefore unlikely to directly substitute for calcium at the precipitating surface (Thomas et al. 2017). As such, incorporation of Li into the otolith most likely occurs through random trapping in interstitial spaces of aragonite crystal during daily increment formation, and may reflect ambient concentrations (Izzo et al. 2016, Thomas et al. 2017). Lithium incorporation could also be facilitated by increased interstitial space following the substitution of calcium for elements with larger atomic radii, such as Sr or Ba (de Vries et al. 2005). However, there were no systematic changes in these elements with Li concentrations. Another possibility is that the elevated Li signature may have been maternally transferred, and in which case, the larvae were the progeny of a common spawning female (Thorrold et al. 2006, Starrs et al. 2013). Regardless of the source of Li, spatio-temporal differences in otolith chemistry remained similar when Li was excluded from multi-elemental analyses (see Appendix A).

4.4.5 IMPLICATIONS AND FUTURE DIRECTIONS

A common application of otolith chemistry is to analyse the natal signatures of juvenile or adult fish to estimate the number of source populations that contribute to recruitment (e.g. Gillanders & Kingsford 1996, Standish et al. 2011, Tanner et al. 2012). Whilst such studies provide considerable insight into population connectivity and stock structure, they cannot identify the source populations from where larvae originated and the degree to which populations rely on larval production from elsewhere. This was the situation with King George whiting in South Australia. There was evidence to suggest that recruits in Spencer Gulf and Gulf St. Vincent originated from different source populations (Rogers et al. 2019b; Chapter 3). In the present study, we determined that the recognised spawning area throughout southern Spencer Gulf and Investigator Strait constitutes two source populations based on the elemental composition of larval otoliths. The next step is to explore the relationships between the different spawning grounds and nursery areas to understand population dynamics and inform stock structure. One possible approach is to simulate larval dispersal using a biophysical model (Fowler et al. 2000b, Jenkins et al. 2000). This technique has been successfully applied to simulate larval transport of western king prawns in Spencer Gulf (McLeay et al. 2016), and has the capacity to be applied to King George whiting. Alternatively, otolith chemistry has the potential to empirically quantify larval movement between populations. This can be achieved by collecting larvae from all potential source populations, characterising the elemental signatures of their otoliths, and then comparing them to the natal signatures of juveniles in different nursery areas. In this way, otolith chemistry can be used to address ecological questions not currently approachable using other techniques.

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Statement of Authorship

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Publication Details	Rogers TA, Redondo-Rodriguez A, Fowler AJ, Doubell MJ, Drew MJ, Steer MA, Matthews D, James C and Gillanders BM. Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish. Submitted for consideration for publication in *Fisheries Oceanography*.

Principal Author

Name of Principal Author (Candidate)	Troy Rogers		
Contribution to the Paper	Contributed to the design of the study, assisted with sample collection and processing, developed the biological model (larval development and behaviour, spawning and settlement areas, dispersal scenarios), prepared and collected the otolith data, collated and analysed the biophysical model data, wrote the manuscript and acted as corresponding author.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	14/11/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ana Redondo-Rodriguez		
Contribution to the Paper	Developed and operated the biophysical model, assisted with data interpretation and revised the manuscript. Produced figures 5.5, 5.6 and 5.7.		
Signature		Date	18/11/19

Name of Co-Author	Anthony Fowler		
Contribution to the Paper	Contributed to the design of the study, assisted with sample collection and data interpretation, and revised the manuscript.		
Signature		Date	14/11/19

Name of Co-Author	Mark Doubell		
Contribution to the Paper	Contributed to the design of the study, developed and operated the biophysical model, and assisted with data interpretation.		
Signature		Date	19/11/19

Name of Co-Author	Michael Drew		
Contribution to the Paper	Assisted with sample collection, processed the majority of plankton samples, identified the eggs, and assisted with data interpretation.		
Signature		Date	18/11/19

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Contribution to the Paper	Contributed to the design of the study, assisted with sample collection and data interpretation, and revised the manuscript.		
Signature		Date	17/11/19

Name of Co-Author	Damian Matthews		
Contribution to the Paper	Assisted with sample collection and processing, Produced figures 5.1, 5.8, 5.10, and provided the base map layer for other figures.		
Signature		Date	18/11/19

Name of Co-Author	Charles James		
Contribution to the Paper	Developed and operated the biophysical model.		
Signature		Date	18/11/19

Name of Co-Author	Bronwyn Gillanders		
Contribution to the Paper	Contributed to the design of the study and revised the manuscript.		
Signature		Date	17/11/2019

Chapter 5 - Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish

ABSTRACT

Most demersal marine fish species depend on a dispersive larval stage that connects geographically discrete sub-populations. Understanding connectivity between these sub-populations is necessary to determine stock structure, which identifies the appropriate spatial scale for fishery management. Such ontogenetic connectivity is poorly understood for King George whiting (*Sillaginodes punctatus*; Perciformes) in South Australia's gulf system, even though spawning grounds and nursery areas are adequately defined. In response to declines in commercial fishery statistics and modelled estimates of biomass, this study aimed to determine the most important spawning grounds and nursery areas to recruitment, and to determine the connectivity between them, by simulating larval dispersal using a biophysical model. The model was seeded with particles according to the spatial distribution of eggs collected throughout the recognised spawning area in 2017 and 2018. Despite inter-annual differences in the origins of larvae, the predicted patterns of dispersal and the nursery areas to which the larvae settled remained consistent between years. Settlement was highest to nursery areas only short distances from regional spawning grounds, which indicated that population processes were localised within each gulf. However, the model also predicted that later in the spawning season, larvae that originated in southern Spencer Gulf contributed to recruitment in Gulf St. Vincent. The within-season shift in dispersal pathways corresponded to the breakdown of thermohaline frontal systems at the entrance of each gulf, and is consistent with spatial and temporal patterns in the otolith chemistry of larvae. Consequently, the most parsimonious explanation is that the populations of King George whiting in South Australia's gulf system constitute a single, panmictic stock, which has implications for fishery management.

This chapter is in review at *Fisheries Oceanography* as:

Rogers TA, Redondo-Rodriguez A, Fowler AJ, Doubell MJ, Drew MJ, Steer MA, Matthews D, James C and Gillanders BM. Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish.

5.1 INTRODUCTION

The life-history of most demersal marine fish species depend on the transport of larvae from offshore spawning grounds to suitable inshore nursery areas (Jenkins et al. 1997, Houde 2008, Teodosio et al. 2016). Throughout the larval stage, which can vary in duration from weeks to months, the inherently small larvae occupy the pelagic environment and reside within a mixed planktonic community (Blaxter 1969, Houde & Hoyt 1987, Cowen & Sponaugle 2009). As such, the distribution and abundance of larvae is heavily influenced by physical oceanographic processes, which provides the potential for larval transport over large spatial scales and the opportunity for exchange between geographically discrete sub-populations (Norcross & Shaw 1984, Cowen et al. 2000, Houde 2008). Most demersal marine fish species conform to a meta-population structure, whereby relatively sedentary adults form spatially discrete sub-populations that are connected by a dispersive larval stage (Levins 1969, Bailey 1997, Secor & Rooker 2005, Cowen & Sponaugle 2009). For these species, understanding the degree to which different sub-populations are connected through larval dispersal is necessary to determine the spatial scale over which the life-history operates, which identifies the most appropriate scale for management (Begg et al. 1999, Cowen & Sponaugle 2009).

Historically, it was assumed that larvae were passive propagules and that larval dispersal was entirely driven by physical oceanographic processes. As such, populations of marine fishes were considered demographically open systems that were largely independent of local reproduction (Hjort 1914, Caley et al. 1996, Houde 2008). However, it is now widely-accepted that larvae develop swimming and sensory abilities during ontogeny that enable them to actively influence dispersal. Late-stage larvae of many marine fish species have well-developed swimming abilities, with most capable of sustained swimming speeds which exceed the prevailing currents (Fisher 2005, Leis 2006, 2010). Furthermore, late-stage larvae can use a range of sensory cues and behavioural techniques to select currents and promote dispersal towards settlement areas (Leis 2010, Teodosio et al. 2016). It is most likely that large-scale transport from offshore spawning grounds to coastal areas is predominantly driven by physical oceanographic processes that disperse essentially passive propagules. Once developed and near the coast, late-stage larvae use a suite of visual, auditory and olfactory cues to establish orientation and actively move towards potential nursery areas (Jenkins et al. 1997, Leis 2010, Teodosio et al. 2016). As such, patterns of larval dispersal, which subsequently influence recruitment, are driven by the complex interactions of numerous physical, biological, and ecological factors (Houde 2008).

Recent developments in biological oceanography have led to biophysical models becoming a leading approach to predict patterns of larval dispersal and investigate population connectivity in marine ecosystems (Treml et al. 2008, Swearer et al. 2019). This technique couples a physical hydrodynamic model, forced by oceanographic, topographic and atmospheric data, with a biological model, which

incorporates larval development and behaviour, to simulate transport throughout the larval stage (Leis 2007, North et al. 2008, Swearer et al. 2019). Highly-resolved biophysical models can comprehensively describe larval dispersal patterns under various meteorological and behavioural scenarios, and are being increasingly advocated as informative tools to support ecosystem-based management (Treml et al. 2008, Kough et al. 2013, Gallego et al. 2016). The theoretical predictions of larval dispersal can be compared with the results of other techniques that retrospectively investigate population connectivity, such as genetic parentage analysis or elemental analyses of hard structures, to assess the accuracy of the model predictions and best interpret population connectivity (Schunter et al. 2011, Nolasco et al. 2018, Bode et al. 2019).

King George whiting (*Sillaginodes punctatus*: Perciformes) is a demersal marine finfish species that is endemic to temperate coastal waters of southern Australia, where it supports important commercial and recreational fisheries (Kailola et al. 1993, Steer et al. 2018). South Australia is in the geographic centre of this distribution and has historically provided the highest State-based catches (Mobsby 2018). However, recent trends in commercial catch statistics and modelled estimates of fishable biomass indicate that the populations of King George whiting in South Australia's gulfs are in decline, and have been classified as 'transitional depleting' (Fowler et al. 2014, Steer et al. 2018). This stock status means that the population is not yet overfished, but that management intervention is required to prevent the population from being depleted to an overfished state (Flood et al. 2016). Despite extensive research into the life-history of this species (Jenkins & May 1994, Fowler & Short 1996, Jenkins et al. 1997, Fowler et al. 2000b, Fowler et al. 2002, Jenkins 2005, Jenkins et al. 2016), there remains considerable uncertainty about the specific locations of key spawning grounds and their connectivity with nursery areas. King George whiting conforms to the general bi-partite life-history of many demersal marine fish species. Adults spawn during the austral autumn and winter at low-profile reefs and shoals in areas of moderate to high wave energy (Fowler et al. 1999, Fowler et al. 2000a). Across south-eastern Australia, the only recognised spawning area is throughout southern Spencer Gulf and Investigator Strait (Fig. 5.1). The developing eggs and larvae disperse over a long larval duration (80-130 d), before the larvae settle to shallow seagrass beds in protected bays (Jenkins & May 1994, Fowler & Short 1996, Jenkins et al. 1997, Rogers et al. 2019b; Chapter 3). In South Australia, the majority of these nursery areas are in Spencer Gulf and Gulf St. Vincent (Fowler et al. 2000b, Fowler & Jones 2008, Rogers et al. 2019b) (Fig. 5.1). The juveniles develop in the vicinity of the nursery areas to which they settled for several years, before they move offshore to replenish the populations of spawning adults (Fowler et al. 2000b, Fowler et al. 2002, Jenkins et al. 2016).

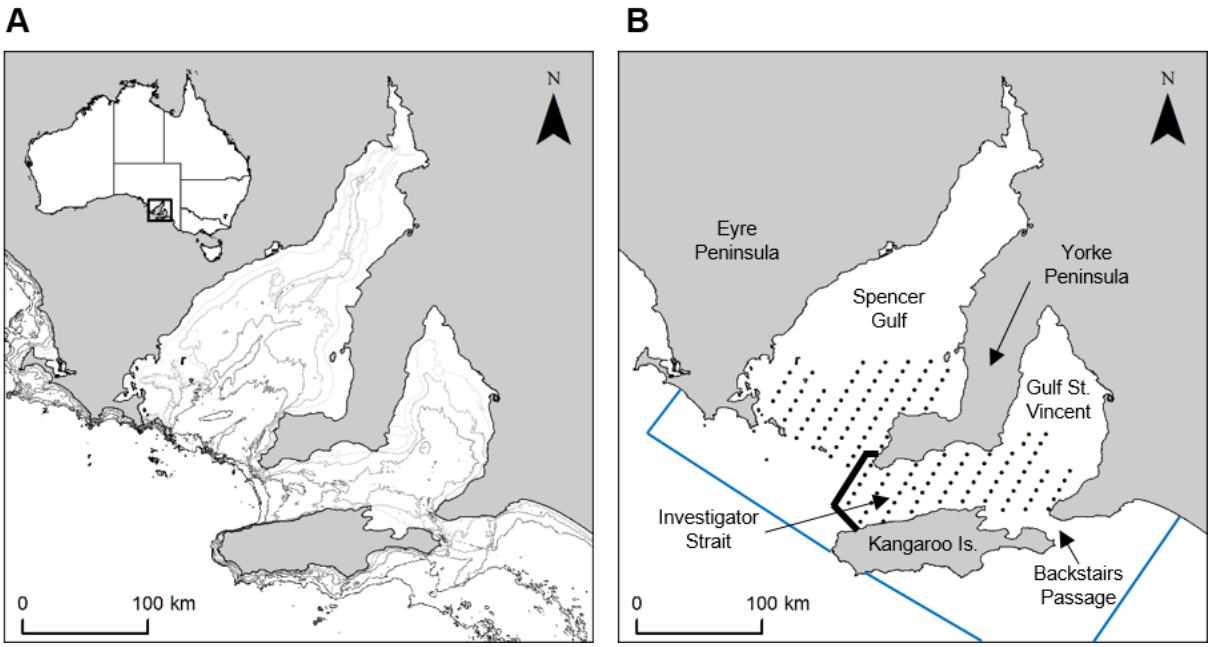

Fig. 5.1. (A) Bathymetry throughout South Australia's gulf system. Contours show 10 m intervals to 60 m depth. Inset – map of Australia showing the study region along the southern coastline. (B) Map of South Australia's gulf system showing the geo-referenced stations where plankton samples were collected in 2017 and 2018. The bold line separates plankton stations in southern Spencer Gulf and Investigator Strait. The blue line shows the boundaries of the Two Gulfs Model (TGM).

In response to the declines in fishery statistics and estimated biomass in Spencer Gulf and Gulf St. Vincent, there was a need to understand the connectivity between key spawning grounds and nursery areas to underpin the development of future management strategies. (Fowler et al. 1999, Fowler et al. 2000a). A recent study identified two spatially-segregated groups of larvae within the large spawning area that had different otolith chemistries. This indicated that southern Spencer Gulf and Investigator Strait supported two independent spawning populations (Rogers et al. 2019c; Chapter 4). Additionally, the larvae that settle to nursery areas in Spencer Gulf have different otolith chemistry than those in Gulf St. Vincent, which suggests that each gulf is replenished by a different spawning population (Rogers et al. 2019b; Chapter 3). However, the relationships between the two spawning grounds and the nursery areas in the Spencer Gulf and Gulf St. Vincent remain poorly understood. As such, the aim of this study was to investigate the demographic relationships between spawning grounds and nursery areas for King George whiting in South Australia's gulf systems by simulating larval dispersal using a biophysical model. The specific objectives were to: (1) identify the spawning grounds and nursery areas that contribute most to settlement; (2) investigate the relationships between the key spawning grounds and nursery areas; and (3) evaluate the effects of larval behaviour on the projected dispersal pathways and settlement success of larvae. The biophysical model simulated larval dispersal based on the spatial distribution and abundance of eggs collected throughout the recognised spawning area in 2017 and 2018.

5.2 MATERIALS AND METHODS

5.2.1 STUDY REGION

This study investigated the dispersal of larval King George whiting throughout Spencer Gulf, Gulf St. Vincent and Investigator Strait, South Australia (Fig. 5.1). The physical environmental characteristics of this region are predominantly influenced by its complex topography, shallow bathymetry and local meteorology, rather than through exchange of water with the continental shelf (Bye & Kampf 2008). Spencer Gulf and Gulf St. Vincent are semi-enclosed seas that are classified as inverse estuaries because they display a positive salinity gradient from mouth to head (35 to 50 ppt) (Nunes & Lennon 1986). This salinity gradient forms because evaporation exceeds precipitation, and there are no permanent freshwater inputs from creeks or rivers (Petrusevics 1993). Throughout the year, both gulfs demonstrate considerable fluctuations in water temperature that reflect a seasonal reversal in temperature gradients. The largest temperature fluctuations (~12-24 °C) are near the heads of the gulfs because of their shallow bathymetry and irregular flushing, whilst temperature fluctuations are moderate (~15-20 °C) toward the mouths and in Investigator Strait because of the increased depth and seasonal exchange with shelf water (Petrusevics 1993, Middleton & Bye 2007). The general clockwise movement of water within the gulfs is largely driven by local wind forcing and density-driven thermohaline circulation (Bye & Kampf 2008).

During the austral summer, temperature and salinity increase in the gulfs which leads to the formation of thermohaline frontal systems at the entrance to Spencer Gulf and in Investigator Strait (Nunes & Lennon 1986, Petrusevics 1993, Petrusevics et al. 2011). These 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 (Bruce & Short 1990, Fowler et al. 2000b, Petrusevics et al. 2011). 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 (Fowler et al. 1999), which may affect the relationships between important spawning grounds and nursery areas.

5.2.2 BIOPHYSICAL 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. 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 included behaviour and settlement sub-models that were developed from published biological data. Three dispersal scenarios were simulated with different larval behaviours and development rates, which were repeated using environmental forcing from 2017 and 2018.

5.2.2.1 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 (Song & Haidvogel 1994, Shchepetkin & McWilliams 2005). 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 1500 m in the horizontal with 15 sigma levels in the vertical, and is run with a 200 s time step to solve 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 (BRAN3; Oke et al. 2013). 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 (Saha et al. 2014), and tidal forcing was provided by TPXO8 (Erofeeva & Egbert 2014). Predictions of the TGM were compared against the measured sea level height, tidal current velocity and residual current velocity at an observational buoy (SAM8SG) of the South Australian Integrated Marine Observing System (SAIMOS).

5.2.2.2 Particle tracking model

Particle tracking was undertaken using the larval transport model (LTRANS) (North et al. 2006, North et al. 2008). 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 (North et al. 2008, Schlag & North 2012). Hourly outputs (external time step) from the Two Gulfs Model were used to run the larval transport model 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} (Visser 1997). Boundary conditions were imposed on particle trajectories at each internal time step of the larval transport model as described by North et al. (2008). In brief, 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.

5.2.3 SPAWNING AREAS

The model was seeded with particles according to the spatial distribution and density of King George whiting eggs throughout southern Spencer Gulf and Investigator Strait. Eggs were collected on two research cruises from 25 April to 1 May 2017 and 24 to 30 April 2018 aboard the RV *Ngerin* at 126 geo-referenced stations arranged in a 2 × 4 nm grid pattern (Fig. 5.1). Plankton samples were collected at each station using an oblique tow with paired bongo nets of 0.57 m diameter with 500 µm mesh. The nets were lowered to within 5 m of the seabed and retrieved at ~45° at ~1 m s^{-1}. Each net was fitted with a flowmeter and calibrated using factory coefficients to estimate the distance travelled by each net for each tow (General Oceanics 2030; FL, USA). Plankton samples were preserved in 100% ethanol and refrigerated at 4 °C prior to sorting.

All teleost eggs were sorted from the plankton using a modified Sedgwick-Rafter sorting tray under a dissecting microscope (Olympus SZX7; Tokyo, Japan). Eggs were separated into 'possible' and 'unlikely' King George whiting eggs following the morphological descriptions by Fowler (2000) and Ham and Hutchinson (2003). The main diagnostic features of King George whiting eggs were: spherical eggs ranging in diameter from 0.84 to 0.94 mm; smooth chorion; narrow perivitelline space; and a single oil globule ranging in diameter from 0.21 to 0.34 mm. Morphological identifications were verified using an *in situ* hybridisation (ISH) molecular technique. This technique uses a horseradish peroxidase (HRP) enzyme conjugated oligonucleotide probe that binds specifically to mitochondrial 16S ribosomal RNA of King George whiting and generates a blue colour through oxidisation with a HRP reactive substrate (Oxley et al. 2017) (Fig. 5.2). The chorion of each 'possible' egg was mechanically pierced to expose the embryonic tissue to the molecular probe. A total of 1,485 King George whiting eggs (n = 541 in 2017 and n = 944 in 2018) were confirmed using the ISH technique.

The density of King George whiting eggs at each station was calculated by the equation:

$$E_d = \frac{C \times D}{V} \qquad \text{Eq. (5.1)}$$

where E_d is egg density (eggs m^{-2}), C is the number of eggs in each sample, D is the maximum depth (m) to which the net was deployed, and V is the volume of water filtered (m^3). V was calculated as the area of the paired nets ($2 \times \pi r^2$) multiplied by the distance travelled according to the flowmeter readings. During the particle tracking model, the number of particles released at each station was the calculated egg density multiplied by 100.

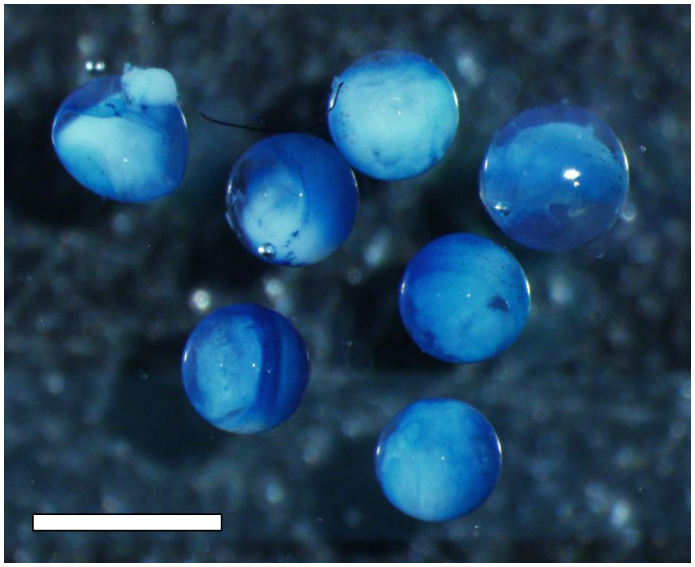

Fig. 5.2. King George whiting eggs following verification using the *in-situ* hybridisation (ISH) molecular technique. The chorion of each egg was mechanically pierced to expose the embryonic tissue to the ISH probe. Eggs were viewed at 20× magnification under a dissecting microscope. The scale bar is 1 mm.

5.2.4 SETTLEMENT AREAS

Settlement areas have been identified by the presence of recently-settled larvae during intermittent recruitment surveys between 1977 and 2018 (Jones et al. 1990, Fowler & Jones 2008, Rogers et al. 2019b). Settled larvae have been found at 25 sites within the boundaries of the hydrodynamic model (Fig. 5.3A; Table S5.1). In general, settlement areas are semi-enclosed embayments that support shallow, sub-tidal beds of the seagrass *Zostera* spp. The 25 settlement areas were divided into six 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 (Fig. 5.3B).

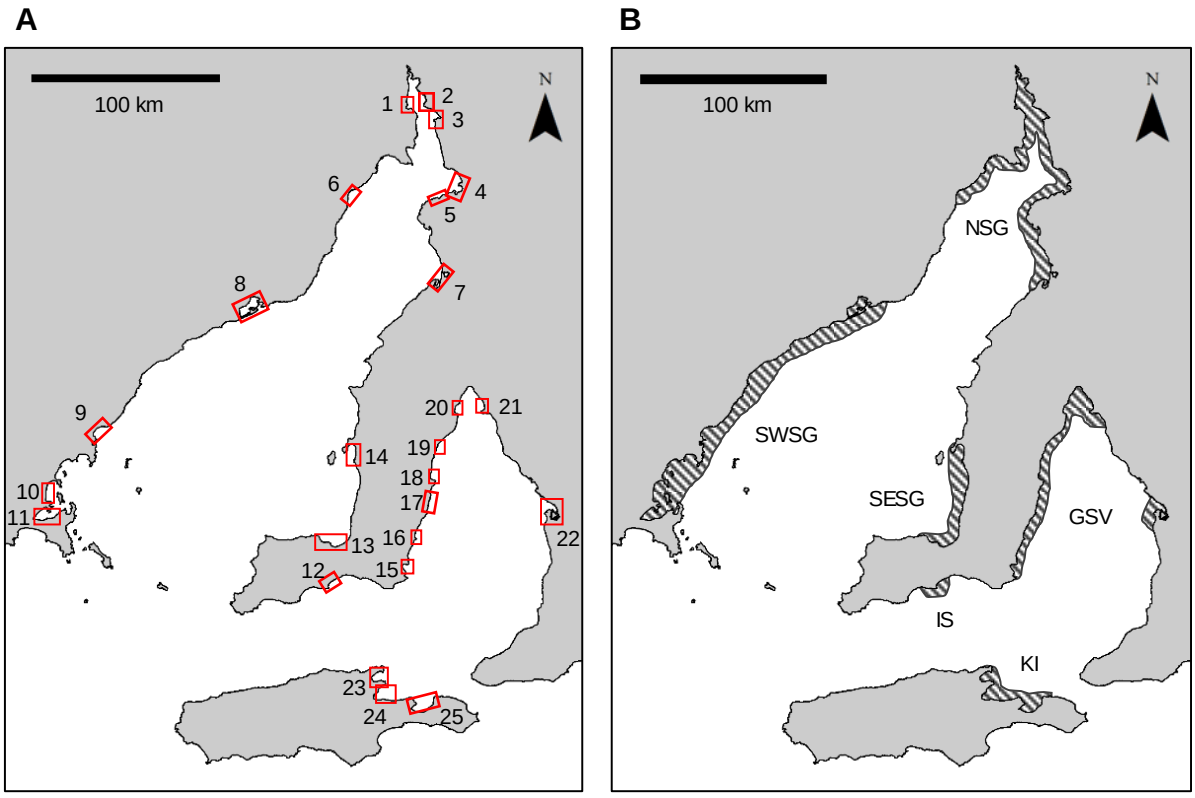

Fig. 5.3. (A) Locations of settlement areas (1-25) incorporated into the larval transport model. Settlement areas were included based on the presence of recently-settled larvae from recruitment surveys between 1977 and 2018. Site names are in Table S5.1. (B) Dashed areas show the broader settlement regions. 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.

5.2.5 LARVAL DEVELOPMENT AND BEHAVIOUR

The larval behaviour sub-model was developed from published field observations and a larval rearing study. Diagnostic characteristics of larval King George whiting are: shallow-bodied, elongate larvae with a small head; small fins; and a moderate to long uncoiled gut (Bruce 1995). Their swimming ability is poor compared to larvae of other demersal marine fish species. The critical swim speed (Ucrit) of recently-settled larvae is ~6 cm s^{-1} (Jenkins & Welsford 2002), which is considerably less than similar-sized larvae of tropical reef fishes (20-35 cm s^{-1}), and is at the lower range of larvae of temperate species (5-10 cm s^{-1}) (Leis 2010). Critical swimming speed does not reflect general swimming speed in the ocean, which is likely to be much lower (Leis 2010). Because the prevailing currents are considerably stronger than the swimming capabilities of the larvae, it is unlikely that larval King George whiting are able to actively influence dispersal through horizontal swimming (Jenkins & Welsford 2002; G.P. Jenkins & J.M. Leis, pers. comm.). However, their swimming abilities are sufficient to move vertically through the water column, which can significantly affect larval dispersal (Jenkins & Welsford 2002, Leis 2007). The speed of vertical movement has not been measured and critical swimming speed has

only be measured for settled larvae (Jenkins & Welsford 2002). Larval swimming ability generally increases proportionally with length (Fisher 2005, Leis 2007), and therefore we used a linear relationship to estimate swim speed at length (Fig. S5.1). In the absence of a relationship between larval development and temperature, and because of variation in the growth rates of larvae, behaviour was directly related to larval development at length (Table 5.1).

Table 5.1. Larval development and behavioural characteristics of larval King George whiting used in the biophysical model. Behaviour was directly related to length. DVM – diurnal vertical migration; RDVM – reverse diurnal vertical migration. Summary of larval behaviour for each dispersal scenario showing the predicted growth rate (mm d^{-1}), age (dph; days post hatch) and duration (d) of each development stage. Descriptions of each dispersal scenario are in Methods 5.2.5.

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)			DVM	DVM	DVM
	Surface (0-5 m)	Surface (0-5 m)	Surface (0-5 m)			RDVM (<10 m)
	Passive (drift)	Passive (drift)	Passive (drift)			Settle
Vertical movement				1 cm s^{-1}	2 cm s^{-1}	2 cm s^{-1}
Scenario 1 - passive						
Age (dph)	-	0-6	6-24	24-31	31-92	92-131
Duration (d)	2	6	18	7	61	39
Scenario 2 – average growth rate						
Growth (mm d^{-1})		0.15	0.15	0.15	0.15	0.15
Age (dph)	-	0-6	6-24	24-31	31-92	92-131
Duration (d)	2	6	18	7	61	39
Scenario 3 – daily growth rate						
Growth (mm d^{-1})		0.10	0.09-0.15	0.16	0.18	0.18
Age (dph)	-	0-9	9-29	29-35	35-82	82-112
Duration (d)	2	9	20	6	47	30

King George whiting eggs are buoyant and remain at or near the surface during embryonic development (Ham & Hutchinson 2003), and so were modelled as passive particles that remain in the surface layer (0-5 m) (Table 5.1). The duration of the egg phase was 2 d based on the mean time until hatching of eggs reared at 19 °C (Ham & Hutchinson 2003), which best reflected the water temperature at the time of collection (range 17.8 to 19.9 °C). The newly hatched larvae (2.1 mm SL) carry a buoyant yolk sac and have no discernible swimming ability, so their movement was modelled as passive in the surface layer (0-5 m). After 5 to 8 d, yolk-sac absorption is complete and the swim bladder begins to develop (Bruce 1995).

During the flexion stage, 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 (Bruce 1995). Reared larvae began vertical movement at this stage (Ham & Hutchinson 2003), and field observations of inflated swim bladders for larvae collected at night indicate that they undergo diurnal vertical migration (DVM) (Bruce 1995). Subsequently, during the flexion stage, larvae were modelled as having DVM behaviour at 1 cm s^{-1} (Table 5.1). The DVM scheme assumed that the larvae swim down when the light level exceeded a threshold of 0.0166 E m^2 s. To calculate the light level, the model first calculated the surface irradiance using estimates of day length, 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.

Larval King George whiting experience a prolonged post-flexion stage before they are competent to settle. During this 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 5.1). Larvae were able to settle to the pre-defined settlement areas during the settlement stage (15.0 to 20.5 mm SL). Larvae were considered competent to settle at > 15 mm SL, the smallest size that settled larvae have been collected from nursery areas (Jenkins & May 1994, Jenkins et al. 1998b, Fowler et al. 2000b, Rogers et al. 2019b). Similarly, very few pre-settlement larvae have been captured > 20.5 mm SL (Hamer & Jenkins 1997). During the settlement stage once in the vicinity of settlement grounds, larvae exhibit reverse diurnal vertical migration (RDVM) behaviour (Jenkins et al. 1998b). They remain near the surface during the day and are distributed throughout the water column at night (Jenkins et al. 1998b). 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 did not settle during the settlement stage, it was considered dead.

5.2.6 DISPERSAL SCENARIOS

Larval dispersal was simulated using three scenarios to assess the effects of biological inputs (larval stage duration and behaviour) on the modelled projections of dispersal. Larval behaviour was directly related to length-at-age (Table 5.1), which was determined from the estimated growth rates of recently-settled larvae that recruited to Barker Inlet in 2017 (Rogers et al. 2019a). 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 d.

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

$$\frac{L_c - L_o}{a} \quad \text{Eq. (5.2)}$$

where L_c is length at capture, L_o is length at hatch (2.1 mm; Bruce, 1995), and a is age (d). The mean average growth rate was 0.15 mm d^{-1} (± 0.01), which was used to estimate length-at-age for scenarios 1 and 2 (Fig. 5.4). For each scenario, the model was initialised at midnight of 1 May and ran until the modelled larvae were > 20.5 mm SL (approximately mid-September).

Scenario 1 – passive movement

Previous studies of hydrodynamic modelling for larval King George whiting simulated the larvae as passive particles (Fowler et al. 2000b, Jenkins et al. 2000). This was because their swimming ability is extremely limited (Jenkins et al. 1998a, Jenkins & Welsford 2002), and the temporal and spatial distribution of late-stage larvae was accurately reproduced when larvae were considered passive (Jenkins & Black 1994, Jenkins et al. 1997, Jenkins et al. 1999). As such, in scenario 1, larvae were projected as passive particles that were neutrally buoyant and moved vertically due only to diffusion or turbulence. Larvae were capable of settlement between 92 to 131 days post hatch (dph) (Table 5.1).

Scenario 2 – average growth rate

Scenario 2 used the average growth rate of recently-settled larvae to estimate length-at-age and the duration of each larval stage. The larval behaviour incorporated into the biological model is described in Table 5.1. Larvae were passive until flexion (24 dph) when DVM began and continued until the pre-settlement stage (92 dph). During the settlement stage, larvae were able to settle to the pre-defined settlement areas for 39 d (92-131 dph).

Scenario 3 – daily growth rate

Whilst the average growth rate provided an estimate of length-at-age throughout the larval period, it is highly unlikely that larval growth is linear. Fowler and Short (1996) identified a significant ontogenetic shift in the daily growth rates of recently-settled King George whiting larvae throughout the settlement season. As such, scenario 3 used the mean daily length-at-age calculated from otolith increment widths to determine the duration of each larval stage (Fig. 5.4). Behaviours were applied at the same larval stage as scenario 2 (Table 5.1). Retrospective daily growth rates (mm d^{-1}) and length-at-age were estimated from otolith increment widths using the 'back-calculation with biological intercept algorithm' (Campana 1990, Campana & Jones 1992). The preparation of otoliths for microstructure examination followed Rogers et al. (2019a; Chapter 2). Daily increments were measured from the primordium to posterior margin to the nearest 0.1 µm, and the size of each larva on successive days was estimated by the equation:

$$L_a = L_c + (\frac{(O_a - O_c)(L_c - L_o)}{(O_c - O_o)}) \quad \text{Eq. (5.3)}$$

where L_a is the length at age a, L_c is length at capture, L_o is length at hatch (2.1 mm; Bruce, 1995), O_a is the otolith radius at age a, O_c is the otolith radius at capture, and O_o is otolith radius at hatch. Here, O_o was defined as the distance from the closest increment to the primordium, and O_a was calculated from accumulating successive increments (Fowler & Short 1996). Larvae were passive until flexion (29 dph) when DVM began and continued until the settlement stage (82 dph). Larvae were able to settle for 30 d (82-112 dph).

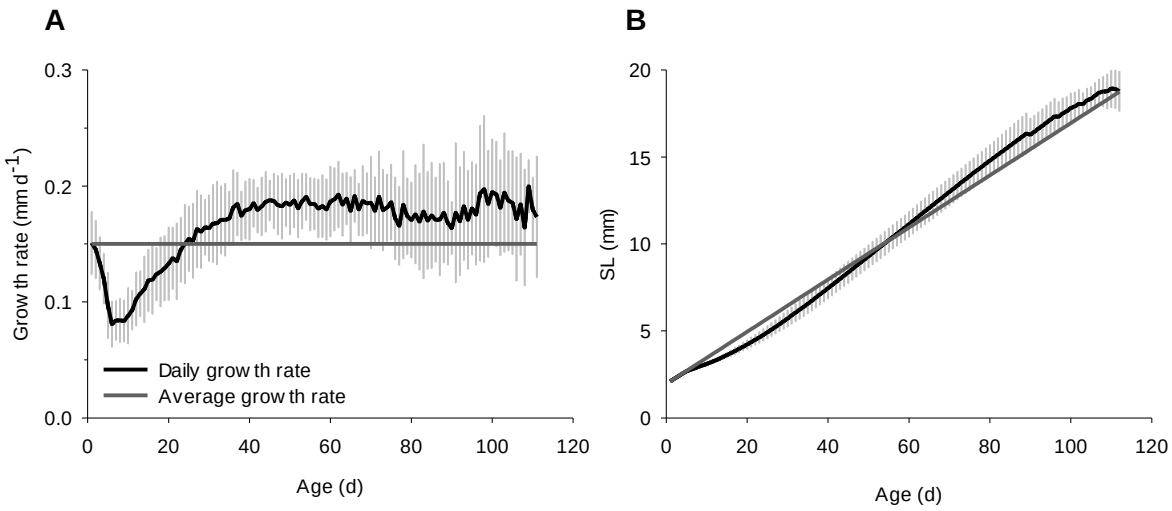

Fig. 5.4. Comparison of (A) daily growth rate (mm d^{-1}) and (B) length-at-age (mm) calculated from the average growth rate from hatch to capture (grey), and daily growth rates estimated from otolith increment widths (black; mean ± 1 SD). Length-at-age was used to determine the timing of larval behaviour for each dispersal scenario. Growth rates were calculated from recently-settled larvae to Barker Inlet that hatched in late April 2017 (Rogers et al. 2019a).

5.3 RESULTS

5.3.1 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 (Fig. 5.5). The model predictions captured neap and spring tides, and the interaction between the four main tidal constituents. Simulated tidal currents varied geographically throughout the study area. Tidal currents averaged ~0.3 to 0.4 m s^{-1} in Spencer Gulf and Investigator Strait, and ~0.2 to 0.3 m s^{-1} in Gulf St. Vincent (Fig. 5.6). 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 to 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} (up to 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 (Fig. 5.7). 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.

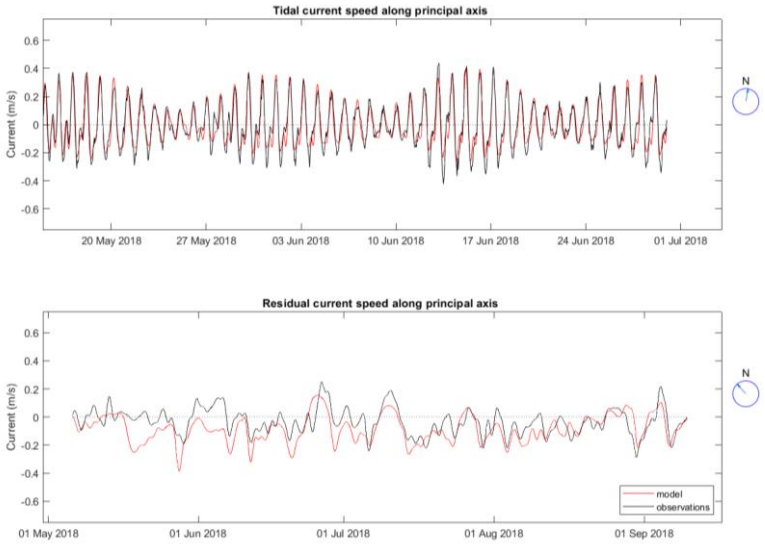

Fig. 5.5. Comparison of (top) vertically averaged tidal current velocity (m s^{-1}; currents with low-pass filtered currents removed) and (bottom) residual current velocity (m s^{-1}) between the hydrodynamic model (red) and measurements at an observational buoy (SAM8SG) of the South Australian Integrated Marine Observing System (SAIMOS) (black) in 2018. Arrows to the right of each plot show the major current direction (i.e. the principal axis along which the currents were resolved). Only seven weeks of tidal currents are plotted to improve the resolution of the comparison.

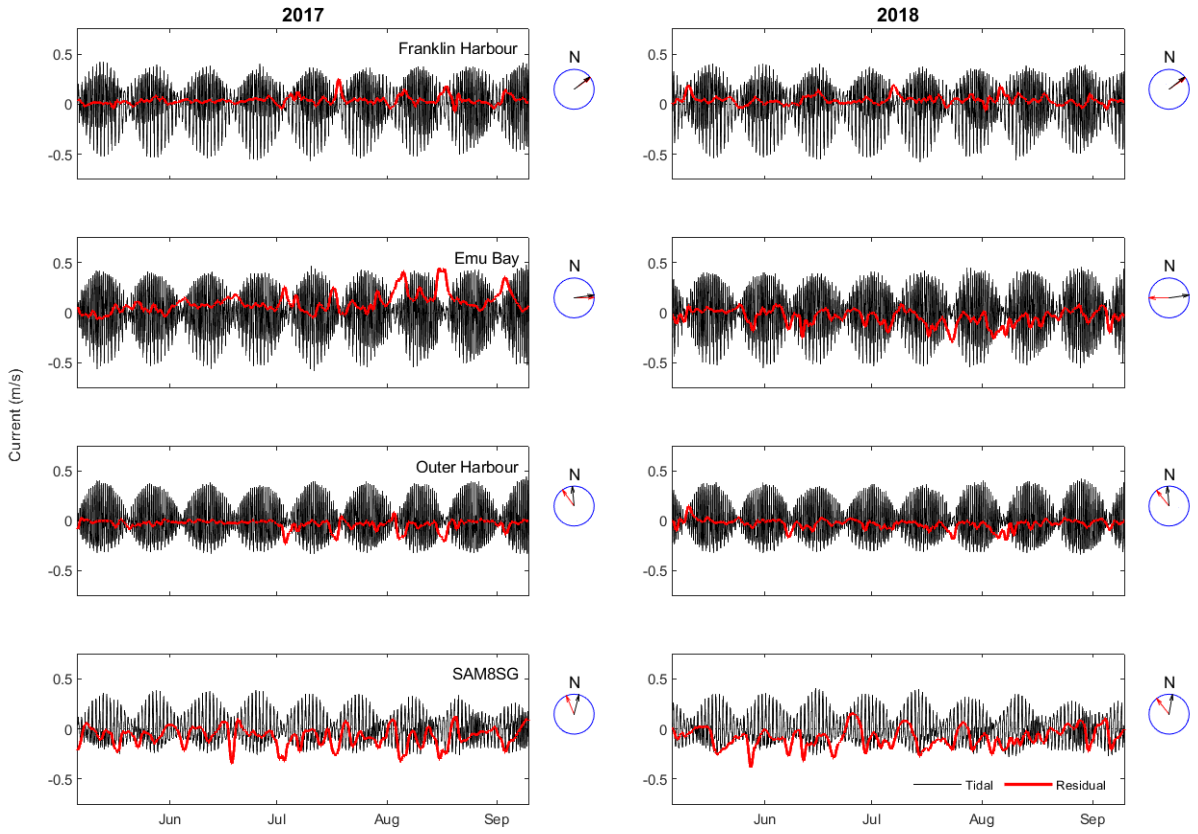

Fig. 5.6. Comparisons of vertically averaged tidal current velocity (m s^{-1}; black) and residual current velocity (m s^{-1}; red) of the Two Gulf Model (TGM) at four sites throughout the study area in 2017 and 2018. Arrows in each plot show the major current direction (i.e. the principal axis along which the currents were resolved). Location of each site is shown in Fig. 5.7.

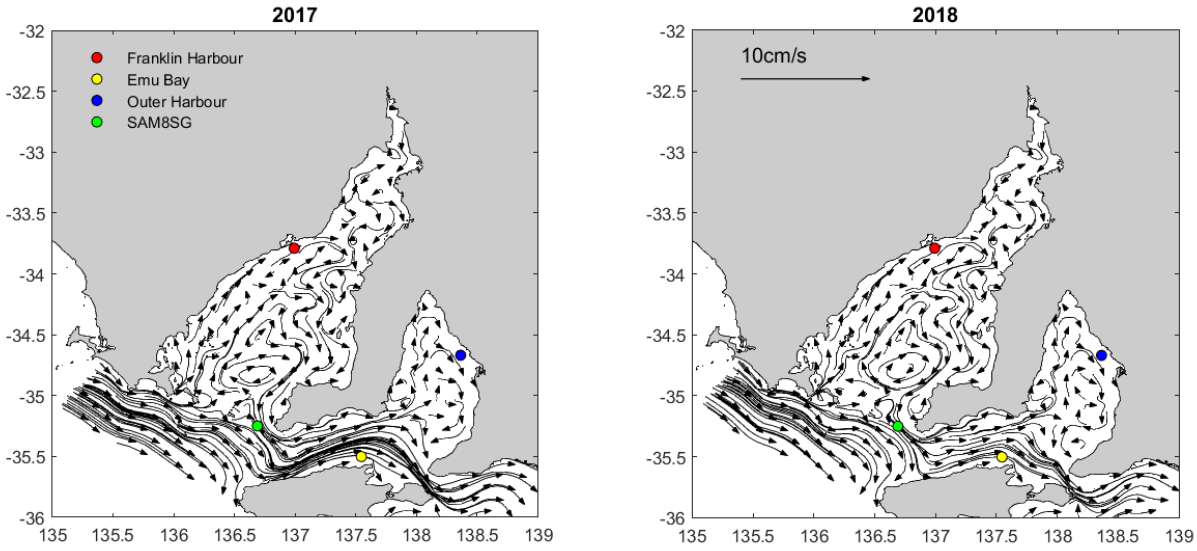

Fig. 5.7. Mean depth-averaged residual circulation throughout South Australia's gulf systems predicted by the hydrodynamic model. Vectors show the direction and velocity of residual currents. Coloured circles identify the locations of the four sites presented in Fig 5.6.

5.3.2 SPATIAL DISTRIBUTION AND DENSITY OF EGGS

In total, 1,485 King George whiting eggs were collected throughout the study (*n* = 541 in 2017 and *n* = 944 in 2018). There were considerable differences in the spatial distribution and density of eggs between years that influenced where particles were released in the larval transport model. In 2017, 349 eggs were collected at 43 stations in southern Spencer Gulf, and 192 eggs were collected at 37 stations in Investigator Strait. Egg densities were considerably higher in southern Spencer Gulf, with 18.0% of stations having > 5.0 eggs m^{-2} compared to 9.2% of stations in Investigator Strait (Fig. 5.8A). The highest egg densities were at spawning areas 2 and 4 in the centre of southern Spencer Gulf, which accounted for 23.6% and 15.9% of particles released, respectively (Fig. 5.8B). The next highest egg densities were at spawning areas 8 and 10 along the northern coast of Kangaroo Island, which contributed 13.6% and 11.7% of particles released, respectively. In 2017, almost double the number of particles were released from stations in southern Spencer Gulf (*n* = 17,167) compared to Investigator Strait (*n* = 9,430) (Table 5.2).

There was a considerable shift in the distribution of eggs in 2018, with 630 eggs collected at 49 stations in Investigator Strait compared with 314 eggs at 46 stations in southern Spencer Gulf. Egg densities were highest in the middle of Investigator Strait, with 6 stations in this area having > 10.0 eggs m^{-2} which accounted for 35.9% of the total particles released (Fig. 5.8A). In comparison, only 2 stations in southern Spencer Gulf had > 5.0 eggs m^{-2}. The highest densities of eggs were in spawning areas 7, 8, 9 and 10 in Investigator Strait, which were responsible for 61.7% of all particles released (Fig. 5.8B). As such, in 2018 there were considerably more particles released from stations in Investigator Strait (*n* = 14,910) than southern Spencer Gulf (*n* = 7,671) (Table 5.2).

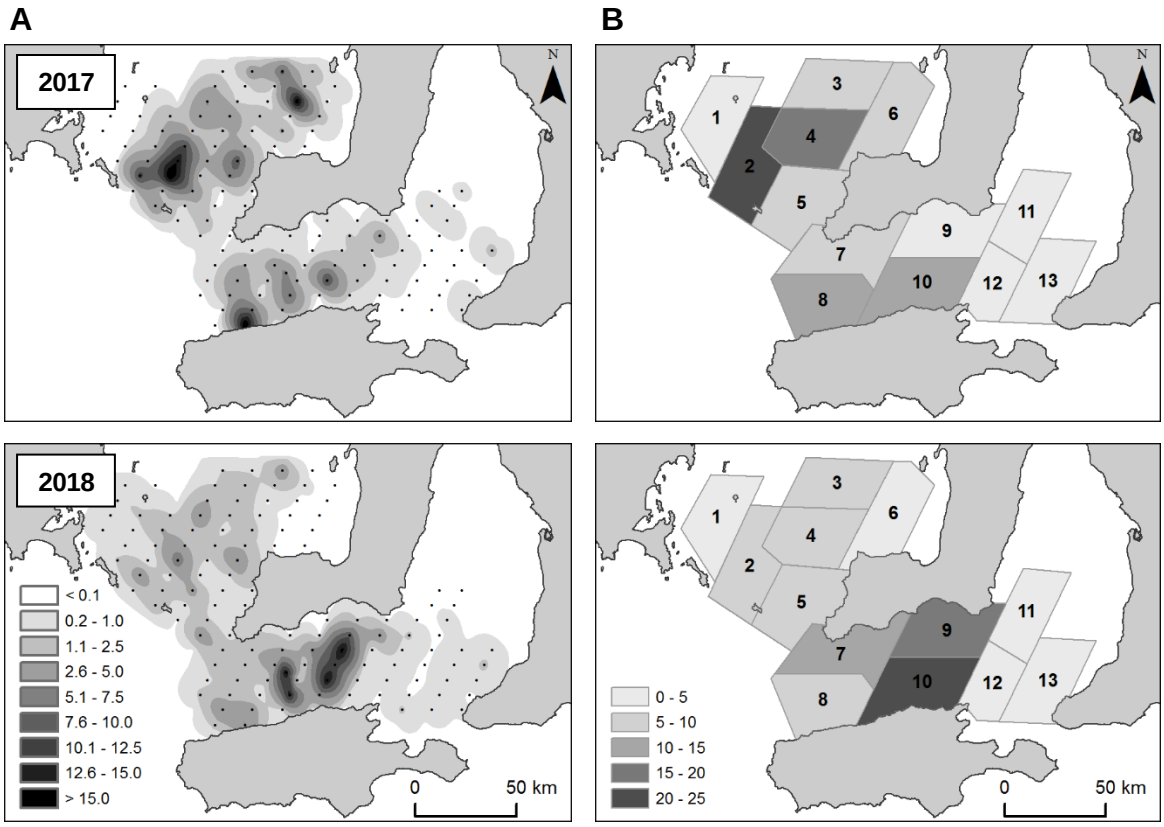

Fig. 5.8. (A) Spatial distribution and density (per m^{-2}) of King George whiting eggs collected from southern Spencer Gulf and Investigator Strait, South Australia, in 2017 (top) and 2018 (bottom). (B) Percent contribution of each spawning area (1-13) to the total number of particles released in the larval transport model. The number of particles released at each station was the observed egg density multiplied by 100.

Table 5.2. Total number of particles released and overall settlement success (%) for each spawning area (1-13) predicted by the biophysical model in 2017 and 2018. Settlement success values are the percentage of particles that settled from each spawning area. Descriptions of each scenario are in Methods 5.2.5. The number of particles released at each station was the calculated egg density multiplied by 100. Stations - # plankton stations in each area.

		2017				**2018**			
Region (area)	Stations	# seeded	Scen. 1	Scen. 2	Scen 3	# seeded	Scen. 1	Scen. 2	Scen 3
Spencer Gulf	**61**	**17167**	**0.7**	**1.8**	**1.4**	**7671**	**0.8**	**1.2**	**1.1**
1	9	0				622	2.6	2.3	1.6
2	10	6265	0.9	1.3	1.2	2134	1.0	1.0	1.4
3	9	2356	0.4	2.8	2.4	1491	0.3	1.5	1.6
4	11	4237	0.9	1.7	1.2	2175	0.5	1.3	0.7
5	11	1692	0.6	1.3	0.9	1226	0.7	0.3	0.3
6	11	2617	0.5	2.3	2.0	23	0.0	4.4	0.0
Investigator Strait	**65**	**9430**	**0.5**	**0.3**	**0.2**	**14910**	**0.7**	**1.4**	**1.3**
7	9	1410	1.1	0.4	0.4	3034	1.5	1.5	0.9
8	10	3619	0.3	0.1	0.1	2194	0.1	0.7	1.3
9	9	751	1.6	0.9	0.5	3662	1.1	3.0	2.9
10	10	3118	0.0	0.0	0.0	5053	0.1	0.5	0.5
11	9	139	5.8	7.9	5.0	157	3.2	2.6	1.9
12	8	158	0.0	0.0	0.0	277	0.7	0.0	0.0
13	10	235	0.0	0.0	0.0	533	0.2	0.0	0.0
Total	**126**	**26597**	**0.7**	**1.2**	**1.0**	**22581**	**0.7**	**1.3**	**1.2**

5.3.3 DISPERSAL PATTERNS

5.3.3.1 Out of bounds

For scenario 1 (passive), the model indicated that in 2017 and 2018, 87-89%, respectively, of particles were transported outside of the open ocean boundaries (Fig. 5.9). In each year, 58-63% of particles were entrained in an eastward current along the north coast of Kangaroo Island that resulted in a high-density outflow through Backstairs Passage and across the eastern boundary. The other 24-31% of particles that exited the model were entrained in a southerly current that flowed out the eastern side of southern Spencer Gulf and across the boundary to the west of Kangaroo Island. Most 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 (Table S5.2). Outputs for scenarios 2 and 3 were very similar. The inclusion of larval behaviour resulted in a decrease in particles exiting the model boundaries from 87% to 72% in 2017, and from 89% to 78% in 2018. This reduction directly related to less particles crossing the western boundary out of Spencer Gulf (from 24-31% to 12-22%).

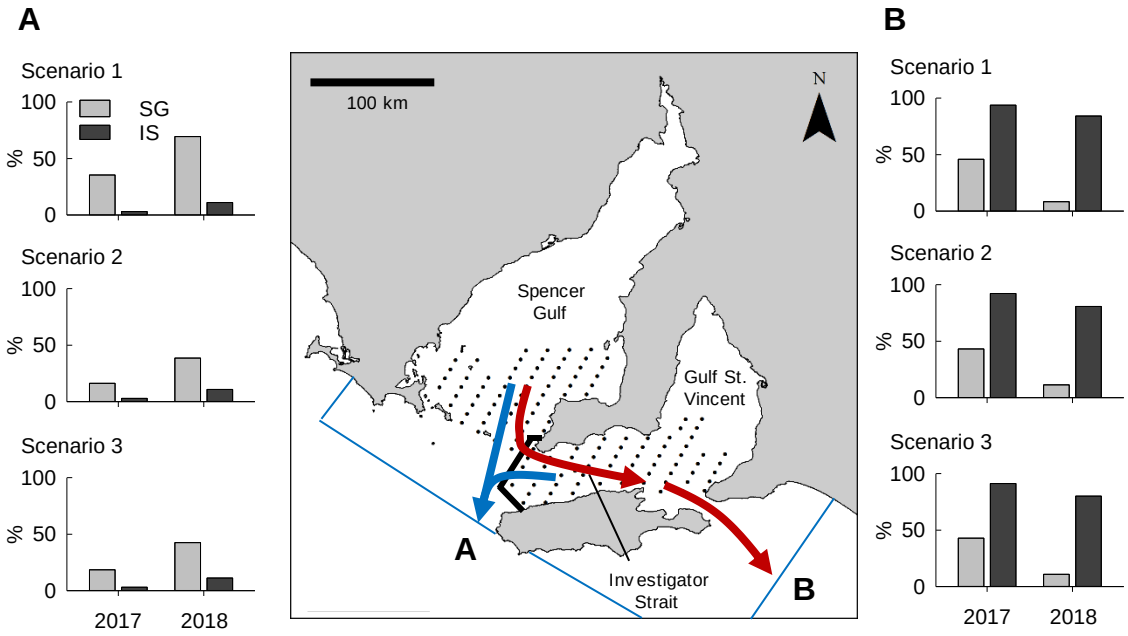

Fig. 5.9. Percentage of total particles released that intersected the open ocean boundaries (blue line) of the larval transport model in 2017 and 2018, which were considered out of bounds and excluded from simulations. The bold black line separates the two spawning regions: SG – southern Spencer Gulf (light); IS – Investigator Strait (dark). (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. Each scenario is described in Methods 5.2.5. The percentages of particles that moved out of bounds from spawning areas 1-13 are presented in Table S5.2.

5.3.3.2 Settlement success

As a consequence of the high percentage of particles that crossed the open ocean boundaries of the model, overall settlement success was very low. Irrespective of the year or model scenario, only 0.7-1.3% of particles settled (Table 5.2). The spawning areas that contributed to settlement differed between years. In 2017, settlement success for scenario 1 was 0.7%, with 53% of the particles that settled originating from spawning areas 2 (32%) and 4 (21%) in the middle of southern Spencer Gulf (Fig. 5.10). The inclusion of larval behaviour improved settlement success to 1.2% for scenario 2, and 1.0% for scenario 3. For both scenarios, the spawning areas that contributed to settlement were very similar, with 85% of settled particles originating from areas 2, 3, 4 and 6 in southern Spencer Gulf. In 2018, settlement success for scenario 1 was the same as in 2017 at 0.7%, but the majority of settling particles originated from spawning areas 7 (28%) and 9 (25%) in Investigator Strait (Fig. 5.10). Settlement success increased to 1.3% for scenario 2, and to 1.2% for scenario 3. Spawning area 9 contributed 37% and 40% of settled particles for scenarios 2 and 3, respectively.

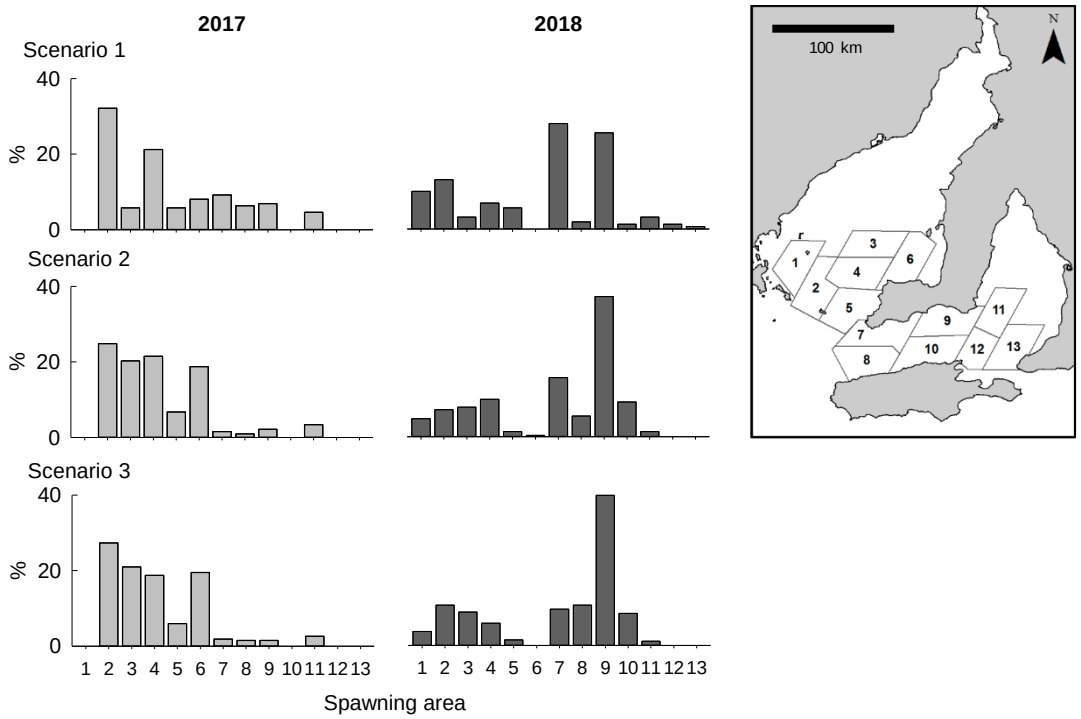

Fig. 5.10. Percent contribution of each spawning area (1-13) to overall settlement in 2017 (light) and 2018 (dark). Scenario 1 - passive; scenario 2 - average growth rate; scenario 3 - daily growth rate. Descriptions of each scenario are in Methods 5.2.5. The map shows the geographic location of each spawning area throughout the study region.

The regions where particles settled differed between model scenarios. For scenario 1, spatial settlement patterns were similar between years. Predicted settlement was highest to Gulf St. Vincent, which accounted for 58% and 65% of settled particles in 2017 and 2018, respectively (Fig. 5.11). Within Gulf St. Vincent, settlement was highest to Stansbury (21%), Pine Point (12%) and Coobowie Bay (10%) (Fig. S5.2). There was a distinct change in the spatial settlement pattern when larval behaviour was incorporated into the model. In 2017, settlement to south-east Spencer Gulf increased from 6% for scenario 1, to 51% for scenario 2 and 46% for scenario 3. Within this region, 37-40% of predicted settlement was to Hardwicke Bay (Fig. S5.2). Similarly, settlement to Kangaroo Island doubled from 10% for scenario 1 to 19-26% for scenarios 2 and 3, whilst settlement to Gulf St. Vincent decreased from 58% to 23%. In 2018, scenarios 2 and 3 predicted settlement to Gulf St. Vincent (52-58%), Kangaroo Island (22-26%), and south-east Spencer Gulf (17-20%) (Fig. 5.11). Within Gulf St. Vincent, 32-36% of particles settled to Coobowie Bay and 12-15% to Barker Inlet.

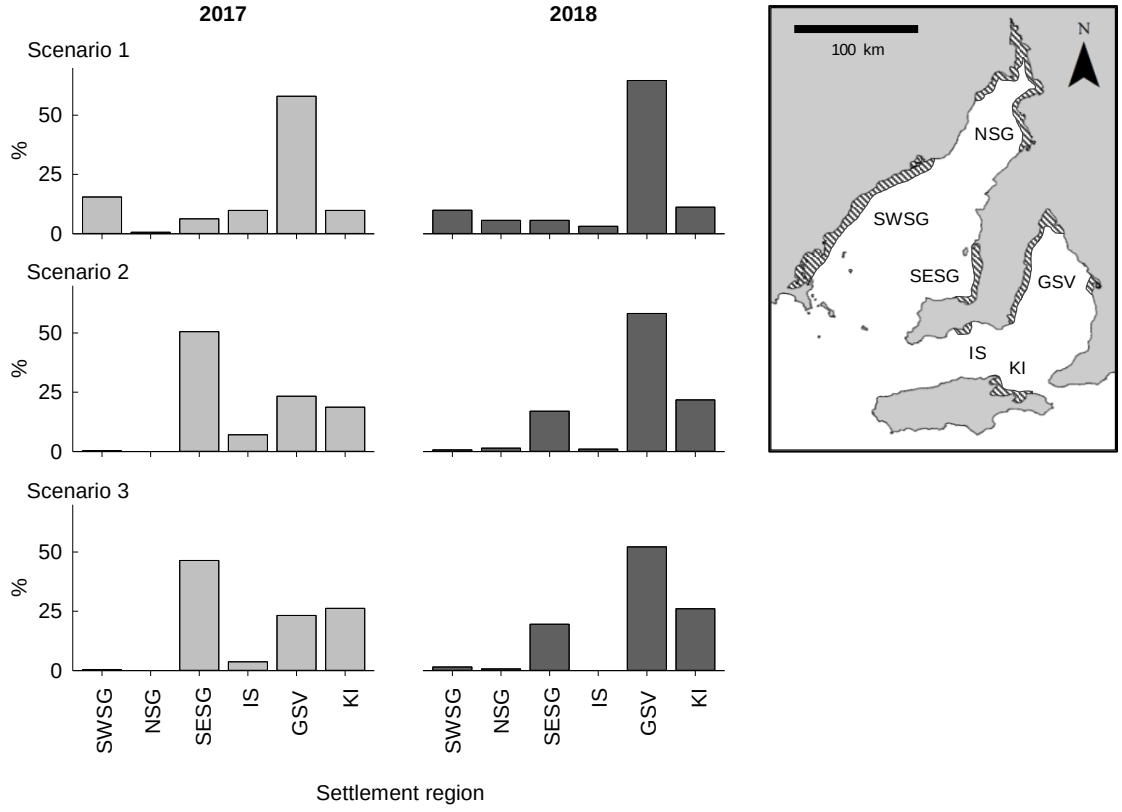

Fig. 5.11. Percent contribution of each settlement region to overall settlement success in 2017 (light) and 2018 (dark). Scenario 1 - passive; scenario 2 - average growth rate; scenario 3 - daily growth rate. Descriptions of each scenario are in Methods 5.2.5. Map shows the geographic location of the settlement regions (dashed). 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. Percent contribution of each settlement area (1-25) to overall settlement success is in the Supplementary Material (Fig. S5.1).

5.3.3.3 Connectivity between spawning and settlement

The most important settlement areas differed between model scenarios, but were similar between years. However, there were inter-annual differences in the spawning areas that seeded the key settlement areas. For scenario 1 (passive), settlement was highest to nursery areas within Gulf St. Vincent. In 2017, the majority of particles originated from spawning area 2 in southern Spencer Gulf. These particles were entrained in a current along the southern coast of Yorke Peninsula and travelled distances of 150-200 km (Fig. 5.12A). In 2018, the settling particles originated from spawning areas 7 and 9 in Investigator Strait and travelled much shorter distances of 50 km up to 150 km (Fig. 5.12B).

For scenario 2, 85% of the particles that settled in 2017 originated from spawning areas 2, 3, 4 and 6 in southern Spencer Gulf. Settlement was highest to Hardwicke Bay (37%) in south-east Spencer Gulf, which involved dispersal distances of 20 to 90 km (Fig. 5.12A; Table S5.3). Settlement was next highest to Coobowie Bay (16%) in Gulf St. Vincent, and American River (14%) on Kangaroo Island. Particles that settled to each area were entrained in an eastward current that flowed through Investigator Strait and travelled distances of 150 to 200 km. In 2018, settlement was highest to the same three nursery areas, but the source of particles differed. The particles that settled to Hardwicke Bay (13%) originated from spawning areas 1-4 in southern Spencer Gulf. Predicted settlement was highest to Coobowie Bay (36%), the particles that settled here were from spawning areas 7 and 9 in Investigator Strait that travelled 40-60 km (Fig. 5.12B). 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). For scenario 3, the most important spawning and settlement areas were the same as scenario 2, and the relationships between them were very similar (Fig. 5.12; Table S5.3).

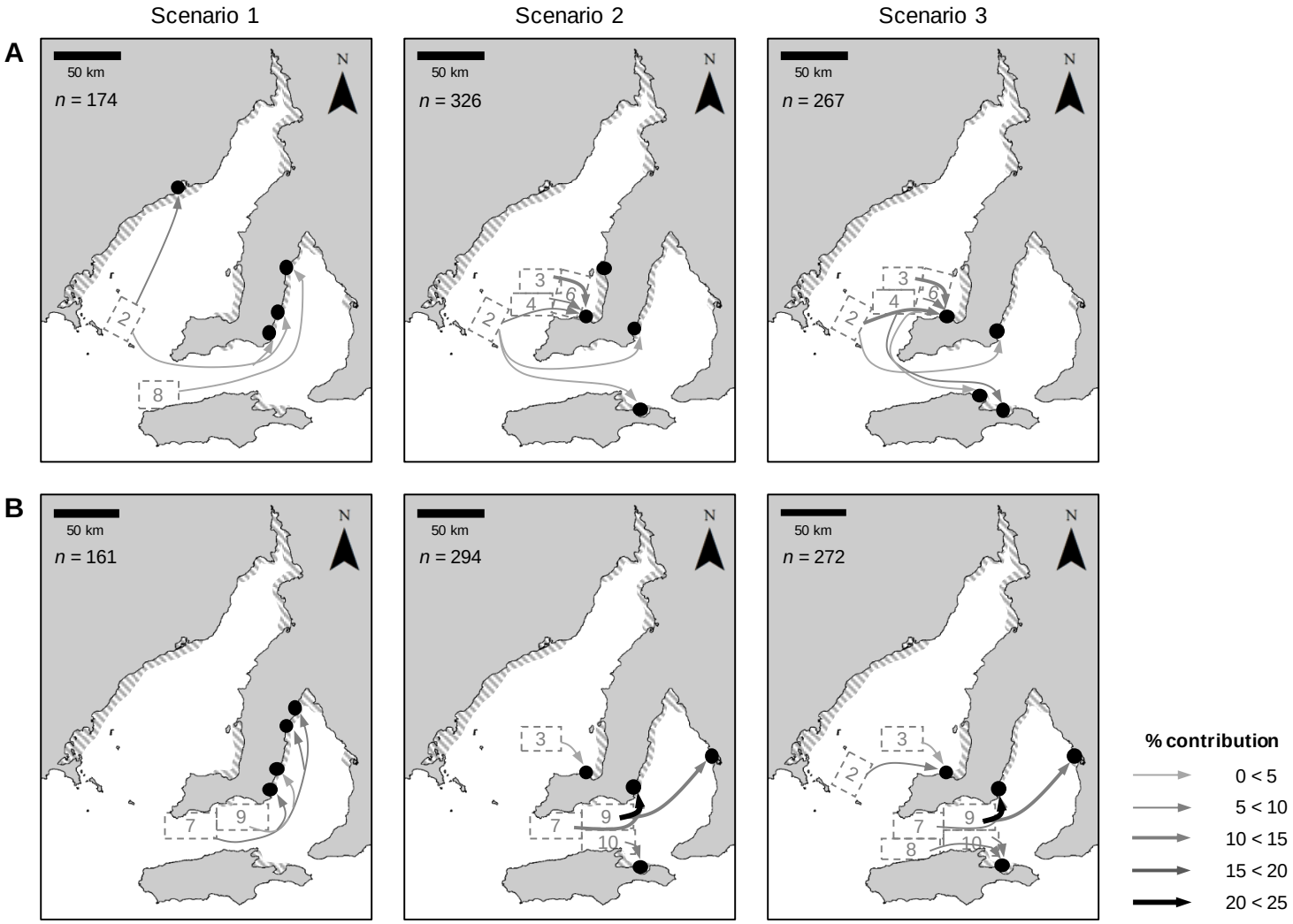

Fig. 5.12. Relationships between spawning areas (1-13) and key settlement areas for each dispersal scenario in (A) 2017 and (B) 2018. Only settlement areas that accounted for ≥ 10% of overall settlement were shown (black circles). The percent contribution of each spawning area to settlement is shown by the colour and width of the connecting arrow. *n* = total number of settled particles. Data are in Supplementary Table S5.3.

5.4 DISCUSSION

This study investigated the larval dispersal and population connectivity of King George whiting in South Australia's gulf system. It aimed to identify the spawning grounds and nursery areas that contribute most to settlement, and to determine the extent of connectivity between them. Outputs of the hydrodynamic model were in strong agreement with meteorological observations, which indicated that the Two Gulfs Model closely replicated the physical oceanography of South Australia's gulf system. There were inter-annual differences in the spatial distributions and densities of eggs that influenced where particles were released in the biophysical model. Despite this, the general patterns of larval dispersal, and the nursery areas that accounted for the highest proportions of settlement, remained similar between years. The demographic relationships between spawning grounds and nursery areas determined by the biophysical model have implications for the interpretation of stock structure, and the spatial and temporal scales at which fishery management should be applied.

5.4.1 KEY SPAWNING GROUNDS AND NURSERY AREAS

In both years, King George whiting eggs were broadly distributed throughout the recognised spawning area in southern Spencer Gulf and Investigator Strait. However, the areas of highest egg production, or spawning 'hot spots', differed between years. In 2017, there were two hot spots of egg production in southern Spencer Gulf, whilst in 2018, the spatial distribution was dominated by a mass of eggs in Investigator Strait. The plankton survey was done once in each year at the time of peak adult reproductive activity and provided a snapshot of spawning activity at that time (late April; Fowler et al. 1999). However, because King George whiting is a multiple batch spawner with a protracted spawning period (Fowler et al. 1999, Fowler et al. 2000a), it is difficult to determine whether the spatial differences in spawning 'hot spots' reflect within-season variation in spawning activity at a fine temporal scale, or broader differences in egg production between years. Therefore, an improved understanding of temporal variation in spawning activity is required to confidently define the most important spawning areas.

In contrast, the most important nursery areas remained consistent despite inter-annual differences in the spatial origins of larvae. In general, predicted settlement was highest to nursery areas in Gulf St. Vincent irrespective of the model scenario, highlighting the importance of this region to recruitment. These model predictions are consistent with field observations that, in general, higher numbers of larvae settle to nursery areas in Gulf St. Vincent than elsewhere (Fowler et al. 2000b; T.A. Rogers, unpub.). Within Gulf St. Vincent, settlement was highest to Coobowie Bay and Barker Inlet, both of which have previously been identified as significant nursery areas (Fowler & Jones 2008, Rogers et al. 2019b;

Chapter 3). When larval behaviour was incorporated into the model, settlement regions became more refined with settlement limited to nursery areas in south-east Spencer Gulf, Gulf St. Vincent, and Kangaroo Island. Larval dispersal pathways suggested that the nursery areas in Gulf St. Vincent and Kangaroo Island were replenished by larvae that had originated from both southern Spencer Gulf and Investigator Strait. In such case, the nursery areas in these regions may be more resilient to spatio-temporal variation in egg production, and therefore, more likely to consistently contribute to recruitment.

5.4.2 CONNECTIVITY BETWEEN SPAWNING GROUNDS AND NURSERY AREAS

Larvae that originated in Investigator Strait followed two different dispersal pathways. Most larvae (84-94%) were entrained in an eastward current along the north coast of Kangaroo Island that flowed through Backstairs Passage and across the model boundary. The fate of these larvae, and whether they eventually settle, is unknown. A small proportion of larvae that were seeded in the northern half of Investigator Strait were entrained in an eastward coastal current that followed the southern coast of Yorke Peninsula, which entered and were then dispersed throughout Gulf St. Vincent. These larvae settled 40 to 100 km from where they were spawned. This supports the hypothesis that spawning grounds and nursery areas are only relatively short distances apart (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3).

The dispersal patterns of larvae that were seeded in southern Spencer Gulf were more diverse and operated over multiple spatial scales. The primary settlement region for larvae that originated from here was south-east Spencer Gulf, which involved dispersal over relatively short distances from 20 to 90 km. These larvae were transported by the prevailing westerly winds to the south-west coast of Yorke Peninsula, where they were retained until settlement. This suggests that recruitment to this region predominantly depended on localised population processes (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3). The model also predicted that spawning in southern Spencer Gulf contributed to settlement in Gulf St. Vincent and the bays of Kangaroo Island. These larvae were firstly entrained in a current that flowed southward out the eastern side of Spencer Gulf and into Investigator Strait. Then, some of the larvae followed the eastward current along the south coast of Yorke Peninsula and were dispersed into Gulf St. Vincent, whilst others were transported along the north coast of Kangaroo Island and settled to nursery areas in the east. Both dispersal pathways involved larval transport over 150 to 200 km. These are consistent with previous projections of dispersal for larvae seeded in southern Spencer Gulf (Fowler et al. 2000b).

The model prediction of two different source populations contributing to recruitment into Gulf St. Vincent could explain the temporal settlement pattern of larvae to Barker Inlet. Here, settlement is

characterised by a bi-modal pattern where larvae from each phase of settlement have different early life-history characteristics and otolith chemistry (Fowler & Short 1996, Rogers et al. 2019a; Chapter 2). The larvae that settle during the second phase of settlement, which contributes the most to recruitment, hatch in May and June (Rogers et al. 2019a; Chapter 2). This corresponds to spawning after the thermohaline fronts at the entrances to Spencer Gulf and Investigator Strait have dissipated, allowing larval transport between the two gulfs (Bruce & Short 1990, Petrusevics 1993, Petrusevics et al. 2011). Because of the geographic distances between the two spawning populations and Barker Inlet, it is most likely that recruits in the first phase of settlement originate from spawning in Investigator Strait, whilst those in the second phase originate from southern Spencer Gulf.

5.4.3 INFLUENCE OF LARVAL BEHAVIOUR ON DISPERSAL

It is widely accepted that larvae of marine fish species develop behavioural abilities during ontogeny that influence their dispersal (Fisher 2005, Leis 2007, 2010). The incorporation of vertical swimming behaviour (DVM and RDVM) in Scenarios 2 and 3 increased larval settlement by 43-86% compared to the passive behaviour modelled in Scenario 1. There was a definitive shift in the patterns of larval dispersal when vertical movement was included, with larvae settling to nursery areas that were much shorter distances from where they were spawned. This was particularly apparent for larvae that were seeded in southern Spencer Gulf in 2017. Scenarios 2 and 3 predicted that larvae would remain near the coast and settle to Hardwicke Bay in south-east Spencer Gulf, which is only 20 to 90 km from where the larvae originated. In comparison, the passive model predicted that larvae would disperse over 100's of km and that there would be negligible settlement in this area. Such local retainment of larvae is a common prediction when behaviour is incorporated into dispersal models (North et al. 2008, Wolanski & Kingsford 2014, McLeay et al. 2016).

There were several limitations to the behavioural and biological models developed in this study that are likely to affect predictions of dispersal. The effective swim speed was estimated from recently-settled larvae because there are no data available for *in situ* swimming abilities of larval King George whiting. However, larval swimming abilities can change significantly following metamorphosis associated with settlement (Fisher 2005). Larval King George whiting are markedly poor swimmers compared to larvae of other demersal fish species (Jenkins et al. 1998b, Jenkins & Welsford 2002), and therefore the vertical swimming behaviour incorporated into the model was considered appropriate based on the limited information available (G.P. Jenkins & J.M. Leis, pers. comm.). The model did not include natural mortality from predation, or incorporate any larval development relationships with environmental parameters, such as temperature and salinity. Spatial and temporal variation in environmental characteristics affect larval growth rates and survivorship, which could significantly impact predictions of dispersal and the relationships between spawning grounds and nursery areas (O'Connor et al. 2007,

Rankin & Sponaugle 2014). Therefore, the development of a temperature-dependent growth relationship and an improved understanding of larval swimming abilities could provide more realistic predictions of larval dispersal.

5.4.4 POPULATION CONNECTIVITY AND STOCK STRUCTURE

In South Australia, the understanding of stock structure for King George whiting was that spawning grounds and nursery areas were relatively short distances apart, and that the State-wide biological stock constituted a meta-population composed of multiple small sub-populations (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3). The dispersal patterns simulated by the biophysical model suggest that the demographic relationships between spawning grounds and nursery areas are more complex than previously considered, and that there is a change in population connectivity within the spawning season. Consistent with previous studies, the model predicted that larvae spawned in Investigator Strait disperse eastward and settle to nursery areas in Gulf St. Vincent and on Kangaroo Island. Similarly, larvae that settle to nursery areas in Spencer Gulf originate from spawning grounds in the south and disperse over relatively short distances. In both gulfs, the simulated connectivity between spawning grounds and nursery areas is supported by similarities in otolith chemistry between larvae and recruits collected from these regions, and is consistent with the previously hypothesised meta-population stock structure (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3, Rogers et al. 2019c; Chapter 4).

In contrast however, the model also predicted that some larvae spawned in southern Spencer Gulf are entrained in an eastward current that flows through Investigator Strait and ultimately replenishes nursery areas in Gulf St. Vincent and on Kangaroo Island. The transport of larvae between the two gulfs is inhibited until the thermohaline frontal systems near their mouths dissipate in late autumn (Petrusevics 1993, Petrusevics et al. 2011). This is consistent with the temporal settlement pattern and change in otolith chemistry of larvae that recruit to Barker Inlet (Fowler & Short 1996, Rogers et al. 2019a; Chapter 2). Consequently, the most parsimonious explanation is that there is a within-season change in the connectivity between the spawning grounds in southern Spencer Gulf and Investigator Strait, and the nursery areas in Spencer Gulf, Gulf St. Vincent and on Kangaroo Island. That is, the larvae that settle to nursery areas in Gulf St. Vincent and Kangaroo Island early in the season (March to May) originate from spawning in Investigator Strait, whilst those that settled later in the season (May to June) are predominantly spawned in southern Spencer Gulf. The mixing of larvae from the two different spawning grounds would maintain genetic homogeneity between the two gulf populations (Kent et al. 2018). This strongly suggests that the populations in Spencer Gulf and Gulf St. Vincent represent a single, inter-mixed stock.

Over 60% of the larvae that were seeded in the biophysical model crossed the open ocean boundary to the east of Kangaroo Island. These larvae were entrained in an eastward current that flowed through Investigator Strait along the north coast of Kangaroo Island, and resulted in a high-density outflow through Backstairs Passage. Although this study was unable to determine the spatial scale of dispersal for these larvae, a previous study predicted that they would be transported up to 400 km east of Investigator Strait along the south-east coast of South Australia (Fowler et al. 2000b). There is a long-held hypothesis 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 (Jenkins et al. 2000, Jenkins 2005, Jenkins et al. 2016). The spawning ground is predicted to be located along the western coast of Victoria or the south-east coast of South Australia (Jenkins et al. 2000). Because of the geographic similarities in the projected locations of larval transport from South Australian spawning grounds, and the predicted spawning grounds that replenish Victorian nursery areas, it is possible that there is a population of King George whiting along the south-east coast of South Australia that connects the two State-based populations. That is, the south-east population is replenished by larvae that originate from the recognised spawning grounds in South Australia, and the adults of this population produce the larvae that settle to Victorian nursery areas (Jenkins et al. 2016). Because the relationships between the South Australian and Victoria populations remain unresolved, there is a need to empirically test this hypothesis to determine the demographic processes that maintain the populations of King George whiting throughout south-eastern Australia.

5.4.5 IMPLICATIONS AND FUTURE DIRECTIONS

This study corresponded to the period of peak adult reproductive activity in late April (Fowler et al. 1999). However, the period of spawning that contributes most to recruitment is from mid-May to mid-June (Rogers et al. 2019a; Chapter 2). Consequently, there is a need to understand within-season variation in the spatial distribution of spawning activity. This could be achieved by repeating the plankton survey in late May to determine whether the key spawning grounds change throughout the spawning season. The larvae from such a survey would provide a means to empirically validate the predicted patterns of larval dispersal and enhance the credibility of the biophysical model.

The spatial patterns of larval dispersal predicted by the model are consistent with previous studies of otolith chemistry of larvae (Rogers et al. 2019a; Chapter 2, Rogers et al. 2019b; Chapter 3, Rogers et al. 2019c; Chapter 4), and indicate that the populations of King George whiting in Spencer Gulf and Gulf St. Vincent represent a single, panmictic stock. The demographic relationships between particular spawning grounds and nursery areas changed throughout the long spawning season following the breakdown of environmental barriers to larval transport. Therefore, the spawning grounds that are most important to recruitment change throughout the protracted spawning season. In particular, the model

predicted that spawning in southern Spencer Gulf contributes to recruitment in Spencer Gulf, Gulf St. Vincent and on Kangaroo Island, highlighting the importance of this spawning ground to the populations of King George whiting throughout South Australia's gulf system. As such, this updated understanding of population connectivity and stock structure should be considered in the development of future fishery management strategies.

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Chapter 6 – General Discussion

6.1 OVERVIEW

Population connectivity is a central topic in fisheries science. This is because the spatial and temporal aspects of connectivity inform population dynamics, identify the spatial scale over which the early life-history operates, and are fundamental to understanding the stock structure of fish populations (Secor & Rooker 2005, Cowen & Sponaugle 2009, Jones et al. 2009). As such, this biological information underpins effective fishery management.

In response to declining fishery statistics and estimated biomass, this study investigated the population connectivity and stock structure for King George whiting (*Sillaginodes punctatus*) in South Australia's gulfs. More specifically, the study explored the demographic relationships between different spawning grounds and nursery areas in order to differentiate between hypotheses relating to stock structure. These hypotheses were: (H1) the larvae that recruit to South Australian nursery areas originate from a single spawning population, and therefore the State-wide population constitutes a single, panmictic stock (Jenkins et al. 2016, Kent et al. 2018); or (H2) the larvae that recruit to nursery areas in the different regions of South Australia originate from different spawning populations, and therefore the stock comprises multiple, self-recruiting populations (Fowler et al. 2000b). In this chapter, I discuss the key findings from the empirical studies described in Chapters 2 to 5, and develop an updated understanding of population connectivity and stock structure based on the new biological information presented. Then, I consider the potential implications for fishery management and propose directions for future research.

6.2 SUMMARY OF KEY FINDINGS

6.2.1 TEMPORAL VARIATION OF EARLY LIFE-HISTORY CHARACTERISTICS

The first step in investigating the early life-history of King George whiting was to understand the temporal nature of recruitment throughout the protracted settlement season (Chapter 2). This was addressed for Barker Inlet, a known significant nursery area in Gulf St. Vincent (Fowler & Jones 2008). A previous study at Barker Inlet in 1993 described a bi-modal settlement pattern where larvae in the two phases differed in size, larval duration and growth rate (Fowler & Short 1996). In contrast to 1993, the temporal settlement patterns in 2016 and 2017 were unimodal each with a distinct peak in late September and early October that accounted for >50% of recruitment. Hatch dates were from March to July, whilst a three-week period from mid-May accounted for the majority of recruitment. The unimodal settlement pattern and corresponding spawning period described here are consistent with other nursery

areas in South Australia (Fowler et al. 2000b, Fowler & Jones 2008, Rogers et al. 2019b; Chapter 3) and Victoria (Jenkins & May 1994, Jenkins et al. 1997).

The sizes, ages and growth rates of larvae systematically decreased throughout the two settlement seasons, which most probably related to the decreasing water temperatures throughout the austral winter (Houde 1989, Fowler & Short 1996, Meekan et al. 2003). It seems counter-intuitive that slower-growing larvae that experienced longer pre-settlement durations contributed most to recruitment, because it is considered that prolongation of the 'critical period' is likely to decrease larval survivorship (May 1974, Houde & Hoyt 1987, Leggett & Deblois 1994, Shima & Findlay 2002, Houde 2008). Nevertheless, there are several plausible explanations. One possibility is that the temporal change in the settlement rate of larvae may reflect a similar temporal shift in egg production, i.e. reproductive output is higher later in the spawning season (late May and early June). This, however, is not consistent with gonadosomatic indices of adult fish which peak in late April, indicating that spawning activity decreases significantly by the start of June (Fowler et al. 1999, Fowler et al. 2000a). Another possibility is that the increase in recruitment corresponds to a temporal shift in plankton composition that improved larval survivorship (Cushing 1990, Black et al. 2016, Jenkins & Black 2019). Water temperature throughout the study region decreases by approximately 5°C during the protracted spawning season (Middleton & Bye 2007, Bye & Kampf 2008), which may influence plankton dynamics and affect the availability of suitable larval food. Alternatively, the within-season settlement pattern may relate to a change in oceanographic circulation patterns that became more favourable for settlement to Barker Inlet later in the settlement season.

Despite there being a single mode of recruits, there were significant differences in the otolith chemistry of larvae that settled at different times throughout the settlement season. The differences were apparent between those larvae that settled in July and August, which were spawned in March and April, and those that settled in September and October, having been spawned in May and June. There are two primary hypotheses to account for this within-season change in otolith chemistry. The first is that the larvae may have originated from a single spawning population, in which case, the differences in otolith chemistry reflect a within-season change in the physico-chemical conditions experienced by larvae that hatched at different times (Gillanders 2002b, Cook 2011, Reis-Santos et al. 2012) (Fig. 6.1A). Previous research suggests that larvae which settle to Barker Inlet originate from the recognised spawning population in Investigator Strait (Fowler et al. 2000b). The temporal change in otolith chemistry coincides with the breakdown of a seasonal thermohaline frontal system in Investigator Strait (Bye & Kampf 2008), which results in a significant change in physical environmental conditions that could be manifested in the otolith chemistry of larvae. Alternatively, the second possibility is that the differences in otolith chemistry represent two different spawning populations that contribute to recruitment at

different stages of the settlement season (Fig. 6.1B). In this case, the spawning source responsible for settlement later in the season provides the largest contribution to annual recruitment.

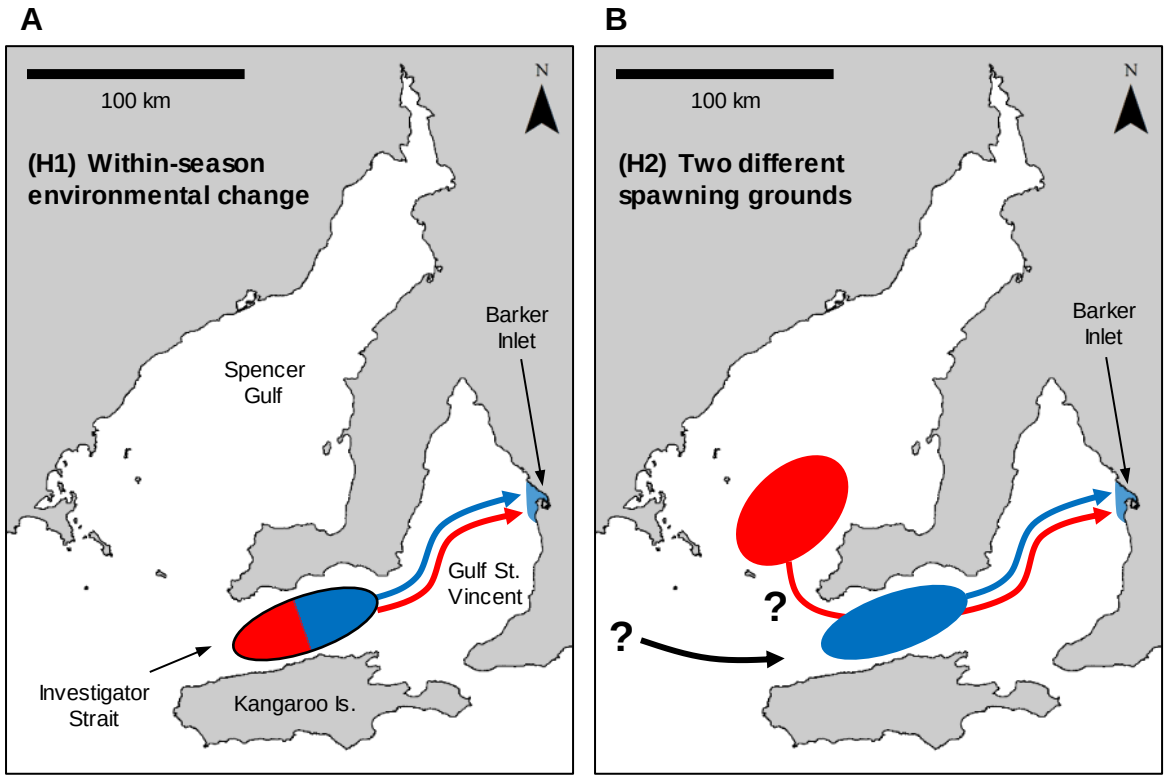

Fig. 6.1. Conceptual models to explain the within-season change in the otolith chemistry of larval King George whiting that settled to Barker Inlet, Gulf St. Vincent, South Australia. (A) A single spawning source and within-season environmental change; or (B) two different spawning populations.

6.2.2 SPATIAL SCALE OF EARLY LIFE-HISTORY

If two different spawning populations do contribute to recruitment in Barker Inlet, then it is likely that larvae from at least one spawning population are transported a considerable distance from the spawning ground, possibly from a population that has not yet been adequately defined. As such, to differentiate between the two hypotheses postulated in Chapter 2 (Fig. 6.1), there was a need to determine the spatial scale of early life-history for larvae that settle to nursery areas in South Australia's gulfs (Chapter 3). King George whiting larvae undergo a long pelagic larval duration (80 to 130 d; Jenkins & May 1994, Fowler & Short 1996, Fowler et al. 2000b, Rogers et al. 2019a; Chapter 2) and have limited larval swimming abilities (Jenkins et al. 1999, Jenkins & Welsford 2002, Hindell et al. 2003). This confers on the larvae the potential for dispersal over considerable distances from where they were spawned by physical oceanographic processes (Norcross & Shaw 1984, Jenkins et al. 2000, Cowen & Sponaugle 2009). Nevertheless, previous predictions of larval dispersal in South Australia using a numerical hydrodynamic model indicated that population processes operated over localised spatial scales (50-200

km; Fowler et al. 2000b), even though King George whiting larvae have been hypothesised to disperse up to 600 km in other parts of the distribution (Jenkins et al. 2000).

Spatial differences in the biological characteristics and otolith chemistry of larvae collected from nursery areas around South Australia suggested the presence of stock structure at multiple scales. At a regional scale, differences in otolith chemistry between larvae that settled to nursery areas on the West Coast of Eyre Peninsula, in Spencer Gulf and in Gulf St. Vincent indicated that larvae which settled in each region originated from a different spawning population and had dispersed through different water masses (Rogers et al. 2019b; Chapter 3). As such, the empirical data supported the hypothesis that the South Australian biological stock of King George whiting is comprised of multiple small, self-recruiting populations (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3) (Fig. 6.2). Although the three regional sub-populations are considered discrete from an ecological perspective, there must be a small degree of exchange between them, through either larval dispersal or adult movement, in order to maintain genetic homogeneity at a State-wide scale (Kent et al. 2018). In such case, the within-season change in otolith chemistry identified in Chapter 2 would reflect a temporal shift in environmental conditions at a single spawning ground that was manifested in the otolith chemistry of larvae (i.e. Fig. 6.1A).

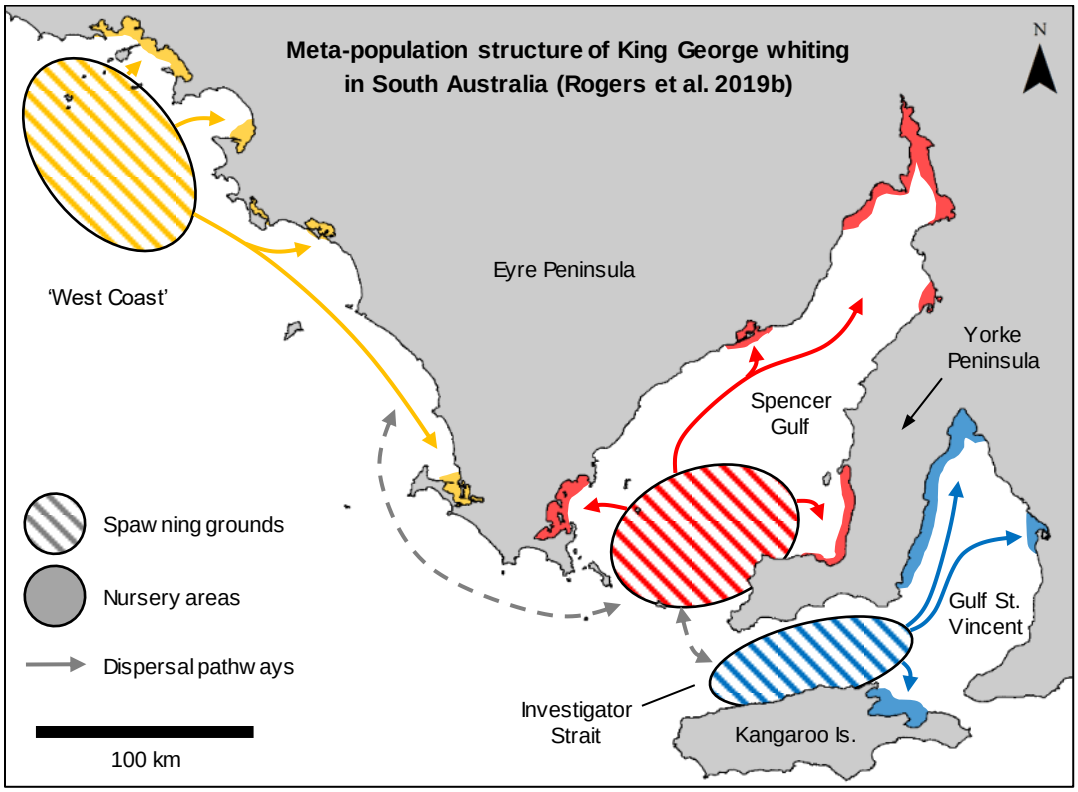

Fig. 6.2. Hypothesised meta-population structure of King George whiting in South Australia from Rogers et al. (2019b; Chapter 3). The three sub-populations are: the West Coast of Eyre Peninsula (yellow); Spencer Gulf (red); and Gulf St. Vincent / Investigator Strait (blue).

6.2.3 IDENTIFYING SOURCE POPULATIONS OF LARVAE

The only recognised spawning area for King George whiting throughout south-eastern Australia is in southern Spencer Gulf and Investigator Strait, which is the water mass that separates the two gulfs (Fowler et al. 1999, Fowler et al. 2000a). The differences in otolith chemistry between the larvae that settled in Spencer Gulf and Gulf St. Vincent indicated that the recognised spawning area may be comprised of multiple discrete spawning populations that replenish nursery areas in the adjacent gulfs (Chapter 3; Rogers et al. 2019b). To assess the potential for different spawning populations within the large spawning area, I investigated the spatial distribution and otolith chemistry of larvae collected from their natal environment (Chapter 4). Otolith chemistry has been successfully applied to discriminate between populations of larvae of various freshwater (Ludsin et al. 2006, Macdonald et al. 2008, Lazartigues et al. 2017), diadromous (Barbee & Swearer 2007), and marine fish species (Warner et al. 2005, Ruttenberg & Warner 2006, Standish et al. 2008, Schaffler et al. 2009, Lazartigues et al. 2016). However, almost all of these studies considered the otoliths of embryonic larvae extracted from substrate-attached egg masses, where embryonic development occurred at a single, fixed location.

The spatial distribution of larvae showed a discontinuity consistent with the locations of the thermohaline frontal systems that form at the entrances to Spencer Gulf and Investigator Strait during the austral summer and autumn (Bruce & Short 1990, Petrusevics 1993, Middleton & Bye 2007, Petrusevics et al. 2011). The frontal systems develop as water temperature and salinity in the gulfs rise relative to the adjacent shelf water, and act as environmental barriers between the gulfs and the waters of the continental shelf, thereby inhibiting plankton transport (Bruce & Short 1990, Fowler 2000). The timing of the frontal systems overlaps with the peak spawning period for King George whiting (Fowler et al. 1999, Fowler et al. 2000a). As such, the eggs and larvae in Spencer Gulf and Investigator Strait would likely remain separated until the fronts dissipate and shelf-gulf exchange resumes.

The discontinuity broadly separated the larvae into two regional groups – those in Southern Spencer Gulf and those in Investigator Strait. The larvae in the two regions did not differ in size, age or hatch date. However, there were significant differences in the otolith chemistry between the two groups. Because larvae in the two regions hatched at the same time, it is most likely that those located in each of southern Spencer Gulf and Investigator Strait had originated from different source populations. The differences in otolith chemistry primarily related to higher concentrations of Ba for larvae from Investigator Strait (Rogers et al. 2019c; Chapter 4). This was consistent with the natal otolith signatures of larvae that settled to nursery areas in Gulf St. Vincent (Rogers et al. 2019b; Chapter 3). The ontogenetic similarities in otolith chemistry suggested that larvae spawned in Investigator Strait dispersed eastward and settled to nursery areas in Gulf St. Vincent, whilst for Spencer Gulf, larvae were spawned in the south and replenished nursery areas in the north. These data provide empirical support

for the hypothesis that spawning grounds and nursery areas are only relatively short distances apart (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3).

6.2.4 CONNECTIVITY BETWEEN SPAWNING GROUNDS AND NURSERY AREAS

Having identified that larvae which settled to nursery areas in Spencer Gulf and Gulf St. Vincent had originated from different spawning grounds (Rogers et al. 2019b; Chapter 3), and that the recognised spawning area is comprised of two different source populations (Rogers et al. 2019c; Chapter 4), Chapter 5 investigated the connectivity between the different spawning grounds and nursery areas using a biophysical model. Despite inter-annual differences in the 'hot spots' of egg production, the patterns of larval dispersal and the nursery areas with the highest settlement remained consistent between years. The model predicted that settlement would be highest in nursery areas that were only short distances from the two spawning grounds. That is, in Spencer Gulf, larvae dispersed eastward and settled in Hardwicke Bay and Port Victoria in the south-east of the gulf, whilst larvae spawned in Investigator Strait were entrained in an eastward current and settled to nursery areas in Gulf St. Vincent and the bays of Kangaroo Island. The predicted relationships between spawning grounds and nursery areas were supported by similarities in otolith chemistry between larvae and recruits (Rogers et al. 2019b; Chapter 3, Rogers et al. 2019c; Chapter 4).

However, the model also predicted that larvae spawned in southern Spencer Gulf were entrained in an eastward current that flowed through Investigator Strait, and would settle to nursery areas in Gulf St. Vincent and the bays of Kangaroo Island. Plankton transport between the two gulfs would be restricted until the thermohaline frontal systems at the entrance of Spencer Gulf and in Investigator Strait breaks down in late autumn (Bruce & Short 1990, Petrusevics 1993, Petrusevics et al. 2011), which is when the biophysical model simulations began. The contributions of two different spawning populations to settlement in Gulf St. Vincent would explain the temporal settlement pattern and otolith chemistry of larvae that recruit to Barker Inlet (Fowler & Short 1996, Rogers et al. 2019a; Chapter 2). In this model, the larvae that settle early in the season would originate from spawning in Investigator Strait, whilst those that settle later would originate from spawning in southern Spencer Gulf. Therefore, the temporal settlement pattern would reflect a change in physical oceanographic processes, and subsequent larval dispersal pathways, which became more favourable for settlement later in the season. The temporal nature of settlement to other nursery areas in Gulf St. Vincent has not been investigated, although high numbers of larvae have been recorded at several nursery areas in October (Fowler et al. 2000b, Rogers et al. 2019c; Chapter 3). Because late season settlement accounts for the majority of recruitment to Barker Inlet (Fowler & Short 1996, Rogers et al. 2019a; Chapter 2), it is plausible that spawning in southern Spencer Gulf is primarily responsible for recruitment in both Spencer Gulf and Gulf St. Vincent.

In summary, the most parsimonious explanation is that the demographic relationships between particular spawning grounds and nursery areas change throughout the protracted spawning season. From March to May, population processes are restricted to each gulf because physical environmental barriers prevent the transport of larvae between southern Spencer Gulf and Investigator Strait (Fig. 6.3A). After the thermohaline fronts breakdown in early May, the physical oceanographic model suggests that larvae from southern Spencer Gulf could be entrained in a current that flows through Investigator Strait, leading to settlement in Gulf St. Vincent and on Kangaroo Island (Fig. 6.3B). The patterns of larval dispersal predicted by the biophysical model are supported by the empirical biological data presented in Chapters 2 to 4 (Rogers et al. 2019a, Rogers et al. 2019b, Rogers et al. 2019c). The mixing of larvae from the two different spawning grounds would maintain genetic homogeneity between populations (Kent et al. 2018), and indicates that the King George whiting in Spencer Gulf, Gulf St. Vincent and Investigator Strait represent a single, panmictic population.

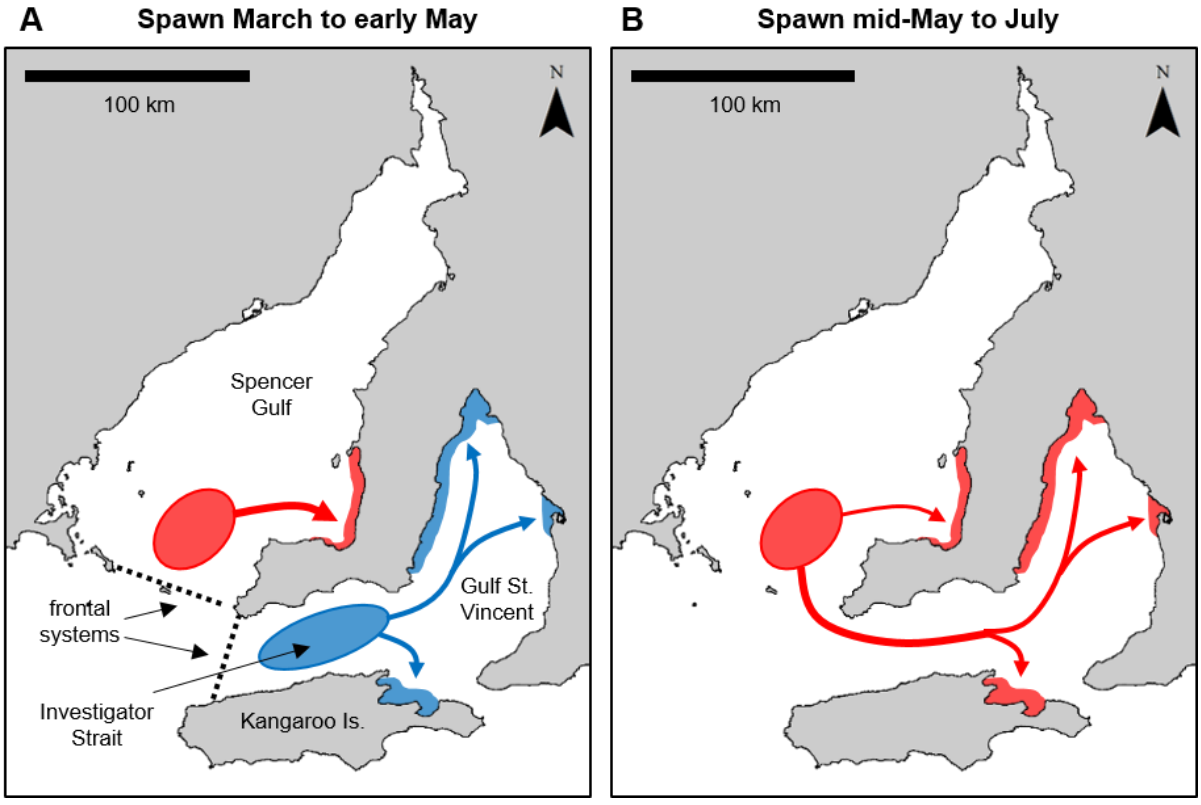

Fig. 6.3. Within-season shift in connectivity between spawning grounds and nursery areas for King George whiting in South Australia's gulfs. (A) From March to early May, thermohaline fronts at the entrances to Spencer Gulf and Investigator Strait inhibit larval transport between the two gulfs, and population processes are localised. (B) After the frontal systems breakdown in May, a density-driven current flows out the eastern side of Spencer Gulf and transports larvae into Investigator Strait. These larvae then contribute to settlement in Gulf St. Vincent and the bays of Kangaroo Island. Red – larvae spawned in southern Spencer Gulf; Blue – larvae spawned in Investigator Strait.

6.3 IMPLICATIONS FOR MANAGEMENT

The results of this study have improved the understanding of the spatial and temporal aspects of early life-history for King George whiting in South Australia. This new biological information has implications for understanding stock structure, i.e. the spatial scale at which fishery management should be applied. Previously, the understanding of stock structure was that the South Australian population constituted a single biological stock that is divided into three regional populations for assessment and management purposes (Jenkins et al. 2016, Steer et al. 2018). The delineation of management units was based on geographic separation and previously described connectivity between spawning grounds and nursery areas, thought to occur at relatively localised spatial scale (Fowler et al. 1999, Fowler et al. 2000b, Fowler et al. 2002). The three regional populations are the West Coast of Eyre Peninsula, Spencer Gulf, and Gulf St. Vincent (Steer et al. 2018). From this study, the level of connectivity based on larval transport suggests that the populations in Spencer Gulf and Gulf St. Vincent are not separated and should be considered a single population. This revised understanding of stock structure should be considered in the development of future fishery management strategies.

In 2017, a spatial closure was introduced in southern Spencer Gulf and Investigator Strait for the month of May to protect spawning aggregations of adult King George whiting (Steer et al. 2018). Based on the temporal nature of recruitment described in Chapter 2, the spatial closure does not align with the period of peak recruitment, i.e. mid-May to mid-June (Rogers et al. 2019a; Chapter 2). Furthermore, it remains to be determined whether the spatial distribution of spawning activity at this time is the same as during the period of peak adult reproductive activity when the plankton surveys were completed (late April; Fowler et al. 1999). Results of this study also suggest that the population connectivity between spawning grounds and nursery areas changes throughout the spawning season, and that larvae which settle to nursery areas in South Australia's gulfs during the peak settlement period likely originate from spawning in southern Spencer Gulf (Chapter 5). Consequently, any revision of the spatial spawning closure should consider these temporal and spatial aspects of early life-history to optimise its effectiveness.

Over the past 40 years, sampling shallow seagrass beds with a small seine net has been a cost-effective and efficient technique to collect recently-settled King George whiting larvae (Bruce 1989, Jones et al. 1990, Jenkins & May 1994, Fowler & Short 1996, Hamer & Jenkins 1997, Fowler et al. 2000b, Jenkins et al. 2016, Rogers et al. 2019a; Chapter 2). Because of the reliability of this method, it may be possible to use larval abundance as an index of recruitment to predict fluctuations in population biomass. Such 'recruitment surveys' are conducted annually in Victoria for recently-settled King George whiting and also for snapper (*Chrysophrys auratus*). In each case, they provide a fishery independent metric to evaluate inter-annual variation in recruitment (Hamer & Jenkins 2004, Kemp et al. 2012). The

predictive power of larval abundance as an index of recruitment for King George whiting in South Australia has previously been questioned because of the small survey area compared to the extensive amount of potential settlement habitat. However, the biophysical model predicted that the same four nursery areas consistently received the highest numbers of recruits (Chapter 5). As such, targeted larval sampling at these nursery areas may provide a useful relative index of annual recruitment that could be incorporated into future management plans.

6.4 DIRECTIONS FOR FURTHER RESEARCH

Several knowledge gaps were identified throughout this study that warrant further investigation to improve understanding the early life-history of King George whiting. The first concerns temporal variation in spawning dynamics and its effect on recruitment. In Chapter 2, I identified a four week difference between the hatch dates of larvae that settled at the time of peak recruitment to Barker Inlet (late May), and the peak reproductive period of adults (late April). Although this discrepancy was identified at Barker Inlet, where the temporal settlement pattern is well described, it may also apply to other nursery areas where there is strong recruitment late in the settlement season (Fowler et al. 2000b, Rogers et al. 2019b; Chapter 3). As such, there is a need to understand the spatial distribution of spawning activity at the time of peak recruitment. One approach that has several potential benefits is to repeat the plankton survey in late May. The potential outcomes include: (1) compare egg distributions between April and May to assess temporal variation in spawning activity; (2) insight into the possible locations of alternative spawning grounds not identified in the April survey; and (3) the distribution of larvae could be used to evaluate the predictions of dispersal from the biophysical model. Another possibility is that the time of spawning for King George whiting may have changed since the reproductive studies of adult fish 20 years ago (Fowler et al. 1999, Fowler et al. 2000a). Because the onset on spawning is related to decreasing water temperatures during the austral autumn, there is the potential that progressively increasing water temperatures from climate change could delay the spawning season (Sims et al. 2004, Crozier & Hutchings 2014). This could be evaluated by analysing the reproductive stages of adult fish from the extensive biological database over this time.

The second knowledge gap concerns the unresolved relationships between the populations of King George whiting on the West Coast of Eyre Peninsula and in South Australia's gulfs. The largest unknown here is the locations of spawning areas on the West Coast. One possibility is to reverse simulate larval dispersal using a biophysical model. This model could incorporate the larval duration of recruits collected from nursery areas with real-time oceanographic conditions to identify potential spawning grounds with some level of probability. Another option is to predict potential spawning areas using a general additive model (GAM). A GAM could be developed between the physical environmental characteristics, benthic spawning habitat, and the spatial distribution of eggs and larvae

from the plankton surveys throughout the recognised spawning area in 2017 and 2018, which could then be used to predict potential spawning areas on the West Coast (Cardinale & Arrhenius 2000, Planque et al. 2007, Bacheler et al. 2010). Targeted plankton sampling of the potential spawning areas could then be undertaken to evaluate the predictions of the two models.

The third area for future research is to develop a better understanding of the connectivity between the South Australian and Victoria populations of King George whiting. In Chapter 5, I presented a hypothesis of an intermediate population that connects the two State-based populations. The hypothesised population along the south-east of South Australia would be replenished by larvae spawned in southern Spencer Gulf and Investigator Strait that are transported eastward by the prevailing coastal current (Middleton & Bye 2007) (Fig. 6.4). The adults of this population would then produce the larvae that replenish Victorian nursery areas (Jenkins et al. 2000). Such connectivity would have implications for cross-jurisdictional management. This hypothesis was previously proposed by Jenkins et al. (2016), and with the improved understanding of early life-history processes presented in this study, is worthy of further consideration. A logical first step would be to sample the limited potential settlement habitat for larval King George whiting along this stretch of coastline. There is no indication in the literature that this has been attempted. And secondly, recreational and commercial catches could be sampled to assess sexual development and the potential for an adult spawning population.

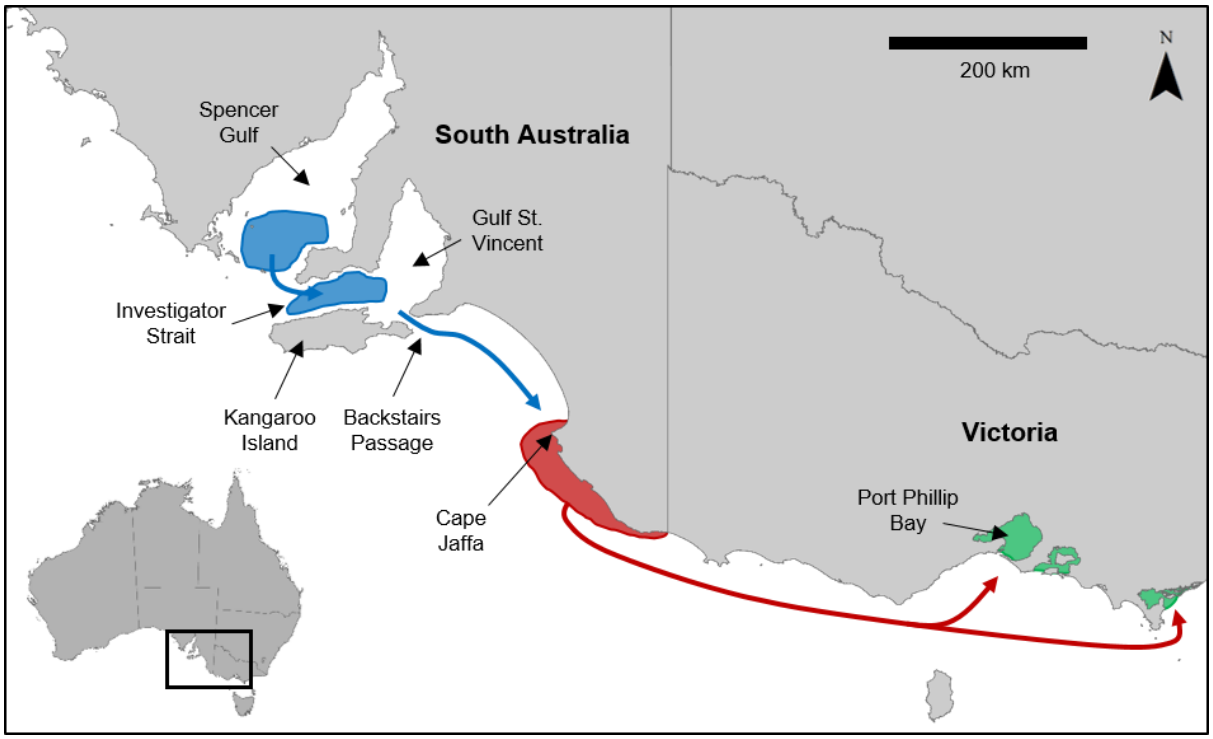

Fig. 6.4. Hypothesised population connectivity of King George whiting in south-eastern Australia. Inset – map of Australia showing this region along the southern coastline. Colours correspond to the different theoretical populations across south-eastern Australia: the South Australian gulfs population (blue); the south-east South Australia population (red); and the Victorian population (green).

The research presented in this thesis, in conjunction with a concurrent project on spawning dynamics (Steer et al. in prep.; FRDC 2016-003), has significantly improved the understanding of early life-history for King George whiting in South Australia's gulf system. The study combined the interdisciplinary techniques of otolith microstructure examination, elemental chemistry analysis, biophysical modelling and a molecular identification method to describe population connectivity and stock structure at a highly-resolved spatial scale. The hypothesised stock structure model of a within-season shift in connectivity between spawning grounds and nursery areas related to a seasonal change in physical oceanography is considerably more complex than the previous understanding of stock structure (Fowler et al. 2000b, Steer et al. 2018). Furthermore, the study has provided highly-resolved spatial and temporal information about spawning activity, early life-history characteristics and the demographic processes that ultimately culminate in recruitment. It is expected that this new biological information will be incorporated into the development of future management strategies to ensure the long-term sustainability of the fishery.

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Supplementary Information

SUPPLEMENTARY INFORMATION S2 - Chapter 2 – Within-season change in the early life-history characteristics and otolith chemistry of recently-settled King George whiting larvae

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Resolving the early life history of King George whiting (*Sillaginodes punctatus*: Perciformes) using otolith microstructure and trace element chemistry

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Abstract. Understanding the early life history processes of fish that lead to recruitment is critical for understanding population dynamics. This study explored the early life history of King George whiting (*Sillaginodes punctatus*) that recruited to an important nursery area in South Australia in 2016 and 2017. The early life history was reconstructed based on the retrospective analysis of otolith microstructure and chemistry for settlement-stage larvae collected fortnightly from July to November. These fish hatched between March and July, but a 3-week period in May led to 52–71% of recruitment. Recruits from successive sampling occasions differed in age, size and growth rate, potentially related to seasonal changes in water temperature and larval food availability. During both years, there were significant changes in otolith elemental chemistry among the groups of recruits that primarily related to changes in Sr : Ca. There are two hypotheses to account for the differences in otolith chemistry: either (1) a single, primary spawning source and within-season environmental change; or (2) multiple spawning sources. Further investigation with oceanographic models of larval dispersal will help differentiate between these. The retrospective analysis of otoliths has improved the understanding of early life history for this important species, with implications for fishery management.

Additional keywords: LA-ICP-MS, laser ablation–inductively coupled plasma–mass spectrometry, recruitment, strontium, temporal.

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Introduction

Understanding the early life history processes of fish that contribute to recruitment is critical for interpreting changes in adult populations (Chambers and Trippel 1997; Cowen and Sponaugle 2009). For marine species with spatially distinct spawning and nursery areas, recruitment can be directly related to larval survivorship during dispersal (Leggett and Deblois 1994; Cowen and Sponaugle 2009). Survival rates can be highly variable because they are affected by environmental factors, including food availability, predator abundance and the prevailing abiotic conditions (Pepin 1991; Leggett and Deblois 1994; Sponaugle *et al.* 2006). Even though larvae are small and their behavioural and sensory abilities are poorly understood, their dispersal is not simply controlled by physical oceanographic processes (Jones *et al.* 2009; Leis *et al.* 2011), and biological factors play an important role in larval survival and subsequent recruitment (Houde 1989; Chambers and Trippel 1997; Rankin and Sponaugle 2014).

Many fish species are batch spawners that produce offspring repeatedly throughout an extended spawning season (Brown-Peterson *et al.* 2011). The larvae that originate from an extensive range of hatch dates can be exposed to different environments throughout their early development (Cargnelli and Gross 1996; Radtke *et al.* 2001). Physical and ecological conditions are dynamic and can vary greatly at different spatial and temporal scales. As such, there is the potential for recruits within a single spawning season, and between years, to experience different environments and have considerably different early life history characteristics (Cargnelli and Gross 1996; Radtke *et al.* 2001; Rankin and Sponaugle 2014). For example, because larval growth is highly correlated with temperature, changes in water temperature during the spawning season may affect larval development rates and their subsequent survival (Pepin 1991; Green and Fisher 2004). Therefore, understanding temporal variation in the biological characteristics of early stage fish may contribute considerably to understanding variation in recruitment.

SUPPLEMENTARY INFORMATION S2 – TABLES

Table S2.1. Biological information for King George whiting recruits collected fortnightly throughout the settlement season at Barker Inlet in 2016 and 2017. Standard length (SL, mm), age (d), and average growth rate (mm d^{-1}) All values are presented in the form mean (range).

Date	*n*	SL	Age	Hatch date	Average growth rate
2016					
7-Jul	18	19.7 (17.7-21.6)	108 (93-132)	21-Mar (26-Feb to 5-Apr)	0.165 (0.135-0.184)
21-Jul	15	19.8 (18.5-21.0)	113 (101-135)	30-Mar (8-Mar to 11-Apr)	0.157 (0.137-0.176)
6-Aug	17	20.3 (18.7-22.0)	124 (107-134)	4-Apr (25-Mar to 21-Apr)	0.147 (0.132-0.173)
21-Aug	18	19.8 (18.8-21.1)	126 (113-147)	17-Apr (27-Mar to 30-Apr)	0.141 (0.122-0.160)
5-Sept	19	19.3 (18.4-20.0)	132 (108-162)	26-Apr (27-Mar to 20-May)	0.132 (0.105-0.159)
22-Sept	20	19.1 (17.5-19.9)	128 (115-151)	17-May (24-Apr to 30-May)	0.133 (0.114-0.147)
7-Oct	20	18.2 (17.2-19.0)	129 (105-165)	31-May (25-Apr to 24-Jun)	0.126 (0.101-0.144)
31-Oct	20	18.5 (16.9-19.3)	151 (117-177)	2-Jun (7-May to 6-Jul)	0.110 (0.096-0.131)
17-Nov	16	21.0 (18.0-22.1)	164 (139-184)	6-Jun (17-May to 1-Jul)	0.116 (0.100-0.137)
2017					
11-Jul	3	20.3 (19.7-20.8)	102 (95-112)	31-Mar (21-Mar to 7-Apr)	0.178 (0.167-0.185)
26-Jul	20	19.0 (17.7-19.5)	103 (92-110)	14-Apr (7-Apr to 25-Apr)	0.165 (0.154-0.184)
12-Aug	20	19.6 (17.9-20.7)	115 (102-134)	20-Apr (31-Mar to 2-May)	0.153 (0.134-0.175)
26-Aug	20	20.0 (18.0-21.0)	124 (103-147)	24-Apr (1-Apr to 15-May)	0.145 (0.129-0.164)
8-Sept	20	19.2 (18.5-19.6)	115 (101-130)	17-May (1-May to 30-May)	0.150 (0.133-0.167)
26-Sept	20	18.6 (17.5-19.3)	125 (110-136)	24-May (13-May to 8-Jun)	0.133 (0.118-0.151)
11-Oct	20	17.0 (16.4-17.7)	114 (103-135)	20-Jun (29-May to 30-Jun)	0.132 (0.112-0.141)
26-Oct	21	18.5 (16.1-20.0)	136 (105-179)	12-Jun (30-Apr to 13-Jul)	0.122 (0.099-0.142)
8-Nov	19	22.8 (19.3-25.3)	158 (141-179)	3-Jun (13-May to 20-Jun)	0.131 (0.106-0.150)

Table S2.2. Individual element:Ca ratios (μmol mol^{-1}) for the otolith core of King George whiting recruits collected fortnightly throughout the settlement season at Barker Inlet in 2016 and 2017. Values are mean (range).

Date	*n*	Li	Mg	Mn	Sr	Ba
2016						
7-Jul	18	6.857 (4.614-8.767)	309.7 (111.3-501.4)	0.941 (0.494-3.422)	2644 (2170-3037)	0.658 (0.504-1.062)
21-Jul	15	7.130 (5.969-8.187)	306.8 (148.7-484.4)	1.268 (0.425-3.309)	2627 (2143-3238)	0.850 (0.574-1.339)
6-Aug	17	6.554 (5.329-7.874)	363.2 (60.5-594.9)	1.033 (0.423-3.065)	2701 (2271-3415)	0.860 (0.457-1.723)
21-Aug	18	7.289 (6.103-9.407)	290.9 (108.4-543.9)	0.678 (0.365-1.579)	2819 (2435-3324)	0.899 (0.543-1.625)
5-Sept	19	7.119 (6.132-8.335)	294.3 (88.8-569.4)	0.700 (0.267-1.711)	2975 (2574-3420)	0.986 (0.507-2.347)
22-Sept	20	7.099 (6.103-8.276)	209.6 (62.0-480.2)	0.721 (0.171-2.012)	3169 (2586-3666)	1.020 (0.581-1.813)
7-Oct	20	7.007 (5.448-8.663)	226.9 (65.4-487.8)	0.704 (0.130-1.448)	3188 (2663-3701)	1.031 (0.616-2.181)
31-Oct	20	6.574 (5.373-7.666)	247.3 (90.9-581.3)	0.682 (0.190-2.651)	3072 (2632-3690)	0.944 (0.440-1.700)
17-Nov	16	6.585 (5.358-7.800)	293.5 (123.2-505.7)	0.789 (0.196-2.990)	2988 (2525-3442)	1.051 (0.556-1.745)
2017						
11-Jul	4	7.209 (6.266-8.722)	202.3 (150.4-264.3)	0.586 (0.511-0.683)	2736 (2394-3188)	0.900 (0.701-1.188)
26-Jul	20	7.414 (5.180-9.333)	229.4 (41.9-476.8)	1.031 (0.290-2.407)	3025 (2607-3522)	1.269 (0.740-2.204)
12-Aug	20	6.829 (5.180-8.514)	184.7 (44.0-547.3)	0.830 (0.322-2.130)	2926 (2442-3720)	1.105 (0.562-2.400)
26-Aug	20	7.578 (6.028-9.184)	263.8 (80.7-569.4)	0.833 (0.269-2.670)	2942 (2547-3351)	1.183 (0.594-2.076)
8-Sept	20	7.165 (5.984-8.529)	222.2 (69.7-530.3)	0.983 (0.162-2.538)	3261 (2568-3714)	1.285 (0.721-2.151)
26-Sept	20	7.230 (5.567-8.454)	215.2 (45.6-419.0)	1.108 (0.280-2.914)	3191 (2549-3734)	1.115 (0.566-1.993)
11-Oct	20	6.874 (5.190-8.142)	203.6 (15.6-498.0)	0.919 (0.096-2.820)	3272 (2742-3740)	1.336 (0.591-2.858)
26-Oct	21	6.745 (5.358-8.261)	257.4 (95.6-854.1)	0.875 (0.355-2.200)	3101 (2776-3573)	1.124 (0.530-2.151)
8-Nov	19	6.767 (5.388-7.844)	270.1 (44.9-419.4)	0.806 (0.239-2.670)	3205 (2641-3743)	1.236 (0.563-2.565)

Table S2.3. Individual element:Ca ratios (µmol mol^{-1}) for the otolith mid region of King George whiting recruits collected fortnightly throughout the settlement season at Barker Inlet in 2016 and 2017. Values are mean (range).

Date	*n*	Li	Mg	Mn	Sr	Ba
2016						
7-Jul	18	6.622 (5.210-7.666)	223.9 (48.8-489.9)	1.706 (1.045-2.226)	2158 (1969-2347)	0.768 (0.613-1.107)
21-Jul	15	6.765 (5.805-7.933)	191.4 (73.5-320.4)	1.763 (1.198-2.613)	2187 (1833-2589)	0.815 (0.649-1.226)
6-Aug	17	6.236 (5.358-7.829)	220.7 (47.5-427.0)	1.585 (1.145-2.369)	2265 (2082-2589)	0.807 (0.712-1.013)
21-Aug	18	6.565 (5.641-7.978)	246.2 (49.6-383.7)	1.189 (0.658-1.907)	2430 (2264-2577)	0.701 (0.560-0.911)
5-Sept	19	6.570 (5.492-7.844)	215.6 (56.1-492.5)	0.987 (0.436-2.192)	2513 (2374-2687)	0.666 (0.509-0.940)
22-Sept	20	6.452 (5.716-7.189)	91.4 (30.7-226.5)	0.815 (0.511-1.036)	2616 (2384-3006)	0.695 (0.499-0.910)
7-Oct	20	6.237 (4.867-7.279)	128.8 (18.3-338.2)	0.880 (0.306-1.510)	2517 (2192-2950)	0.693 (0.529-0.948)
31-Oct	20	5.605 (4.986-6.296)	132.3 (23.4-329.7)	0.986 (0.500-1.658)	2552 (2228-2993)	0.687 (0.510-0.851)
17-Nov	16	5.539 (4.287-6.847)	170.5 (39.5-329.7)	1.213 (0.421-2.010)	2470 (2281-2793)	0.744 (0.602-0.958)
2017						
11-Jul	4	6.202 (4.882-7.353)	86.8 (75.6-101.6)	1.086 (0.705-1.356)	2210 (2117-2291)	0.674 (0.643-0.700)
26-Jul	20	6.960 (4.971-9.496)	103.3 (16.5-233.7)	1.036 (0.637-1.940)	2439 (2314-2618)	0.750 (0.628-0.938)
12-Aug	20	6.303 (5.061-8.187)	82.9 (19.3-201.8)	0.946 (0.250-1.634)	2392 (2126-2753)	0.703 (0.577-0.918)
26-Aug	20	6.691 (5.656-8.320)	139.1 (20.0-400.3)	1.041 (0.703-1.803)	2446 (2152-2624)	0.723 (0.552-0.878)
8-Sept	20	6.480 (5.582-7.800)	76.0 (17.1-178.9)	0.871 (0.589-1.290)	2521 (2255-2839)	0.733 (0.569-1.248)
26-Sept	20	6.293 (5.016-7.561)	117.7 (16.3-330.6)	0.711 (0.417-1.049)	2623 (2326-2944)	0.655 (0.533-0.889)
11-Oct	20	5.994 (4.733-7.338)	67.3 (11.1-236.7)	0.678 (0.399-0.957)	2738 (2439-3518)	0.665 (0.535-0.865)
26-Oct	21	5.757 (5.061-7.338)	109.6 (18.4-363.3)	0.695 (0.295-1.175)	2677 (2261-3062)	0.659 (0.527-0.826)
8-Nov	19	5.521 (4.629-6.385)	186.6 (67.3-331.0)	0.966 (0.553-1.863)	2694 (2160-3104)	0.695 (0.557-0.833)

Table S2.4. Leave-one-out classification success for the temporal comparison of trace element chemistry for the otolith core of King George whiting recruits collected from Barker Inlet in 2016 and 2017. Data represent the percentage (%) of individuals from the date of capture (row) classified to each sample occasion (columns).

2016

Date	7-Jul	21-Jul	6-Aug	21-Aug	5-Sep	22-Sep	7-Oct	31-Oct	17-Nov
7-Jul	**6**	35	24	24	0	0	0	0	12
21-Jul	36	**43**	7	14	0	0	0	0	0
6-Aug	12	18	**41**	6	6	0	0	12	6
21-Aug	26	0	11	**37**	11	11	5	0	0
5-Sep	4	0	17	17	**21**	17	0	17	8
22-Sep	0	0	0	17	9	**22**	39	0	13
7-Oct	0	0	0	13	4	26	**17**	22	17
31-Oct	0	0	4	4	13	13	17	**30**	17
17-Nov	5	5	10	10	10	0	15	30	**15**
Overall	**25**								

2017

Date	11-Jul	28-Jul	12-Aug	26-Aug	8-Sep	26-Sep	11-Oct	26-Oct	8-Nov
11-Jul									
28-Jul		**9**	13	35	17	0	9	17	0
12-Aug		9	**41**	14	14	0	9	14	0
26-Aug		30	15	**40**	10	0	0	5	0
8-Sep		8	4	8	**33**	0	29	17	0
26-Sep		15	5	10	35	**0**	20	15	0
11-Oct		8	4	4	33	0	**29**	21	0
26-Oct		26	9	4	9	0	22	**30**	0
8-Nov		7	21	0	36	0	21	14	**0**
Overall	**24**								

Table S2.5. Leave-one-out classification success for the temporal comparison of trace element chemistry for the otolith mid region of King George whiting recruits collected from Barker Inlet in 2016 and 2017. Data represent the percentage (%) of individuals from the date of capture (row) classified to each sample occasion (columns).

2016

Date	7-Jul	21-Jul	6-Aug	21-Aug	5-Sep	22-Sep	7-Oct	31-Oct	17-Nov
7-Jul	**17**	50	22	6	0	0	0	0	6
21-Jul	43	**29**	21	7	0	0	0	0	0
6-Aug	12	12	**53**	18	0	0	0	0	6
21-Aug	0	6	17	**39**	28	6	0	0	6
5-Sep	0	4	4	25	**21**	8	29	0	8
22-Sep	0	0	0	0	13	**63**	21	0	4
7-Oct	0	4	9	9	35	26	**4**	13	0
31-Oct	0	0	0	9	0	9	13	**39**	30
17-Nov	0	0	15	15	5	0	0	30	**35**
Overall	**33**								

2017

Date	11-Jul	28-Jul	12-Aug	26-Aug	8-Sep	26-Sep	11-Oct	26-Oct	8-Nov
11-Jul									
28-Jul		**42**	21	13	17	0	0	4	4
12-Aug		17	**54**	8	17	0	0	4	0
26-Aug		33	29	**19**	10	5	0	0	5
8-Sep		17	29	8	**17**	13	17	0	0
26-Sep		4	17	4	4	**26**	22	13	9
11-Oct		4	0	0	13	8	**63**	4	8
26-Oct		0	13	0	13	4	26	**26**	17
8-Nov		0	14	0	0	7	0	50	**29**
Overall	**35**								

SUPPLEMENTARY INFORMATION S3 - Chapter 3 - Spatial connectivity during the early life-history of a temperate marine fish inferred from otolith microstructure and geochemistry

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Spatial connectivity during the early life history of a temperate marine fish inferred from otolith microstructure and geochemistry

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ABSTRACT

Connectivity during the ontogenetic development of fishes identifies the spatial scale over which populations function, which is the appropriate scale for conservation and management. For many marine species, spawning grounds and nursery areas are spatially segregated and larval dispersal is an obligate process that connects life history stages. This study investigated the spatial scale of early life history for one such species, the King George whiting (*Sillaginodes punctatus*; Perciformes), through the retrospective analysis of otolith microstructure and elemental chemistry of recently-settled larvae. The aim was to determine whether the South Australian population constitutes a single panmictic stock, or if it comprises multiple sub-populations. Sizes (15.1–25.1 mm SL), ages (85–183 d) and hatch dates (24-Apr to 1-Aug) of larvae varied considerably between nursery areas at different spatial scales. Regional differences in multi-elemental otolith signatures indicated that multiple spawning grounds contribute to recruitment, and larvae that settled in each region dispersed through different water masses. Within each region, there were differences in hatch dates and otolith chemistry indicative of finer-scale relationships between particular spawning grounds and nursery areas, consistent with local oceanographic circulation patterns. Although multi-elemental signatures were year-specific, concentrations of Ba and Mn were largely responsible for spatial differences and assigned larvae to regional groups with 52–66% accuracy. The results suggest the State-wide stock is replenished by three putative source populations, and provide an example of how otolith chemistry can discriminate among geographically-close, yet-ecologically separated groups of fish in coastal marine ecosystems.

1. Introduction

A common feature in the life history of marine fishes is coastal spawning followed by larval ingress to nursery areas, which is strongly influenced by physical oceanographic processes (Norcross and Shaw, 1984; Beck et al., 2001; Teodosio et al., 2016). For such species, nursery areas provide critical habitats for larvae to settle and then develop into juveniles, before they move to adjacent coastal populations during their ontogenetic development (Cowen et al., 2000; Beck et al., 2001; Cowen and Sponaugle, 2009). Larval transport provides the potential for extensive dispersal and the opportunity for mixing between geographically segregated populations (Cowen and Sponaugle, 2009; Leis et al., 2011). As such, understanding connectivity during ontogenetic development is necessary to determine the spatial scale over which fish populations operate, which informs population dynamics, stock structure, and ultimately underpins effective conservation and management strategies.

The extent of mixing between larvae from different spawning grounds during dispersal determines whether fish populations are essentially self-recruiting stocks, or if they form part of a larger metapopulation where recruits originate from multiple sources (Bailey, 1997). Historically it was assumed that marine fish populations were open systems and that recruitment was largely independent of local reproduction (Hjort, 1914; Caley et al., 1996; Cowen et al., 2000). However there has been a growing body of evidence that connectivity between habitats and life stages is far more complex than originally considered, and that life history processes can occur over much finer spatial scales (Swearer et al., 1999; Thorrold et al., 2001; Jones et al., 2005). Empirically quantifying larval movement is logistically challenging because of the inherently small size and difficulty in marking larvae. However, the refinement of analytical techniques has facilitated the use of biological structures as 'natural tags'. Of those considered (reviewed by Gillanders, 2009), analysis of the incremental structure and chemistry of otoliths has become a leading approach for assessing

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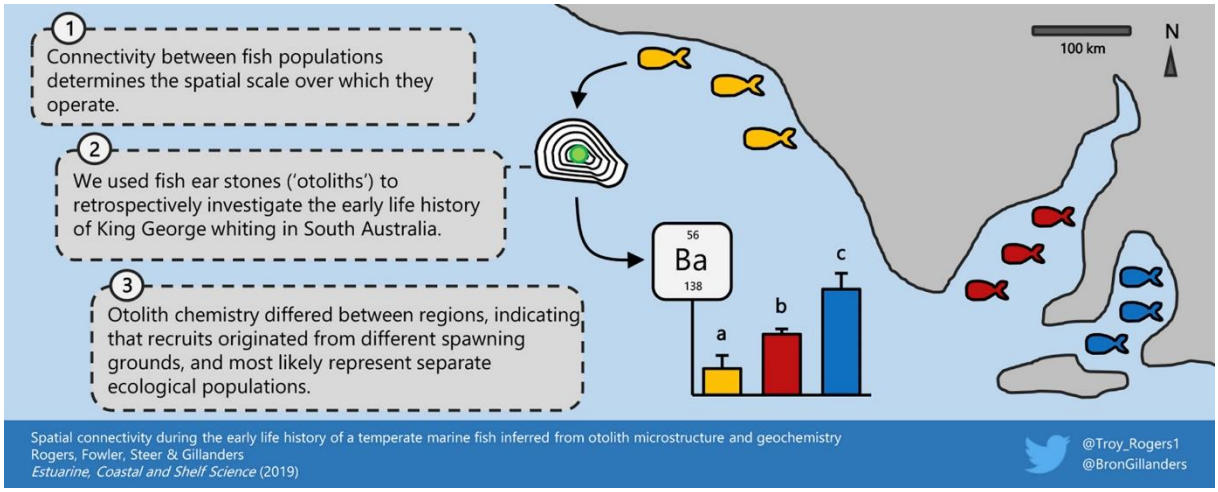
GRAPHICAL ABSTRACT



SUPPLEMENTARY INFORMATION S3 – TABLES

Table S3.1. Classification success for the spatio-temporal comparison of multi-elemental chemistry related to the otolith core and mid of recently-settled King George whiting larvae in 2016 and 2017. Data represent the percentage (%) of fish from the region of capture (row) allocated to each region (columns). Bold values are correctly allocated.

	Core						Mid					
Year	Region	SG16	GSV16	WC17	SG17	GSV17	Region	SG16	GSV16	WC17	SG17	GSV17
2016/17	SG16	**41**	34	14	4	6	SG16	**32**	29	20	9	11
	GSV16	29	**37**	11	10	13	GSV16	18	**53**	3	8	19
	WC17	15	0	**62**	18	5	WC17	5	0	**63**	25	7
	SG17	13	4	40	**20**	23	SG17	16	5	24	**40**	14
	GSV17	11	16	19	17	**36**	GSV17	10	40	4	19	**26**
	Overall	**37**					Overall	**42**				

2016	Region	SG	GSV
	SG	**61**	39
	GSV	42	**58**
	Overall	**60**	

	Region	SG	GSV
	SG	**64**	36
	GSV	32	**68**
	Overall	**66**	

2017	Region	WC	SG	GSV
	WC	**67**	22	11
	SG	36	**33**	31
	GSV	21	18	**60**
	Overall	**52**		

	Region	WC	SG	GSV
	WC	**64**	25	11
	SG	25	**49**	26
	GSV	5	23	**72**
	Overall	**62**		

Table S3.2. Classification success for the spatio-temporal comparison of multi-element chemistry related to the otolith core of recently-settled King George whiting larvae by site for 2016 and 2017. Data represent the percentage (%) of fish from the site of capture (row) classified to each site (columns). Bold values are allocated classified. Site names in Table 1.

2016

Site	TV	SB	CB	TB	FH	FB	PV	PT	PH	VN	PP	BI	BR
TV													
SB													
CB													
TB													
FH					**18**	9	14	9	23	5	5	9	9
FB					6	**11**	6	11	6	0	6	6	50
PV					11	0	**11**	11	22	0	11	33	0
PT					5	14	10	**38**	5	0	0	19	10
PH					0	0	21	5	**53**	0	21	0	0
VN					18	5	18	0	14	**14**	5	14	14
PP					4	17	4	13	30	0	**13**	13	4
BI					0	9	13	9	17	0	4	**35**	13
BR					5	38	0	14	0	5	10	10	**19**
Overall	**24**												

2017

Site	TV	SB	CB	TB	FH	FB	PV	PT	PH	VN	PP	BI	BR
TV	**6**	0	18	0	6	18	0	6	0	6	6	29	6
SB	0	**12**	24	0	6	12	6	18	6	6	12	0	0
CB	0	0	**19**	0	0	24	0	14	10	14	10	5	5
TB	0	11	17	**0**	0	17	0	17	6	11	0	17	6
FH	0	6	0	0	**11**	22	0	11	11	11	11	11	6
FB	0	5	25	0	10	**25**	0	5	0	5	0	20	5
PV	0	18	18	0	6	6	**0**	12	18	6	6	12	0
PT	0	6	6	6	17	6	0	**39**	17	0	0	6	0
PH	0	5	0	0	0	0	0	0	**42**	42	0	5	5
VN	0	10	0	5	14	0	0	5	38	**10**	10	0	10
PP	11	21	11	5	0	0	0	11	26	0	**5**	11	0
BI	0	0	4	0	4	17	0	17	13	8	0	**38**	0
BR	13	13	7	0	13	13	0	0	20	0	7	7	**7**
Overall	**17**												

Table S3.3. Classification success for the spatio-temporal comparison of multi-element chemistry related to the otolith mid of recently-settled King George whiting larvae by site for 2016 and 2017. Data represent the percentage (%) of fish from the site of capture (row) classified to each site (columns). Bold values are correctly allocated. Site names in Table 1.

2016

Site	TV	SB	CB	TB	FH	FB	PV	PT	PH	VN	PP	BI	BR
TV													
SB													
CB													
TB													
FH					**0**	9	4	13	26	0	0	22	26
FB					0	**27**	5	27	5	18	5	5	9
PV					0	0	**44**	33	0	11	0	11	0
PT					0	5	14	**59**	0	14	0	5	5
PH					18	5	5	5	**50**	0	5	9	5
VN					0	13	4	13	17	**17**	4	13	17
PP					5	5	0	32	18	5	**14**	9	14
BI					9	0	23	9	9	5	5	**18**	23
BR					9	9	0	13	26	0	22	4	**17**
Overall	**27**												

2017

Site	TV	SB	CB	TB	FH	FB	PV	PT	PH	VN	PP	BI	BR
TV	**12**	35	6	6	0	6	12	0	0	0	6	12	6
SB	16	**53**	0	0	0	11	0	5	5	5	5	0	0
CB	10	20	**10**	35	5	10	5	0	0	0	5	0	0
TB	16	5	16	**16**	11	5	5	0	5	0	5	16	0
FH	0	0	0	0	**18**	6	18	12	0	12	0	35	0
FB	10	19	10	0	0	**29**	5	0	0	10	5	10	5
PV	6	6	12	12	6	0	**35**	0	6	18	0	0	0
PT	0	17	0	6	17	6	22	**11**	6	6	0	6	6
PH	0	5	0	0	0	0	5	5	**63**	5	5	11	0
VN	10	0	5	5	10	0	5	0	29	**24**	5	5	5
PP	0	5	11	0	16	21	0	0	16	16	**5**	5	5
BI	0	0	0	13	0	0	8	0	17	0	0	**63**	0
BR	6	13	6	13	6	13	0	0	13	6	6	19	**0**
Overall	**27**												

SUPPLEMENTARY INFORMATION S3 – FIGURES

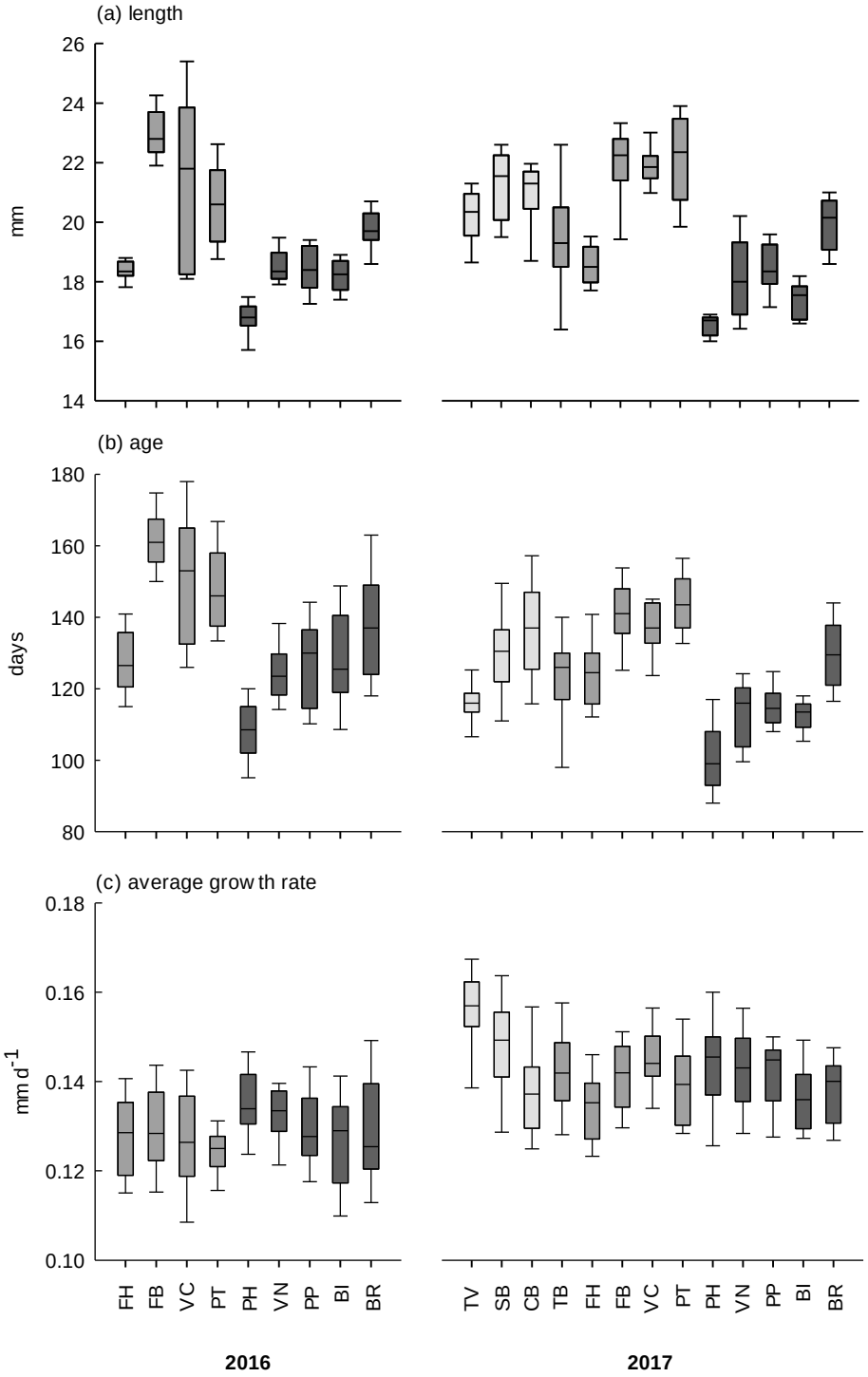


Figure S3.1. Comparisons of mean standard length (mm), age (days) and average growth rate (mm d^{-1}) between sites for recently-settled King George whiting larvae in 2016 and 2017. Box and whisker plots show median, box 25-75%, error bars 10 and 90%. Site names in Table 3.1.

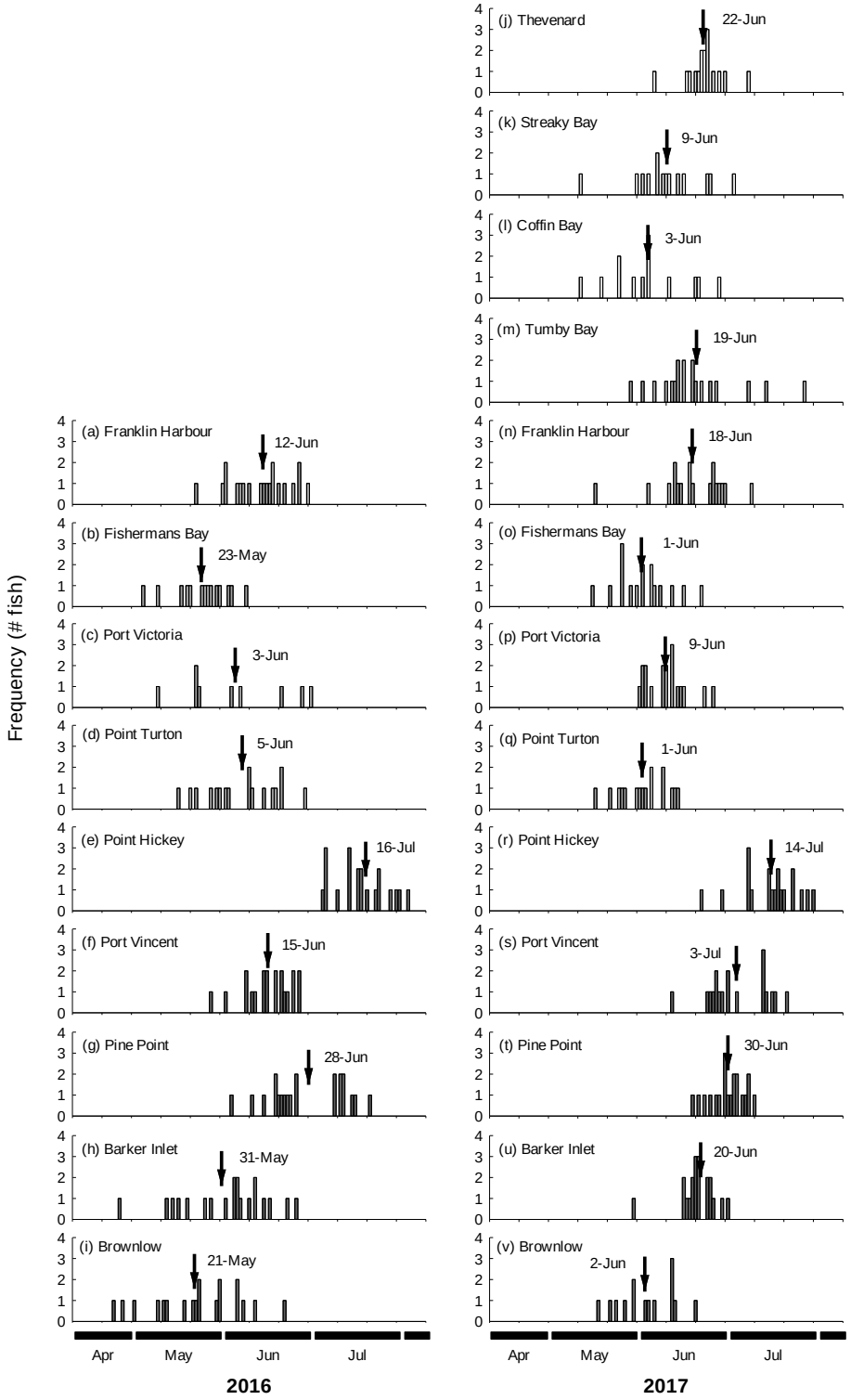


Figure S3.2. Frequency histograms showing the number of aged King George whiting larvae from each site that hatched on the nominated Julian day in 2016 and 2017. Arrows identify mean hatch day.

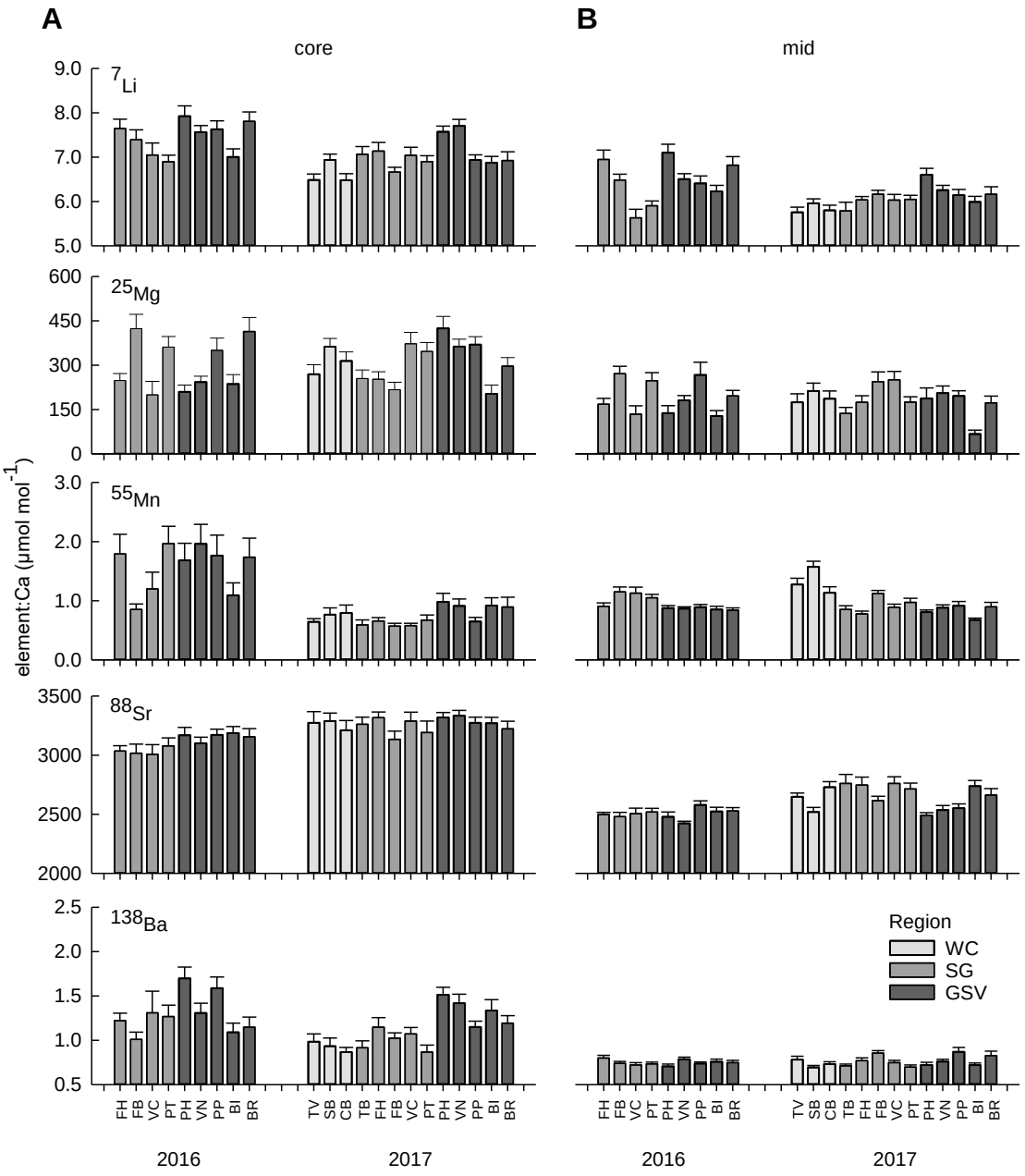


Figure S3.3. Mean element:Ca ratios (µmol mol^{-1}) for the otolith (A) core and (B) mid of recently-settled King George whiting larvae grouped by site from 2016 and 2017. Error bars are +1 SE. Shading corresponds to the different regions: WC – West Coast; SG – Spencer Gulf; GSV – Gulf St. Vincent. Site names in Table 3.1.

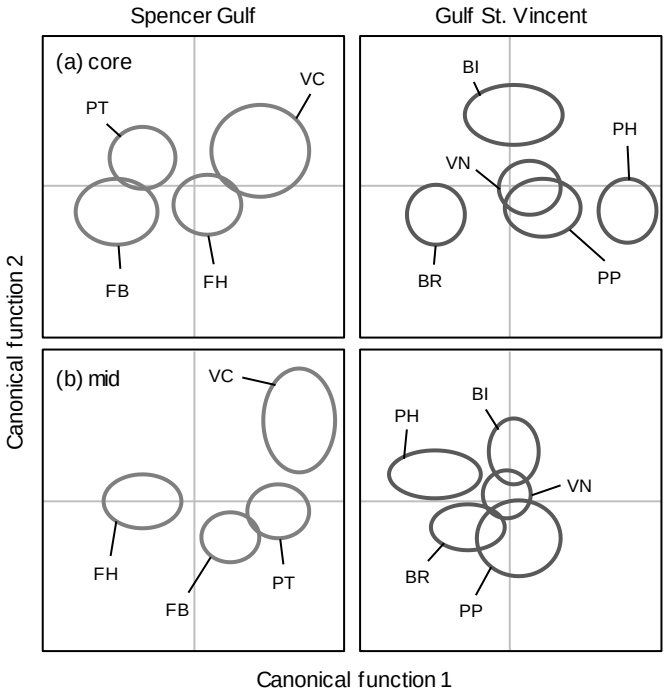


Figure S3.4. Canonical variate plots for the multi-elemental chemistry of the otolith core and mid of recently-settled King George whiting larvae sampled in 2016 grouped by site. Ellipses represent 95% confidence around group centroids. Figures were separated by region for clarity. Site names in Table 3.1.

SUPPLEMENTARY INFORMATION S4 - Chapter 4 – Discriminating natal source populations of a temperate marine fish using larval otolith chemistry

frontiers in Marine Science

ORIGINAL RESEARCH
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Discriminating Natal Source Populations of a Temperate Marine Fish Using Larval Otolith Chemistry

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The life cycles of many marine species depend on a dispersive larval stage that connects spatially segregated populations. However, quantifying larval movement among populations remains one of the greatest challenges in marine ecology. Such movement determines whether a population is essentially a self-recruiting stock, or if it forms part of a larger meta-population where recruits originate from multiple sources. Previous research has struggled to differentiate between such stock structure models for King George whiting (*Sillaginodes punctatus*; Perciformes) in southern Australia, largely due to difficulties in identifying the source populations of dispersing larvae. In this study, pelagic larvae were collected throughout the only recognized spawning area in South Australia in 2017 and 2018. First, we identified that the distribution of larvae was broadly divisible into two groups – those in southern Spencer Gulf and those in Investigator Strait. Then, the incremental structure and elemental composition of otoliths of larvae from the two regions were compared to determine if they had originated from a common source population. There were no spatial differences in the sizes (3.0–5.0 mm SL), ages (5–21 days), hatch dates (April 7–24) or average growth rates (0.09–0.21 mm d^{-1}) of larvae. However, multi-elemental (Li, Mg, Mn, Sr, and Ba) otolith signatures differed significantly between the two regions, primarily driven by differences in concentrations of Li and Ba. Although otolith signatures were year-specific, larvae were assigned to their region of capture with 70–82% accuracy. Larvae in each region hatched at the same time yet had significantly different otolith chemistry, providing strong evidence that those in southern Spencer Gulf and Investigator Strait originated from spatially segregated water masses. This study has demonstrated the ability of otolith chemistry to discriminate source populations of pelagic larvae in a fully marine environment, which provides a basis to quantify larval movement between fish populations.

Keywords: larvae, connectivity, otolith chemistry, microstructure, LA-ICP-MS, early life history, lithium, King George whiting

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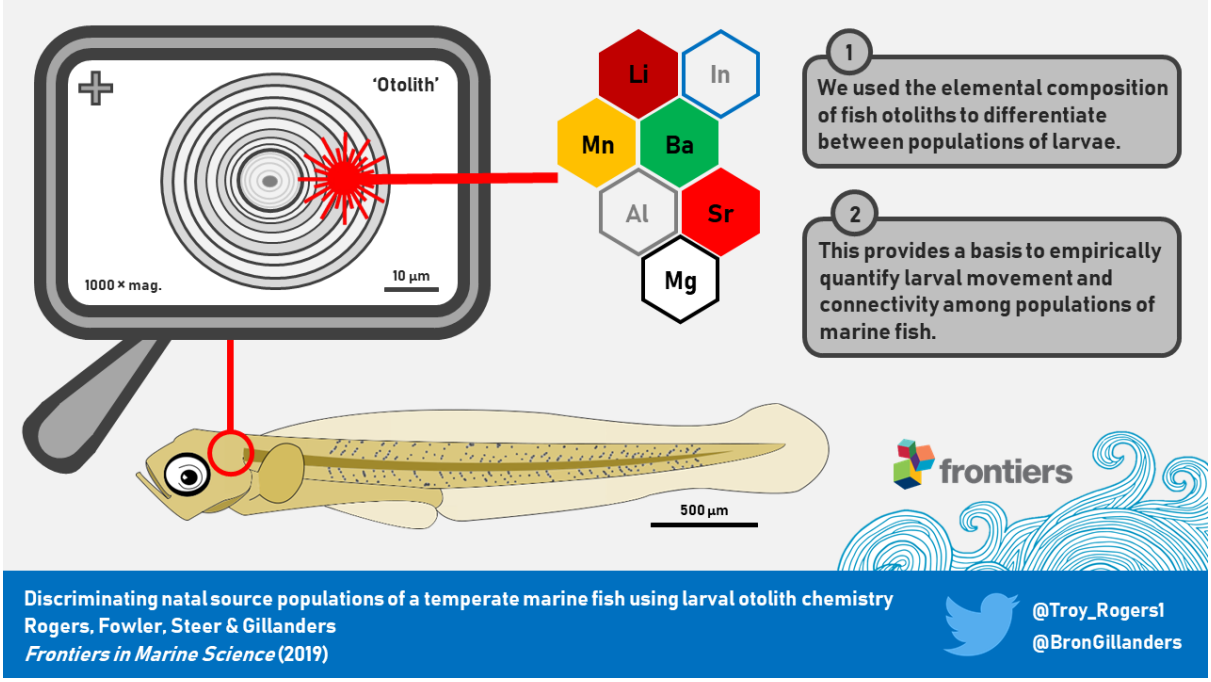
INTRODUCTION

Many marine species conform to a bipartite life cycle whereby spawning grounds and nursery areas are spatially segregated, and larval transport is an obligate process that connects life history stages (Cowen et al., 2000). The dispersal of larvae in marine environments is heavily influenced by physical oceanographic processes, which results in a high probability of mixing between larvae

GRAPHICAL ABSTRACT



SUPPLEMENTARY INFORMATION S4 – TABLES

Table S4.1. Summary of results for two-way ANOVAs for the effects of year and region on the total length (mm), age (d), average growth rate (mm d^{-1}) and otolith diameter (µm) of larval King George whiting. Year and region were fixed factors. There were no significant differences between means.

		Total length		Age		Growth rate		Otolith diameter	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Year	1	0.03	2.16	0.57	3.15	<0.00	<0.00	49.09	0.73
Region	1	0.01	0.57	0.11	0.62	<0.00	0.04	96.31	1.44
Year × Region	1	0.01	0.34	0.35	1.96	<0.00	0.84	192.23	2.87
Residuals	129	0.01		0.18		<0.00		67.01	

Table S4.2. Summary of results for repeated-measures analysis of variance (RM-ANOVA) used to compare individual growth trajectories between regions and years. There were no significant differences between regions, years, or the interaction between the two factors.

	df	MS	*F*	*P*
Intercept	1	10.28	1957.92	< 0.001
Year	1	0.01	1.44	0.236
Region	1	< 0.01	0.01	0.975
Year × Region	1	0.01	2.02	0.161
Error	122	0.01		

Table S4.3. Classification success for the spatio-temporal comparison of multi-elemental chemistry for the (a) primordial and (b) non-primordial area of larval King George whiting otoliths in 2017 and 2018. Data represent the percentage (%) of larvae from the region of capture (row) allocated to each region (column). Bold values are correctly assigned. SSG – southern Spencer Gulf; IS – Investigator Strait. κ = Cohen's Kappa.

		2017		2018	
		SSG	IS	SSG	IS
(a) primordial					
2017	SSG	**72**	17	11	0
	IS	33	**56**	4	7
2018	SSG	20	0	**57**	23
	IS	21	3	16	**61**
	Overall	**60**			
	κ	0.47			
(b) non-primordial					
2017	SSG	**78**	0	6	17
	IS	26	**67**	0	7
2018	SSG	23	0	**40**	37
	IS	26	0	34	**39**
	Overall	**52**			
	κ	0.36			

SUPPLEMENTARY INFORMATION S4 – FIGURES

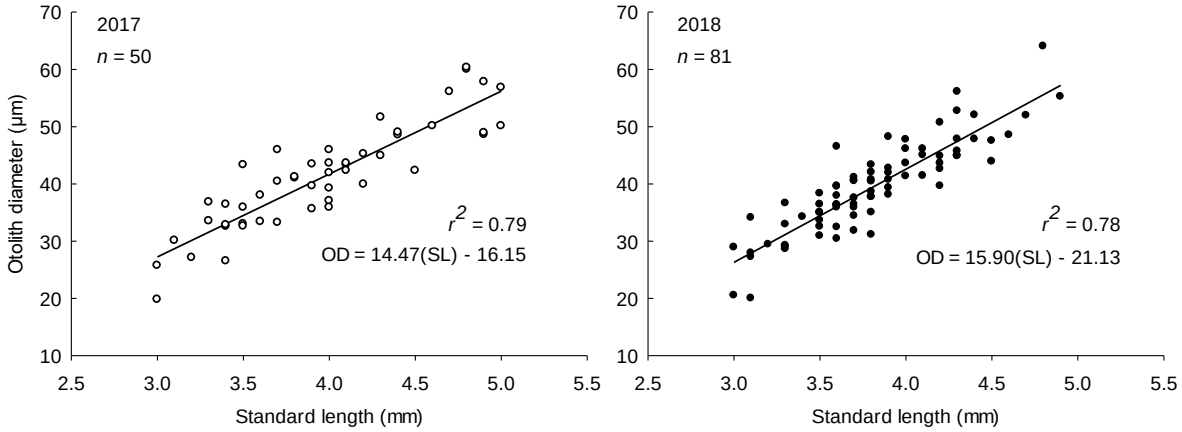


Figure S4.1. Relationship between standard length (mm) and otolith diameter (µm) for King George whiting larvae in 2017 and 2018. There were strong linear relationships in both years (r^2 = 0.79 and 0.78 in 2017 and 2018, respectively).

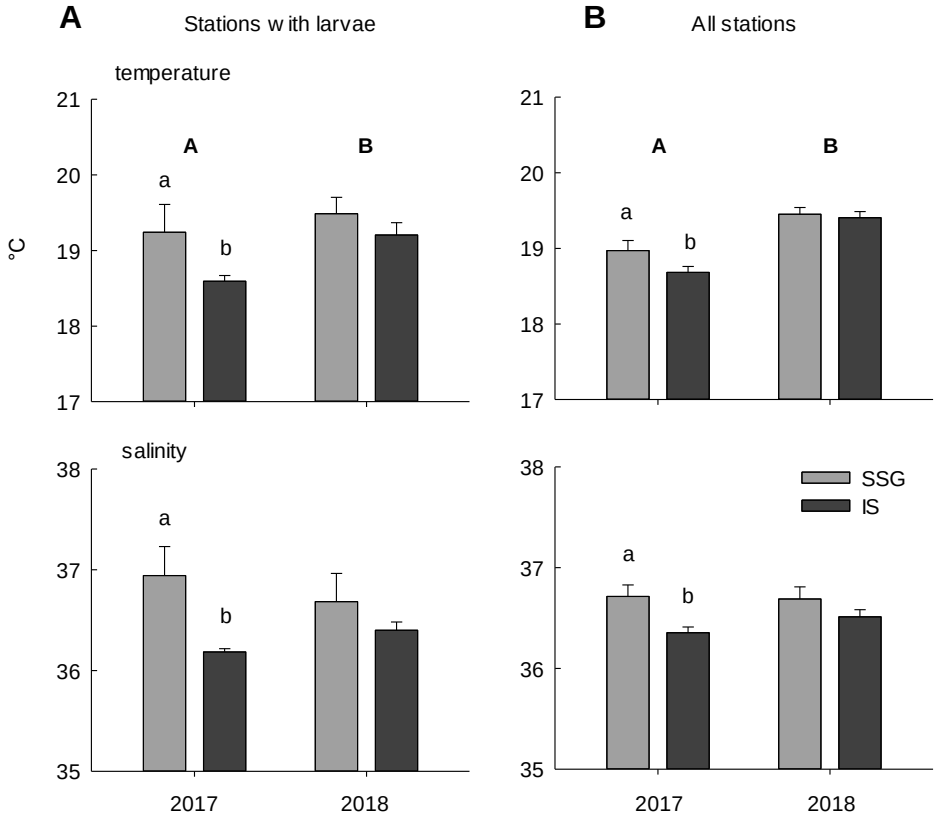


Figure S4.2. Comparison of mean temperature (°C) and salinity between southern Spencer Gulf (SSG) and Investigator Strait (IS) in 2017 and 2018. (A) Stations where larvae were sampled (used for otolith chemistry); (B) all stations. Letters identify significant differences (ANOVA; $P < 0.05$; capitals = between years; lower case = between regions). Error bars are + 1 SE.

Appendix 4A.

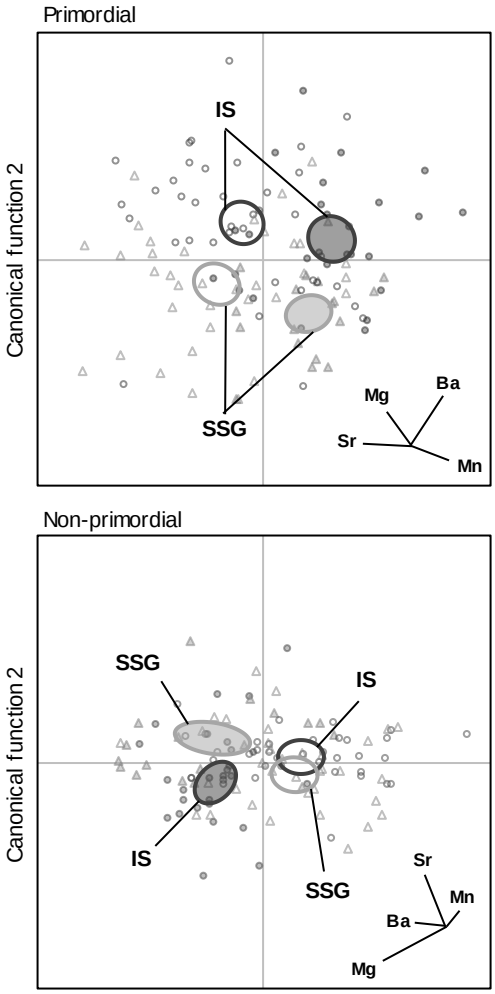


Fig. 4A.1. Canonical variate plots for the multi-elemental otolith chemistry of King George whiting larvae in 2017 (shaded) and 2018 (open) excluding Li (elements included were Mg, Mn, Sr and Ba). Ellipses show 95% confidence around group centroids. SSG – southern Spencer Gulf (▲; light); IS – Investigator Strait (●; dark).

Table 4A.1. Summary of results for two-factor PERMANOVAs for the effect of year and region on individual and combined element:Ca ratios excluding Li (elements included were Mg, Mn, Sr and Ba) of larval King George whiting otoliths. Year and region were fixed factors. *P < 0.05; **P < 0.01; ***P < 0.001.

		Mg		Mn		Sr		Ba		All elements	
	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(a) primordial											
Year	1	6.70	7.32**	12.48	13.65***	23.05	28.45***	5.76	7.43**	47.99	14.06***
Region	1	3.64	3.97*	0.01	0.01	0.15	0.19	19.88	25.65***	23.68	6.94***
Year × Region	1	0.99	1.08	0.36	0.40	1.61	1.99	0.08	0.11	3.04	0.89
Residuals	127	0.92		0.91		0.81		0.78		3.41	
(b) non-primordial											
Year	1	30.48	41.48***	<0.01	<0.01	2.39	2.56	4.66	4.85*	37.54	10.26***
Region	1	0.23	0.32	<0.01	<0.01	2.62	2.79	2.87	2.99	5.74	1.57
Year × Region	1	0.47	0.64	<0.01	<0.01	8.25	8.81**	0.01	0.01	8.74	2.39
Residuals	127	0.73		1.03		0.94		0.96		3.66	

SUPPLEMENTARY INFORMATION S5

Chapter 5 – Using a biophysical model to investigate demographic connectivity between spawning grounds and nursery areas of a temperate marine fish

SUPPLEMENTARY INFORMATION S5 – TABLES

Table S5.1. Names of the settlement areas in included in the larval transport model. Settlement areas were included based on the presence of recently-settled larvae from intermittent recruitment surveys since 1977.

	Region (area)
Northern Spencer Gulf (NSG)	
1	Blanche Harbour
2	Chinamans creek
3	Yatala Harbour
4	Pt. Pirie creeks
5	Pt. Davis creeks
6	Cowleds Landing
7	Fishermans Bay
South-west Spencer Gulf (SWSG)	
8	Franklin Harbour
9	Tumby Bay
10	Boston Bay
11	Proper Bay
Investigator Strait (IS)	
12	Point Davenport
South-east Spencer Gulf (SESG)	
13	Hardwicke Bay
14	Pt. Victoria
Gulf St. Vincent (GSV)	
15	Coobowie Bay
16	Stansbury
17	Pt. Vincent
18	Pine Point
19	Ardrossan
20	Price creek
21	Pt. Wakefield creek
22	Barker Inlet
Kangaroo Island (KI)	
23	Bay of Shoals
24	Brownlow
25	American River

Table S5.2. Percentage of overall particles released from each spawning area (1-13) that intersected the open ocean boundaries of the larval transport model in 2017 and 2018. The particles were considered out of bounds and excluded from simulations. OB – total percentage of particles released that were out of bounds; OB_W – percentage of particles out of bounds to the west of Kangaroo Island; OB_E – percentage of particles out of bounds to the east of Kangaroo Island.

2017		Scenario 1			Scenario 2			Scenario 3		
Spawning area	# seeded	OB	OB_W	OB_E	OB	OB_W	OB_E	OB	OB_W	OB_E
Spencer Gulf	**17167**	**81.3**	**35.4**	**45.9**	**59.6**	**16.4**	**43.2**	**61.1**	**18.3**	**42.8**
1	0									
2	6265	76.5	30.5	46.0	60.1	17.7	42.4	61.3	19.0	42.3
3	2356	85.1	42.4	42.8	49.5	10.3	39.2	49.5	10.9	38.7
4	4237	80.4	36.0	44.4	59.4	17.3	42.1	63.1	20.0	43.0
5	1692	84.2	26.8	57.3	74.2	18.4	55.7	74.0	19.7	54.3
6	2617	88.9	45.2	43.7	58.5	16.0	42.4	59.4	19.6	39.8
Investigator St	**9430**	**97.0**	**3.1**	**93.9**	**95.2**	**2.9**	**92.3**	**94.3**	**3.0**	**91.3**
7	1410	94.9	3.5	91.4	91.1	3.8	87.3	89.1	3.8	85.2
8	3619	98.6	6.9	91.7	98.2	6.2	92.0	97.6	6.4	91.2
9	751	88.5	0.0	88.5	78.2	0.0	78.2	75.8	0.0	75.8
10	3118	100.0	0.0	100.0	99.8	0.0	99.8	100.0	0.0	100.0
11	139	48.2	0.0	48.2	39.6	0.0	39.6	27.3	0.0	27.3
12	158	100.0	0.0	100.0	100.0	0.0	100.0	100.0	0.0	100.0
13	235	100.0	0.0	100.0	96.6	0.0	96.6	96.6	0.0	96.6
Total	26597	86.9	24.0	62.9	72.2	11.6	60.6	72.9	12.9	60.0

2018		Scenario 1			Scenario 2			Scenario 3		
Spawning area	# seeded	OB	OB_W	OB_E	OB	OB_W	OB_E	OB	OB_W	OB_E
Spencer Gulf	**7671**	**77.8**	**69.5**	**8.3**	**50.3**	**38.7**	**11.5**	**53.1**	**42.4**	**10.7**
1	622	36.5	32.5	4.0	9.2	5.3	3.9	10.8	6.9	3.9
2	2134	61.1	53.9	7.2	30.4	23.1	7.3	32.3	24.4	7.9
3	1491	92.3	83.4	8.9	63.0	43.8	19.2	68.6	53.6	15.0
4	2175	86.3	75.7	10.6	52.0	37.5	14.4	55.0	41.1	13.9
5	1226	94.5	86.9	7.6	86.9	78.6	8.3	88.3	80.1	8.2
6	23	100.0	87.0	13.0	60.9	52.2	8.7	73.9	60.9	13.0
Investigator St	**14910**	**95.2**	**11.0**	**84.2**	**91.7**	**10.9**	**80.8**	**91.1**	**11.0**	**80.1**
7	3034	89.1	15.0	74.1	82.2	15.0	67.2	81.0	15.3	65.7
8	2194	99.1	53.9	45.3	98.4	53.0	45.4	97.5	53.6	43.9
9	3662	91.8	0.0	91.8	88.0	0.1	87.8	87.8	0.0	87.8
10	5053	99.8	0.0	99.8	98.8	0.0	98.8	98.6	0.0	98.6
11	157	72.0	0.0	72.0	52.9	0.0	52.9	53.5	0.0	53.5
12	277	98.6	0.0	98.6	93.9	0.0	93.9	91.7	0.0	91.7
13	533	97.0	0.0	97.0	86.5	0.0	86.5	84.2	0.0	84.2
Total	22581	89.3	30.8	58.4	77.6	20.3	57.3	78.2	21.7	56.5

Table S5.3. Percent contribution of each spawning area (1-13) to overall settlement success for each scenario. Only settlement areas (1-25) that accounted for ≥ 10% of overall settlement for each model run were included. *n* = total number of particles that settled in each scenario. Corresponds to Fig. 5.12.

2017	Scenario 1 (*n* = 174)				Scenario 2 (*n* = 326)				Scenario 3 (*n* = 267)			
	Settlement area				Settlement area				Settlement area			
Spawning area	8	15	16	18	13	14	15	25	13	15	23	25
1												
2	8.0	4.6	4.6	1.1	8.6	4.6	4.6	4.3	11.2	4.1	3.4	3.4
3	0.6	0.6	0.6		10.1	4.3	2.5	0.3	11.6	3.0	1.5	1.9
4	3.4	1.1	3.4	1.1	6.7	3.1	4.3	3.7	6.7	2.2	1.9	5.6
5	1.1		1.1	1.1	2.1	0.3	0.9	2.5	1.5	1.9	0.4	1.9
6		2.3	3.4	0.6	9.5	0.9	1.5	3.1	9.4	3.4	4.1	0.7
7		0.6	2.3	2.3			1.2			1.1		
8			2.3	2.9	0.3		0.3	0.3		0.4		0.7
9			2.3	0.6			0.3			0.4		
10												
11		1.1	1.1	1.7								
12												
13												
Total (%)	13.2	10.3	21.3	11.5	37.4	13.2	15.6	14.1	40.4	16.5	11.2	14.2

2018	Scenario 1 (*n* = 161)				Scenario 2 (*n* = 294)				Scenario 3 (*n* = 272)			
	Settlement area				Settlement area				Settlement area			
Spawning area	15	16	18	19	13	15	22	25	13	15	22	25
1					2.7				1.5			
2	0.6				3.1	1.4		0.3	7.4	0.4		0.4
3					4.1	1.4		1.4	4.4	1.1		0.4
4					2.7	1.0		1.4	2.9			1.1
5		1.2	0.6	0.6	0.7			0.3		0.4		0.4
6												
7	5.6	4.3	4.3	7.5		10.5	0.3	0.3		8.1	0.4	
8	0.6	0.6						4.8				9.9
9	3.7	3.7	6.8	3.7		20.7	11.2			22.1	14.0	
10						0.3		7.8				8.1
11	0.6		1.2	0.6		1.0				0.4	0.7	
12	0.6	0.6										
13												
Total (%)	11.8	10.6	13.0	13.7	13.3	36.4	11.6	16.3	16.2	32.4	15.1	20.2

SUPPLEMENTARY INFORMATION S5 – FIGURES

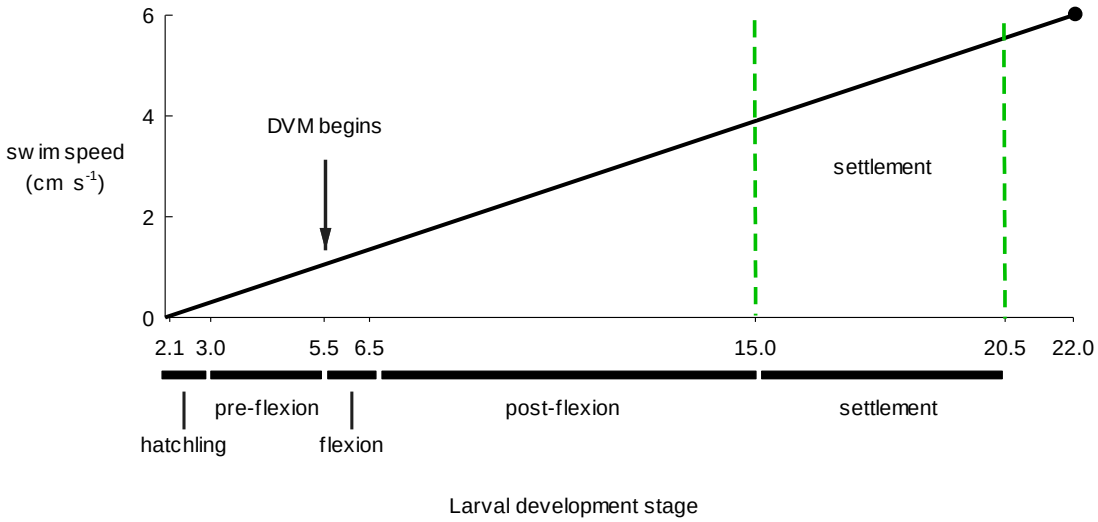


Figure S5.1. Theoretical linear relationship between body length and swim speed of larval King George whiting. Swim speed at length was estimated based on the critical swim speed of ~6 cm s^{-1} for recently-settled larvae (Jenkins & Welsford 2002). The size range for each development stage and the onset of vertical movement follow the descriptions by Bruce (1995).

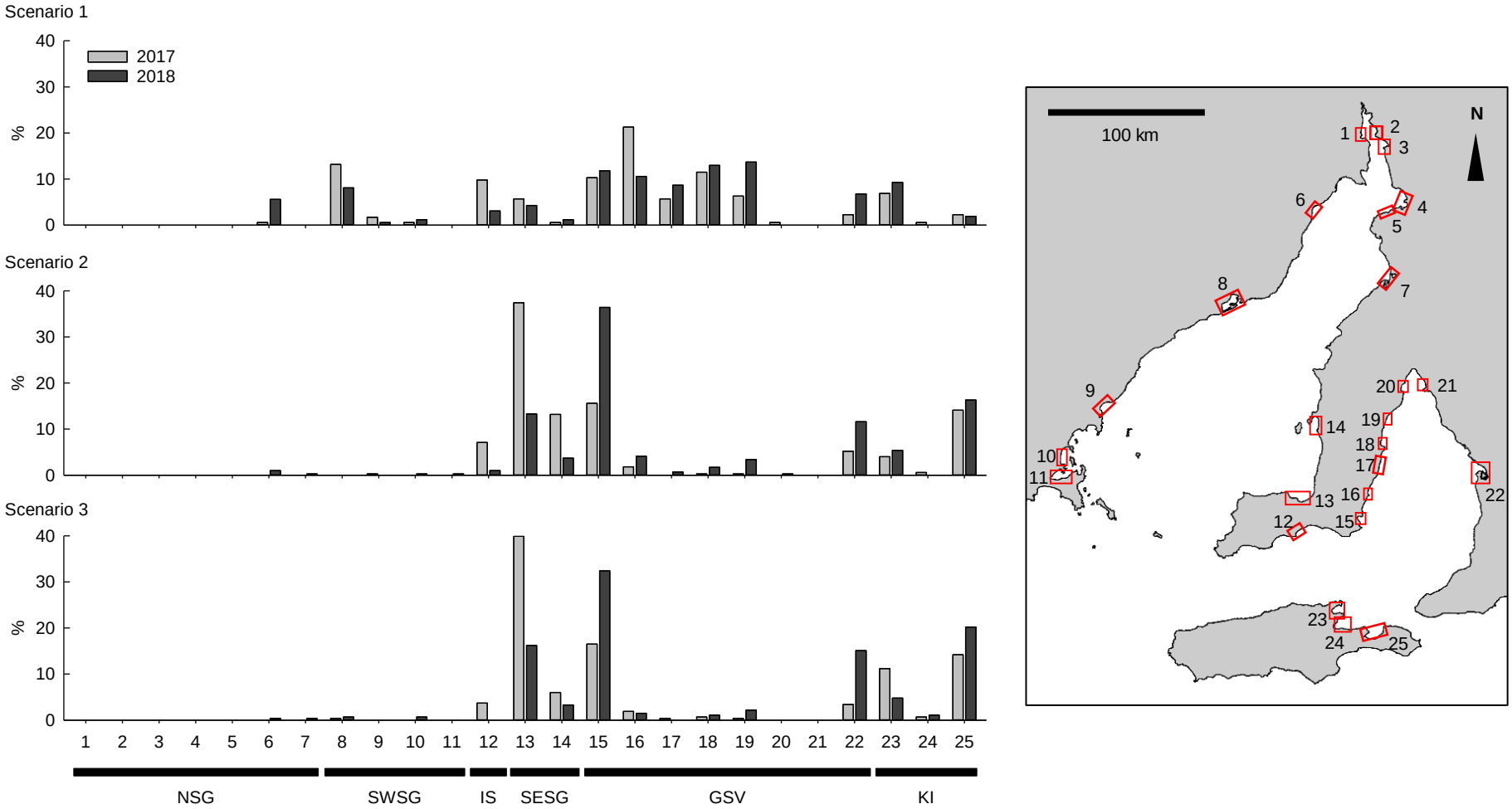


Figure S5.2. Percent contribution of each settlement area to overall settlement success in 2017 (light) and 2018 (dark). Scenario 1 - passive; scenario 2 - average growth rate; scenario 3 - daily growth rate. Descriptions of each scenario are in Methods 5.2.5.