Experimental assessment of constraints for King George whiting (*Sillaginodes punctata*) recruitment in Gulf St Vincent, South Australia

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ABSTRACT

King George whiting *Sillaginodes punctata* (KGW) is one of South Australia's most valuable finfish species highly targeted by commercial and recreational fishers. The response of this species to both anthropogenic and climate changes is currently unknown and may impact on recruitment success. This thesis examined four key areas potentially influencing the recruitment success of KGW in relation to current and future environmental changes. The specific aims were:

- to investigate the molecular response of KGW to acute and extended changes in temperature by examining the production of heat shock proteins.
- to reveal the osmoregulatory ability of KGW by looking at blood plasma ion concentrations, and to assess if current high salinity levels in the gulfs are impacting growth and survival of young recruits.
- to identify the influence of light intensity in regulating growth, survival, behaviour and body colouration.
- to study the dietary interactions of young of the year recruits with estuarine opportunists and permanent resident fish species.

Heat shock proteins (HSP) play an important role in protein folding and cytoprotection. Members of the HSP70 family are sensitive and readily produced in response to thermal stress in many fish species thus serve as a useful stress bio-indicator. KGW inhabit inshore shallow waters as juveniles and are subject to considerable thermal variance before migrating to deeper water towards maturity. Two experiments were conducted to test the hypothesis that whiting approaching

sexual maturity exhibit a decrease in HSP production and that exposure to high temperatures provokes HSP production in juvenile whiting. Both adult and juvenile whiting expressed significant increases in HSP69 in response to temperature shocks of 24, 26, 28 and 30 ºC. Juvenile whiting had significantly higher basal levels of HSP69 than adult whiting and showed significantly more protein expression at 24 and 26 ºC. No mortalities were observed in juvenile fish at 30 ºC while 50% of adults suffered mortality at 30 ºC. Juveniles were exposed to temperature increase at 24, 26 and 28 ºC and HSP69 was measured after 24, 96 and 168 h. The HSP69 peaked at 96 h and by 168 h, exposure and returned to levels similar to 24 h post heat shock. Juvenile whiting elevated HSP induction at 96 h after heat shock. This study indicates juveniles have a better ability to cope with high temperature than adult and supports the fish behavioural pattern that younger fish inhabit near shore shallow water and then migrate to inshore deep water towards maturation.

The impact of salinity on KGW was assessed in an attempt to understand the mechanisms by which salinity could potentially influence habitat selection and growth of King George whiting in southern Australia. The experiment included whiting of two age classes, young of the year (YOY) and 2^+ yr, at three salinities (30, 40, 50 ppt). YOY whiting showed no significant difference in length or weight gain, specific growth rate, feed intake, food conversion ratio or condition factor when exposed to the three salinities for 72 d. Plasma osmolality of YOY whiting was not significantly different at any salinity, though it was significantly lower than that of 2^+ yr whiting. The 2^+ yr whiting showed significantly higher plasma osmolality than the YOY. Blood plasma potassium and chloride levels of 2^+ yr fish at 50 ppt were significantly higher than those at 30 ppt and 40 ppt. Blood sodium levels at 50 ppt

were significantly higher than at 30 ppt but the sodium level at 40 ppt was not different from 30 ppt or 50 ppt. Haematocrit of 2^+ whiting was significantly higher at 30 than at 50 ppt while haematocrit at 40 ppt was not different from 30 or 50 ppt. The 2^+ yr old whiting had a more pronounced increase in plasma osmolality and plasma ions at high salinities, indicating poorer osmoregulatory capacity in older fish. This study provides physiological evidence to partially explain habitat occupancy and growth in relation to salinity of different age groups of whiting in southern Australia.

Light is an important environmental factor regulating physiological process and ecological activities in fishes. This research aimed to examine the effect of light on the growth, distribution and body colour of juvenile KGW when exposed to different light levels under laboratory conditions. In this study, the fish were exposed to three light levels (25, 500, and 1000 lux) in triplicate for two months. Comparisons were made for growth, body colour and behavioural changes between treatments. During the experimental period, there were no statistical differences in specific growth rate, survival, growth efficiency and condition factor between the light levels. Although light did not affect swimming speed or the encounter rates, it did influence group flight activity and fish distribution in the tanks. Whiting in low light preferred to spend more time grouped at the bottom of the tank than at medium (500 lux) or high light (1000 lux). Likewise, after having been exposed to low light, whiting exhibited a brighter colour and had fewer body markings and counter-shading than fish in medium and high light intensities. The differences in fish distribution and body colour between medium and high light intensities were not detected. This study offers a new insight into understanding the impact of light on

behaviour and body colour of fish, which may contribute to the spatial distribution, predator avoidance and recruitment of fish in inshore coastal waters.

Adult fish stocks are highly dependent on the recruitment success of juveniles in the nursery grounds. A nursery site for post larval KGW was sampled to assess the potential for food competition with other species at King's Beach in the Barker Inlet, South Australia. All fish species were collected using seine nets with a 1-mm mesh along with concurrent sampling of both pelagic and benthic food resources. Post larval whiting <60 mm consumed primarily harpacticopid copepods and had high (>0.6) diet overlap with Gobidae and Syngnathidae families. Electivity index of fish species indicates that whiting post larvae preferred harpacticoid copepods and amphipods and Clinidae and Scorpaenidae fishes targeted larger prey items such amphipods. The difference in food preference between whiting and Clinidae and Scorpaenidae species was due to mouth morphology. Whiting showed an ontogenetic shift in diet with fish larger than 60 mm consuming less copepods and more amphipods and whiting larger than 120 mm consuming polycheate worms. Competition for food resources between whiting and other fish species in the Barker Inlet nursery ground is likely compromised through differences in temporal and spatial feeding behaviours, mouth morphology, and ontogenetic shift in prey consumption. However, habitat destruction caused by climate change and anthropogenic factors will increase competition for food resources by reducing spatial separation and food availability.

STATEMENT OF DECLARATION

I certify that this thesis is a presentation of my original research work and that it does not contain without acknowledgement, any material previously submitted for a degree or diploma in any university. To the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

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Craig Meakin

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Craig Meakin

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This thesis is dedicated to my children Arion and Lily who give me a reason to try and succeed.

Chapter 1 General introduction

1.0 Introduction

Human activity has induced environmental changes that are becoming increasingly influential to the recruitment and survival of many ocean species (Brander 2007). Pollution, recreational use, urbanization, storm water runoff, dredging and the introduction of invasive species are all human induced changes that have had devastating effects on the natural state of fish nursery areas (Duarte 2002). With intensive use of fossil fuels and vast deforestation, the atmospheric concentrations of carbon dioxide have significantly increased. The accumulation of these 'greenhouse' gases is impacting conditions on earth as they absorb heat from the earth and radiate it back causing overall warming of the planet (Watson et al., 1996). As a result, global mean temperatures are expected to rise by 1–3.5°C by the end of the next century creating the most rapid warming event the planet has experienced in 10,000 years (Watson et al., 1996). The consequences of climate change and increased atmospheric CO_2 are likely to result in alterations to ocean salinity, temperature, pH and sea levels, all of which will impact on many aquatic plants and animals within marine ecosystems (Short and Neckles 1999).

Most aquatic fauna require a specific range of environmental parameters in order to thrive, as they have limited tolerance to rapid change. However, the environmental changes, resulting directly from anthropogenic effects and climate change are occurring faster than many species can adapt (Dulvy et al. 2003). These environmental changes will impact fish populations directly through the physiological responses of fish to sub-optimal conditions. In addition, fish will also be impacted indirectly through the loss of seagrasses and fish nursery habitats as the

abundance of aquatic flora is also altered by environmental changes. While larval and juvenile fish are likely susceptible to natural threats such as predation, competition and food availability, loss of habitat resulting from changes in environmental conditions and anthropogenic destruction will certainly exacerbate these threats. In this introduction chapter, I will (1) review how changes in environmental factors including temperature, salinity and light that can impact larval fish recruitment and fish populations changes in the wild, (2) discuss the importance of resource competition between fish larvae in relation to regulating fish population change and recruitment success in a threatened environment with a particular focus on King George whiting (*Sillaginodes punctata*), (3) discuss the biology and distribution of King George whiting (KGW) as well as the status of the KGW fishery in order to illustrate the importance of understanding the mechanisms underlying KGW recruitment, (4) describe the background of the study site including its location and physical characteristics, and (5) introduce the aims of this thesis and the thesis structure.

1.1 Environmental Factors

1.1.1 Temperature

As fish are ectothermic, their body temperature must conform to the surrounding environment, making temperature one of the most important physical factors influencing fish. Temperature increases due to climate change are predicted to have a considerable impact on fish distribution, spawning sites, timing of migration events and overall productivity/survival (Drinkwater 2005). Furthermore, climate changes can alter ocean current patterns resulting in altered dispersal patterns of fish (Doney et al. 2012). Estuaries are critically important to juvenile fish and are also greatly influenced by environmental factors. Therefore environmental changes

are most likely to influence susceptible juvenile fish during estuary residence (Attrill and Power 2002)

The stressful impacts of high temperature on fish are comprehensively studied although the tolerance and threshold temperatures that initiate stress vary between species. Negative impacts resulting from climate change affect fish directly via biological means and indirectly through ecological factors such as geographic distribution (Drinkwater 2005; Booth et al. 2011), effects on habitat (Micheli et al. 2008; Marba and Duarte 2010), and food sources (Mackas et al. 2007; Kotta et al. 2009). Up to an optimal point, increasing water temperature leads to an increase in fish metabolic activity and fast growth rates (Clarke and Johnston 1999; Johnston 1999). Exceeding optimum temperatures further, increases metabolic demand causing fish to struggle with the maintenance of respiration rates and cardiac function. These stresses will ultimately result in reduced fish growth rates and death (Secor and Gunderson 1998; Neuheimer et al. 2011). Even sub-lethal temperatures cause stress to an individual and lead to increases in hormones such as cortisol which are related to reductions of growth and immune response making fish more susceptible to disease (Snieszko 1974; Pickering and Pottinger 1989).

In considering current climate trends, we must question the likelihood that larval and juvenile fishes inhabiting nursery grounds subject to extreme water and air temperatures are either already experiencing thermal stress, or are likely to experience thermal stress in the future. Heat shock proteins are a useful tool when studying thermal tolerance among populations (Schulte 2007). At high temperatures, aquatic organisms synthesise heat shock proteins (HSPs) to cope with temperature induced stress. The earliest observations of enhanced protein synthesis following heat shock were reported in *Drosophila* cells (Ritosser 1962; Tissiere et al. 1974).

Subsequently, heat shock proteins have since been used in many animal species to examine the stress response of environmental temperature change (Currie and White 1983; Jornot et al. 1991; Lesser and Kruse 2004). Temperature stress can challenge the homeostasis of cells and lead to unfolding of proteins. Heat shock proteins initiated by temperature stress are able to act as molecular chaperones and are responsible for cytoprotection by preventing protein unfolding and aiding in cell recovery (Hendrick and Hartl 1993). These proteins are highly conserved between simple and complex organisms and can be measured within minutes of organisms experiencing sub-lethal environmental disturbances making these proteins commonly utilised as biomarkers.

When utilising heat shock proteins it is important to take into acclimatisation consideration. Differences in temperature tolerance are often a result of thermal acclimatization (Beitinger et al. 2000). This is observed between intertidal and submerged populations of mussels (Hofmann and Somero 1995), Antarctic and temperate notothenioid fish (Carpenter and Hofmann 2000), and intertidal sculpins where enhanced protein protection increases tolerance to extreme temperatures (Nakano and Iwama 2002). The study species used in this thesis is the King George whiting *Sillaginodes punctata* (KGW). Like many marine migrants, KGW experience different environments throughout their lifespan. The offshore waters inhabited by KGW as adults are far less susceptible to rapid temperature fluctuations compared with the dynamic temperatures experienced by juvenile KGW in shallow water habitats. In considering the assessment of thermal tolerance in marine migrants like KGW, we must first question if adult fish have a different thermal tolerance to juveniles and whether this difference, if any, is related to acclimatization or a result of physiological changes attributed to age, as is observed in fish species such as is

zebrafish *Danio rerio* (Murtha and Keller 2003), and rainbow trout *Oncorhynchus mykiss* (Fowler et al. 2009). Controlled laboratory experiments examining the heat shock protein response of adult and juvenile KGW make a sensible starting place in order to begin establishing an understanding of the thermal tolerance of this species and their molecular response to current and future climatic conditions.

1.1.2 Salinity

Juveniles of many marine species in Australia typically inhabit estuarine regions that have intermediate salinity levels, copious amounts of food and shelter from predators allowing survival of these species in their early life. Most teleost fish live in an environment that differs in osmolarity to their plasma. Salt water fish are required to ingest large amounts of salt water while excreting excess salts through their gills in order to maintain homeostasis (Kidder et al. 2006). The energetic cost of osmoregulation varies between fish species but contributes a significant amount to the standard metabolic rate. The review by Boeuf and Payan (2001) highlighted that osmoregulation accounts for 20-50% of total energy expenditure and that many marine fish species, particularly at the juvenile stage, prefer lower than the full strength salinity (35 ppt). Increases in metabolic rates divert energy away from other functions so it is not a surprise that growth is highly influenced by salinity. For instance, growth performance of gilthead sea bream *Sparus aurata* is optimised at 28 ppt, while the growth of juvenile turbot is maximised at 19 ppt (Gaumet et al. 1995). Even typical stenohaline species like halibut *Hippoglossus hippoglossus* can benefit from environments closer to isoosmotic conditions (Imsland et al. 2008). However, this is not always the case, as seen in Senegalese sole *Solea senegalensis* (Arjona et al. 2009) where the tertiary stress response and growth are maximised at 39 ppt.

Changes in metabolic processes may be responsible for the increased growth in fish at lower salinities (Vargas-Chacoff et al. 2011). For example, both Lambert et al. (1994) and Imsland et al. (2010) observed that food conversion efficiency and growth were improved at lower salinity levels without any increase in food intake. It is likely that the interaction between growth and salinity is more complex however as the impacts of salinity are also influenced by numerous factors including temperature (Imsland et al. 2001), toxic contamination (Cardeilhac et al. 1979), swimming activity (Dutil et al. 1997) and fish age where adults migrate to regions of lower salinity (Madon 2002).

Globally, ocean salinity levels average at 35‰. The most important ions in normal salinity seawater are chloride (19.2‰) and sodium (10.59‰) with a number of different ions including potassium (0.38‰), making up the remainder (Hay et al. 2006). In general, blood osmolalities in teleost fish range from 260 to 400 mOsmol (Jobling 1995). Besides a reduction in growth, exposure to high salinity and an inability to osmoregulate adequately, result in a number of different consequences for fish including congestion, hyperplasia and fusion of the gill lamella (Velasco-Santamaria and Cruz-Casallas 2008), cell damage and potassium intoxication (Cardeilhac et al. 1979), reduced disease resistance (Zhang et al. 2011) and even death (Serrano et al. 2011).

The ecological implications of high salinity in early life stages are known to affect later stages of development in many species (Pechenik 2006). Fish species composition, distribution and abundance are often closely related to parameters such as temperature and salinity and are some of the main issues attributed to climate change (Gibson et al. 1993; Hughes 2000; Lotze et al. 2006). In addition, some regions naturally maintain salinity levels that are above average. This places aquatic

flora and fauna in an environment that is particularly susceptible to the impacts of climate change. Juvenile KGW residing in South Australia's gulfs live in inverse estuaries where the salinity is much higher than typical open ocean salinities (de Silva Samarasinghe and Lennon 1987; Fowler et al. 2002). The southern migration of aging fish means that salinity levels experienced by juveniles are likely to be much higher than those typically experienced by adult fish occupying deeper water. In Gulf St Vincent, salinity levels can exceed 42 ppt in the north and eastern sections of the gulf while in Spencer Gulf, juvenile KGW have been observed in water up to 48 ppt (Ham and Hutchinson 2002).

In evaporation dominated regions such as semi enclosed gulfs, increased temperatures result in high evaporation and leads to increased sea surface salinities (Durack et al. 2010). The implication of this process is something that must be understood in order to assess a species ability to survive future climate changes. This is particularly pertinent for species where transport of larvae is primarily determined by ocean currents resulting in settlement locations being passively selected for. This exposes juvenile fish to ambient salinities that may potentially influence growth rates and recruitment success. The salinity tolerance and osmoregulatory abilities of many species including KGW and the consequences of inhabiting regions with salinity far exceeding the ocean average are relatively unknown. Plasma serum osmolality and ion concentration are readily influenced by changes in ambient salinity and frequently used to assess osmoregulation in fishes (Zhao et al. 2011) serving as a useful marker providing understanding of the implications of high salinity to a particular species. Studying the osmoregulatory abilities of KGW will reveal if current and future environmental conditions have the potential to influence their recruitment, growth and distribution.

1.1.3 Light intensity

Elevations in temperature, salinity and light intensity (UV radiation) are all environmental stressors that will be influenced by climate change (Johnson and Katavic 1984; Watson et al. 1996). Fish have the ability to alter their body colour through the aggregation or dispersal of chromatophores within the skin thus altering the light that is absorbed or reflected from the surface of the skin (Fujii 2000). The aggregation or dispersal of chromatophores is highly influenced by light intensity either directly or indirectly via neural and endocrine regulation as a result of visual stimuli (Fujii 2000). In general, fish exposed to high light intensities exhibit a darkening of the skin (Han et al. 2005; Pavlidis et al. 2008) related to protection from UV radiation (Lowe and Goodman-Lowe 1996). The cryptic colouration of fish plays an important role to survival by providing camouflage for effective predation and predator avoidance through countershading, background matching, or disruptive colouration (Ruxton et al. 2004; Stevens and Merilaita 2009a; Stevens and Merilaita 2009b). The implications associated with changes in environmental light conditions altering fish colouration is another important consideration in order to understand factors influencing successful recruitment. Direct influence of light intensity on shallow water species may alter their cryptic colouration potentially making fish either better camouflaged or more detectable to predation.

Light intensity can also influence fish in numerous other ways. The consequences of low light intensities include a reduction in prey capture rates and feeding efficiency (Benfield and Minello 1996; Fraser and Metcalfe 1997). Importantly, light is also responsible for variations in growth (Wallace et al. 1988; Downing and Litvak 1999), and survival (Soderberg 1990; Tamazouzt et al. 2000; Cerqueira and Brugger 2001). In larval fish, critical stages of development such as

metamorphosis (Puvanendran and Brown 2002) swim bladder inflation (Battaglene et al. 1994) and metabolism (Appelbaum and Kamler 2000) are all influenced by light. Behaviour is highly related to light level. Diel variation in light intensity often triggers changes in distribution, activity levels, feeding and predator avoidance behaviours (Prenda et al. 2000; Sloman et al. 2006). At high intensities, fish species such as minnows show avoidance and shelter seeking behaviour which increases swimming activity (Harden-Jones 1955). Variations in light intensity can also provoke antagonistic behaviours in some fish such as white seabream *Diplodus sargus* (Castro and Caballero 2004) and African catfish *Clarias gariepinus* (Britz and Pienaar 1992). Influence on cannibalistic behaviours is also observed (Appelbaum and Kamler 2000; Han et al. 2005). Other behavioural influences include changes in schooling (Glass et al. 1986), fright reaction, and colour attractiveness (Loukashkin and Grant 1959).

Light intensity is an important environmental factor which affects fish physiology and should therefore be included in studies assessing the impact of climate change to a species (Boeuf and Le Bail 1999). The impact of light intensity on swimming activity, behaviour, growth, survival and body colouration in KGW has not been studied and the effects are currently unknown.

1.2 Competition for food resources

Temperature, salinity and irradiation all have an impact on the local environment. The consequences of climate change will not only result in direct pressure on fish through biological and physiological responses, but also indirectly via prey availability and habitat utilisation. Seagrass beds are important structures providing habitat for numerous fish species (Pollard 1984). Direct anthropogenic

impacts such as physical damage from boats and fishing activity, dredging, coastal development and eutrophication as well as climate impacts such as rising sea levels, increased UV radiation, temperatures, storms and erosion all contribute to the worldwide decline of seagrass meadows and alterations of food webs being currently observed (Duarte 2002). In the Mediterranean, climate change is predicted to cause the functional extinction of beds of *Posidonia* sp. by the year 2050 (Jorda et al. 2012). Prey density is often highly correlated with sea grass density with lower epifaunal prey abundance observed in areas of minimal seagrass cover (Connolly 1994). Loss of food and shelter is responsible for poor recruitment of juveniles, often as a result of increased predation by larger predators or competition for scarce resources between inter and conspecifics (Muehlstein 1989; Pihl et al. 2006; Warren et al. 2010). While each organism may have its own niche within the environment it lives, climate induced changes such as habitat loss will exacerbate interactions between species leading to greater competition for resources such as habitat and food (Suchanek 1994; Toscano et al. 2010). In order to understand how climate change will impact a species, it is imperative to first understand how and where that species fits into the nursery ecosystem in regards to food utilization and competitive interactions.

Nursery systems are home to a wide variety of fish species, some of which are permanent residents while others like KGW are marine migrants that utilize protected habitats as juveniles before returning to offshore waters. In the Gulf St Vincent and Spencer Gulf, KGW juveniles have a strong association to seagrass beds relying on these habitats for protecting from predators as well as providing structure for prey species (Jones 1984). How do KGW and other seagrass associated fish interact in terms of their food utilisation and feeding habits while occupying this

habitat? Currently the only study examining dietary interactions specific to KGW was conducted by (Hyndes et al. 1997) who assessed diet between the six *Sillaginid* species of Australia's coastal waters. However, no study has examined the interaction between the other numerous species that share the same habitat as KGW, particularly at the critical early life stages when they share the same food source.

Competitive interactions between fishes can occur when an individual depletes food before the arrival of a competitor (exploitation competition), when competitors attempt to be first to consume a food source (scramble competition), or through direct aggression between competitors (contest competition) (Ward et al. 2006). Offshore spawners whose offspring recruit and reside in inshore estuaries face competitive interactions impacting upon both growth and survival (Craig et al. 2007). Other ramifications of increased competition during settlement in marine systems include temporary shifts in food preference during periods of depleted resources (Huh and Kitting 1985), increases in antagonistic behaviours between competing species resulting in increased risk of being preyed upon (Toscano et al. 2010), and density-dependent starvation of recent settlers (Olafsson et al. 1994).

Competition between species can however be reduced within an ecosystem through differences in prey preference (Darnaude et al. 2001), habitat selection (Labropoulou and Machias 1998), variation in mouth morphology, ontogenetic differentiation, migration (Hyndes et al. 1997), and diel variations in feeding times (Pereira et al. 2010). Even when fish are consuming similar prey items, competition can be reduced through differences in the contribution of each particular prey type (Lek et al. 2011). Currently, knowledge about competition for food resources with other species in the same nursery ground is lacking for KGW and requires

investigation to further understand the consequences of climate and anthropogenic change and the factors that may constrain recruitment of this species.

1.3 Study species: King George whiting *(Sillaginodes punctata)*

The shallow gulfs of South Australia face considerable stress from both climatic conditions, and human induced changes. The threat of climate change is at the forefront of recent research. The environmental factors discussed above will likely impact upon all species of fish living in seagrasses differently. Fish of commercial value are often the first species studied in terms of their response to environmental change. Changes to population of commercially important species are often just as crucial outside the water as it can have wider impacts to local economies. In South Australia, King George whiting (KGW) is one of South Australia's most valuable finfish species highly targeted by commercial and recreational fishers and makes this species a suitable species to evaluate, although the results from this study can likely be applied to numerous other fish species. The current biological and ecological research on KGW is mainly focused on whiting life history and reproductive biology (Hyndes et al. 1998; Fowler et al. 1999), migration (Fowler et al. 2002), transport and settlement of juveniles (Fowler et al. 2000a) and to a lesser extent, on diet (Connolly 1995; Hyndes et al. 1997), as well as issues pertaining to aquaculture (Kumar et al. 1997; Ham and Hutchinson 2002; Reuter et al. 2003). However, the response of KGW to abiotic stressors is largely unknown.

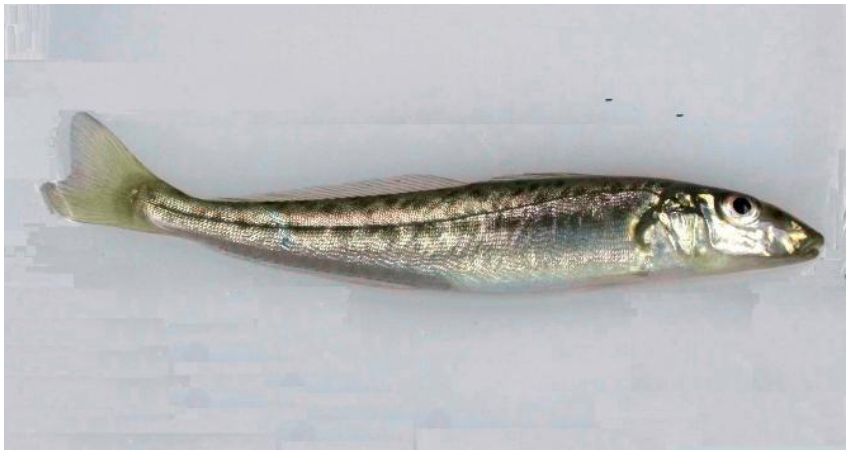

Figure 1.1. A juvenile King George whiting captured from seagrass beds in the Barker Inlet South Australia.

1.3.1 Biology, distribution and life cycle

King George whiting (KGW) is typically characterised by a light brown upper body covered with distinctive brown spots. The lower half of the body is silver and the fins are slightly greenish brown (Gommon et al. 1994). These fish are endemic along the southern Australian coastline ranging from the south west corner of Western Australia, the northern coasts of Tasmania, and as far north as Sydney on Australia's eastern coast (Paxton et al. 1989). Juvenile fish often inhabit shallow, protected waters utilizing cover from seagrass beds (*Zostera* and *Posidonia* spp.) but will also inhabit areas of bare sand (Hyndes et al. 1996; Jenkins et al. 1997). Adult fish migrate into deeper, more exposed waters and are found over broken bottom and rocky reefs (Fowler et al. 2002).

The main spawning areas of KGW are located in the deep offshore waters north of Kangaroo Island at the mouth of the Gulf St Vincent (Fowler et al. 2002). The presence of multiple stages of oocyte development within KGW gonads suggest this species to be a batch spawner with gonad maturation and spawning occurring from early March until late May (Fowler et al. 1999). Larvae are then transported via

ocean currents to the northern parts of the gulf. This larval and pre-settlement stage is comparatively long compared to most fish species and occurs over 80 – 120 days. Post larvae then settle into suitable seagrass habitats in protected bays along the coast (Fowler and Short 1996). Immature KGW remain in these protected habitats for up to 2 yr before beginning to move offshore into deeper water where they inhabit reefs and *Posidonia* spp. seagrass beds (Jones et al. 1990; Fowler et al. 2002).

From 2-4 yr the whiting are capable of migrating several hundred kilometres within a period of a few months. Net movement of whiting from the northern grounds is in a southerly direction as 4 yr old KGW migrate towards the southern spawning grounds and replenish the spawning stocks (Fowler et al. 2002; McGarvey et al. 2003). Fish in the northern waters are typically made up of KGW in the 3-4 yr class. While these fish are of an age and size to facilitate spawning, these fish are not often observed in spawning condition. Contrary to this, fish of the same age in southern waters are observed to spawn and the population contains KGW of varying age classes from 2 to 18 yr. This indicates that migration is unidirectional and that KGW do not return to the northern waters (Fowler et al. 1999; Fowler et al. 2000b).

1.3.2 King George Whiting diet

KGW diet has been studies in all three of mainland Australia's southern states with reasonable similarity. Throughout pelagic transportation, KGW consume primarily planktonic calanoid copepods (Moran et al. 2004). Recently settled post larvae whiting are considered generally weak swimmers and while they are capable of rapid vertical movement, their prolonged swimming ability is poor (Jenkins and Welsford 2002). The occurrence of planktonic prey items decreases with increasing transitional age from settlement. Dominant prey items of recently settled KGW

contain primarily benthic harpacticoid copepods, gammaridian amphipods with small occurrences of calanoid and cyclopoid copepods, ostracods, mysids, tanaids, cumaceans and caridean shrimp (Connolly 1995; Jenkins et al. 1996).

As KGW grow, they begin to occupy less dense seagrass and larger amphipods, prawns, polychaetes and crabs become more common (Robertson 1977; Hyndes et al. 1997). This shift in food preference occurs at 5-6 months (75-124 mm) of age with body size, mouth gape and availability of prey all thought to contribute to the change in food preference (Hyndes et al. 1997). Throughout the 74-224 mm size range 30-35% of gut contents consists of amphipods however as fish grew bigger than this, amphipod contribution reduces to zero. Contrary to this, consumption of polycheates remain high even in the largest fish up to 325 mm. Crabs are only found in largest individuals while 15% of gut volume consists of carid shrimp in whiting only over 175 mm in length. In general, stomach contents analysis of whiting species in WA shows that crustaceans and polychaetes are the most common prey items consumed (Hyndes et al. 1997).

1.3.3 Current whiting fishery and management

The systematic southward migration of maturing KGW exposes this species to heavy fishing pressure. KGW must navigate through the Gulf St Vincent while avoiding a gauntlet of recreational and professional fishing vessels. During the period of 1999-2002 there was a significant downturn in the KGW fishery, resulting in changes to management. This included a reduction of the personal bag limit from 20 to 12 fish per day, and an increase in the minimum size limit from 30 to 31 cm. Boat limits were also reduced from 60, to 36 fish per day. The commercial sector is also restricted in terms of fishing gears and locations (Fowler et al. 2011). South

Australia produces the highest national catches of KGW (ABARE 2010). In South Australia, KGW remain the highest value species per unit weight and is heavily targeted by commercial and recreational fishers (Fowler et al. 2011). Across the state, recreational angling contributes 34% of the total KGW catch while in Gulf St Vincent, the recreational catch makes up 48% of the total catch due to its proximity to metropolitan Adelaide (McGarvey et al. 2005). Exploitation rates on the west coast and Spencer Gulf have declined since 1992 while in Gulf St Vincent, the exploitation rate has increased since 2001. While the fishery is currently classified as stable, there are reports that breeding stock are now being targeted more heavily due to the rise in charter operations further increasing the pressure on this species (Fowler et al. 2011).

1.4 Study area: Barker Inlet, Gulf St Vincent, South Australia

1.4.1 Location and environmental characteristics

South Australia is subject to large seasonal fluctuations in temperature. Average winter temperatures are around 16 °C with minimum temperatures as low as 0 °C. Summer temperatures average around 30 °C with maximum temperatures exceeding 40 °C (BOM 2011). Recent weather in south eastern Australia has seen record heat waves resulting in extended periods where temperatures remain 12-15 °C above average in South Australia and Victoria (National-Climate-Centre 2009). South Australia's climate and shallow gulfs make it an ideal study site to assess the impacts of climate change on seagrass associated fish.

South Australia has two main gulfs, the Spencer Gulf and Gulf St Vincent. Gulf St Vincent is a 7,000 km^2 triangular shaped marine embayment located at latitude 35° S and lies just west of the city of Adelaide. From head to mouth

(Troubridge Shoal-Cape Jervis line) it is 145 km in length, with an average depth of 21 m (de Silva Samarasinghe and Lennon 1987, Cann et al. 2009). At the head it is ~5 m deep while at the mouth it is up to 45 m deep (Petrusevics 1993). Kangaroo Island lies at the mouth of Gulf St Vincent 13 km from the tip of the Fleurieu Peninsula and about 40 km from the Yorke Peninsula (Fig. 1.2).

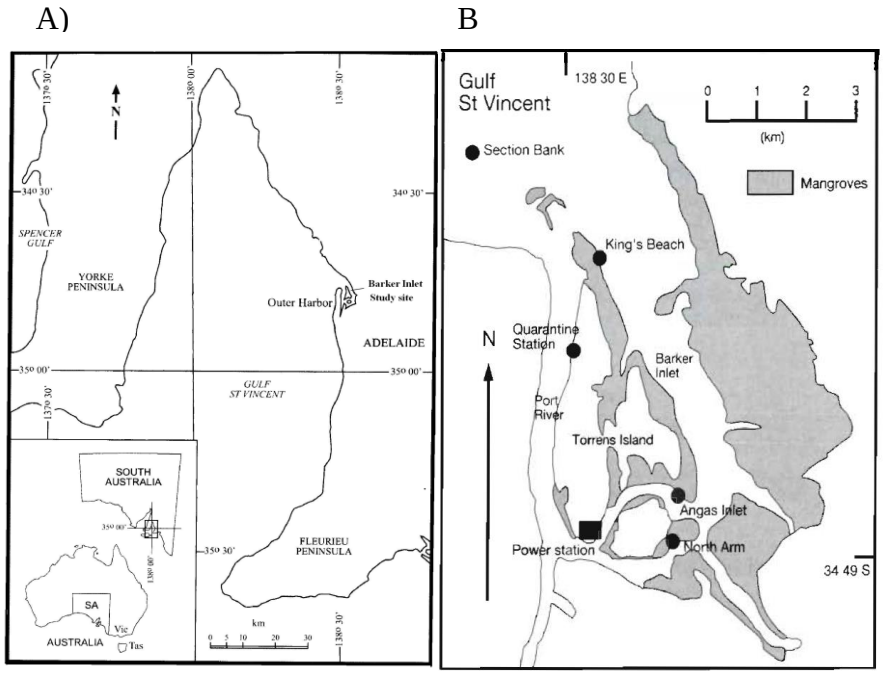

Figure 1.2. Location of Gulf St Vincent (A) and Barker Inlet (B) on South Australia's coastline. Adapted from Cann et al. (2009) and Jackson and Jones (1999).

The gulfs of South Australia have a semi-arid climate with annual precipitation less than 500 mm/yr. Gulf St Vincent has a large surface area and is relatively shallow making it highly influenced by evaporation, rainfall and irradiation (de Silva Samarasinghe and Lennon 1987). Located in the driest state of the driest continent on earth, Gulf St Vincent experiences evaporation rates exceeding precipitation by the order of 400 % (Kampf et al. 2009). In addition, Gulf St Vincent receives very little fresh water as there are no permanent inflows of fresh water from rivers or creeks into the gulf (Petrusevics 1993). These substantial evaporation rates are responsible for the Gulf St Vincent being an inverse estuary with a positive salinity gradient towards the head of the gulf which exceeds salinity levels in the ambient ocean (Kampf et al. 2009). Due to protection from Kangaroo Island, the gulf is relatively sheltered from significant wave action and has tidal movements in the order of 2-3 m. Water flow within Gulf St Vincent is characterised by a clockwise circulation. Water enters the gulf from the northern side of Kangaroo Island and first travels along the western coastline before reaching the head of the gulf where it is then directed southwards flushing highly saline water out of the gulf along the eastern metropolitan coastline (Samarasinghe 1998). Circulation within the gulf is quite slow and flushing of water towards the head of the gulf takes greater than 1 yr allowing ample time for evaporation to impact salinity levels. Salinity levels within the gulf are higher than typical oceanic levels with salinity towards the head reaching over 42 ppt in summer and reducing only as low as 39 ppt in winter. At the mouth of the gulf, salinity levels remain much more stable remaining around 36.5 ppt throughout the year. In addition to this, Gulf St Vincent is also subjected to the unique phenomena known as ‘dodge tides’ where the diurnal constituents (M2 Lunar

and S2 Solar) cancel each other out causing periods where there is little or no tidal movement. These events occur up to twice a month during which time, the dilution and dispersal of salt, pollutants and warn water are considerably reduced (de Silva Samarasinghe and Lennon 1987). Water temperature within the gulf varies considerably with the seasons. Towards the head of the Gulf, winter temperatures can be as low as 11 °C while summer temperatures can reach as high as 26 °C (Dimmlich et al. 2004) (Fig. 1.3).

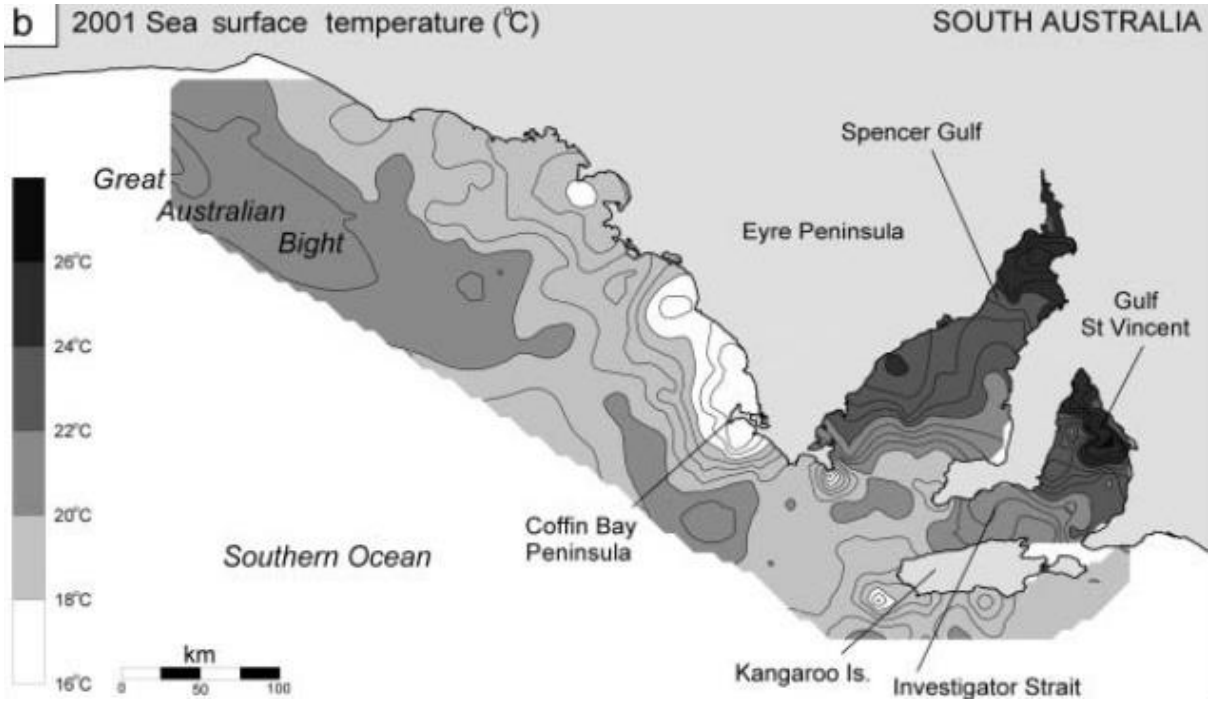

Figure 1.3. Summer time sea surface temperatures in South Australia (Dimmlich et al. 2004).

1.4.2 Port River/Barker Inlet estuary system

The many sheltered embayments within the gulf provide protected nursery grounds for a number of commercially and recreational fisheries including King George whiting (Jones et al. 1990). One important King George whiting nursery area is in Adelaide's largest estuary system called the Port River/Barker Inlet and is where juvenile fish were studied and collected for this thesis. This estuary system is located on the eastern side of the gulf approximately 20 km north of the city of Adelaide and is considered regionally important as a nursery for many fish species in the Gulf St Vincent (Jones 1984) (Fig. 1.2 B). The Port River/Barker Inlet estuary receives intermittent fresh water inflow and comprises of areas of mud flats, samphire marshes and mangroves. Its sediments are fine to course sand with seagrass beds (*Zostera* spp. and *Heterozostera* spp.) providing habitat for juvenile fish (Thomas et al. 1986). The nursery ground on the eastern coast of GSV inclusive of the Port River/Barker Inlet faces considerable environmental stress from treated effluent, stormwater runoff, thermal waste and oily ballast water (Petrusevics 1993). Since the 1960's, this region has considerably changed in regards to its habitat structure and distribution. The discharge of pollution into the gulf is responsible for substantial loss of seagrass beds along the metropolitan shoreline and has imposed additional challenges to the survival of many organisms (Tanner 2005). Within the Port River/Barker Inlet, nutrient concentrations exceed trigger values indicative of biological stress and suggest that the health of the ecosystem is in serious decline (Bryars et al. 2006).

With King George whiting occupying such a dynamic environment in terms of chemical (salinity), meteorological (temperature, radiation) and anthropogenic factors, there are some critical issues that require study in order to increase our knowledge on the biological and ecological implications of current and future environmental condition to this important fish species.

1.5.1 Thesis aims

The overall aim of this thesis is to assess the environmental factors potentially constraining larval recruitment of KGW in Gulf St Vincent through laboratory studies in order to understand the possible impacts of climate change and anthropogenic effects. This thesis attempts to understand how KGW respond to the abiotic stressors currently experienced by this species in the wild and estimate resource competition between KGW and other major species that occupy the same habitat. The results will broaden our understanding of the impacts that current and future environmental conditions will have on the ecological and physiological status and recruitment of this important species. Specific aims of this thesis are as follows:

1. To investigate the molecular response of KGW to acute and long term changes in temperature by examining the production of heat shock proteins in 1 y and 3 y old fish and assess their vulnerability to climate change (Chapter 2).

2. To reveal the osmoregulatory ability of 1 y old and 2 y old KGW by looking at blood plasma ion concentrations in order to assess if (1) the salinity levels currently experienced in the gulfs are impacting growth and survival of 1y old recruits and (2) whether future increases in salinity will have a detrimental impact on fish (Chapter 3).

3. To understand how increases in light intensity will influence or regulate the growth, survival, behaviour and body colouration of 1 y old KGW (Chapter 4).

4.To study the dietary interactions of young of the year KGW recruits with marine migrants and permanent resident species as well as older KGW conspecifics in order to understand how these species may be impacted by seagrass loss induced by climate change and anthropogenic effects (Chapter 5).

1.5.2 Thesis structure

This thesis consists of 6 chapters including a general introduction highlighting background information and the aims of the thesis (Chapter 1), four data chapters presenting original research (Chapters 2,3,4 and 5), as well as a general discussion (Chapter 6) offering conclusions and areas for future research. Each datum chapter is written as an independent manuscript suitable for publication and therefore *may contain slight repetition* between the chapters of the thesis.

Chapter 1 contains background information relevant to this thesis. Information is provided into the biology and life history of King George whiting (*Sillaginodes punctata*) and justifies the need for the research presented as well as the aims of this thesis.

Chapter 2 examines the response of KGW to rising temperatures. Heat shock proteins were used to assess the response to acute and chronic temperature stress. Comparisons were made in the response of two different age classes of whiting (1 and 3 y old) in an attempt to understand the implications of climate warming on this species. This chapter has been peer reviewed and published in C*omparative*

Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 172: 46-51, 2014.

Chapter 3 reveals the growth and survival of young of the year (YOY) KGW reared at three different salinities (30, 40, 50 ppt) for 72 d, as well as the osmoregulatory capacity of 1 and 2 y old fish. This chapter has been peer reviewed and published in *The Journal of Applied Ichthyology* 27: 1316–1321, 2011.

Chapter 4 This studies the impacts of light intensity and offers insight into its effects on growth, behaviour and body colouration of King George whiting in relation to spatial distribution, predator avoidance and recruitment in inshore coastal waters. This study has been accepted for publication in the *New Zealand Journal of Marine and Freshwater Research* 46: 111-123, 2012.

Chapter 5 assesses the potential for competition to impact upon recruitment of post larval King George whiting in the nursery grounds of the Barker Inlet. In this study, I have examined the diet, diet preference, niche breadth and food overlap between King George whiting and a number of other fish species that utilise the same habitat to reveal trophic relationships. This chapter has been resubmitted to *Hydrobiologia* for consideration acceptance after revision (submitted on 28 February 2014).

Chapter 6 summarises the main findings and discusses the broader implications of this study in relation to changes in environmental conditions and their impact on juvenile recruitment of KGW. Suggestions for further research that will enable greater understanding of the factors influencing King George whiting have been made.

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Chapter 2 Thermal tolerance in juvenile King George whiting (*Silliginodes punctata*) reduces as fish age and this reduction coincides with migration to deeper colder water

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2.1 Abstract

Heat shock proteins (HSP) are sensitive and readily produced under thermal stress in many fish species and thus serve as a useful stress bio-indicator. Two experiments were conducted to test the hypothesis that King George whiting *Sillaginodes punctata* (KGW) approaching sexual maturity exhibit a decrease in HSP production and that exposure to high temperatures provokes HSP production in juvenile whiting. Both adult and juvenile whiting expressed significant increases in HSP69 in response to temperature shocks of 24, 26, 28 and 30 ºC. Juvenile whiting had significantly higher HSP69 than adults and expressed more HSP69 at 24 and 26 ºC. No mortalities were observed in juvenile fish at 30 ºC while 50% of adults suffered mortality at 30 ºC. Following exposure of juveniles to 24, 26 and 28 ºC, HSP69 was measured at 24, 96 and 168 h. HSP69 peaked at 96 h and returned to the 24 h level after 168 h exposure. This study indicates that juveniles can cope with high temperatures better than adults, which offers a partial explanation to fish movement patterns in nature where younger fish inhabit near shore waters and then migrate to deep water towards maturation. Further, this work implies that KGW growth and recruitment can be affected by increasing temperatures due to global warming.

2.2 Keywords: temperature, survival, recruitment, migration, global warming

2.3 Introduction

The natural environment is subject to thermal fluctuations that can exert stress on aquatic organisms (Sanders, 1993). Within a temperate zone, seasonal changes in temperature can affect fish physiology, distribution and behaviour in various habitats including rivers, lakes and oceans (Evans and Claiborne, 2006). At high temperatures, organisms synthesise heat shock proteins (HSP) to cope with temperature induced stress. The earliest observations of enhanced protein synthesis following heat shock were reported in *Drosophila melanogaster* cells (Ritosser, 1962; Tissiere et al., 1974). Subsequently, heat shock proteins have been used in many animal species to examine the stress response to environmental temperature change (Currie and White, 1983; Jornot et al., 1991; Lesser and Kruse, 2004). Temperature stress can challenge the homeostasis of cells and lead to unfolding of proteins. Heat shock proteins initiated by temperature stress are able to act as molecular chaperones and are responsible for cytoprotection by preventing protein unfolding and aiding in cell recovery (Hendrick and Hartl, 1993). Heat shock proteins are highly conserved between simple and complex organisms and are rapidly synthesised in response to organisms experiencing sublethal conditions (Lindquist and Craig, 1988). HSPs are an excellent biomarker for environmental change because they can be sampled from non-terminal tissue such as red blood cells (Lund et al., 2003) and do not respond to handling stressors such as cortisol (Vijayan et al., 1997). The temperature to initiate a heat shock response however varies greatly between fish species (Schlesinger, 1990).

There are a number of HSP families including HSP90 (85-90 kDa), HSP70 (68-73 kDa) and proteins of low molecular weights (16-24 kDa) (Evans and Claiborne, 2006). The HSP70 kDa family are highly conserved proteins observed in

all species examined and are induced by a variety of biological stresses including heat stress (Lewis et al., 1999). Heat shock proteins in fish are found in numerous tissues such as the liver, brain, kidney, gill and blood cells (Currie and Tufts, 1997; Das et al., 2005). Using these somatic tissues to assess the effect on heat shock proteins is a terminal process for fish. However, utilising red blood cells offers a means of assessing fish without the need for terminal sampling and in fact red blood cells show a clear pattern of response to temperature stress both *in vivo* and *in vitro* (Currie et al., 2000). Lund et al., (2003) suggested that red blood cells are suitable biomarker tissues of heat stress with either HSP mRNA or HSP protein. The red blood cell HSP mRNA provides a sensitive indicator of heat stress when compared to other tissues such as brain, heart, gill, liver and muscle, whereas HSP proteins offers a more sustained response retaining elevated levels of HSP for a longer period post temperature shock (Lund et al., 2003).

In an unstable environment, organisms tend to produce higher levels of constitutive HSPs than those living in a stable environment. This has been observed between intertidal and submerged populations of bivalves (Hofmann and Somero, 1995; Li et al., 2007), Antarctic and temperate notothenioid fishes (Carpenter and Hofmann, 2000), and intertidal sculpins *Oligocottus maculosus* and *O. snyderi* where enhanced protein protection increases tolerance to extreme temperatures (Nakano and Iwama, 2002). Constituent levels of HSP are involved in ensuring appropriate spatial and folding arrangements of cellular proteins important for growth and development (Hartl, 1996). Furthermore, the age of organisms affects the amount of HSP production and thermal tolerance in drosophila (Sorensen and Loeschcke, 2002), mammals (Soti and Csermely, 2003) and fish (Murtha and Keller, 2003). Basal and induced levels of HSPs in zebrafish *Danio rerio* are greater in juveniles as

opposed to mature fish (Murtha and Keller, 2003) while Fowler et al. (2009) reported an enhanced HSP response in rainbow trout juveniles compared to adults when exposed to high stream temperatures. Given the role HSPs play in cytoprotection, low HSP production indicates that thermal stress may have a more detrimental effect on health in older fish.

Globally, climate warming continues to threaten population size and structure, survival, and species diversity in aquatic communities (Portner and Knust, 2007). Southern Australia has recently been experiencing extreme environmental conditions in regards to drought and record high temperatures (National-Climate-Centre, 2008, 2009). King George whiting (KGW) *Sillaginodes punctata* is the highest value fish species in southern Australia and is heavily targeted by recreational and commercial fishers, particularly in Gulf St Vincent where exploitation rates have been increasing since 2001 (Fowler et al., 2008). If the trend of increasing temperature continues in conjunction with increasing fishing pressure then fish recruitment may be further reduced. Juvenile whiting inhabit inshore shallow coastal waters where extreme heat waves have frequently occurred in the past decade (National-Climate-Centre, 2009). Therefore, it is necessary to understand the physiological responses of KGW to thermal stress to explore potential causes of low recruitment and potential impacts of climate change. KGW spawn in the deep waters of southern Gulf St Vincent, and most post larval recruits begin to arrive in the shallow seagrass nursery grounds in winter (Fowler et al., 2002). Water temperatures in winter range from 11 to 15 °C (Jones et al., 1996), but as winter ends, water temperatures quickly rise to around 26 ºC in the upper Gulf St Vincent (Dimmlich et al., 2004). In nursery areas such as the Port River/Barker Inlet system, young whiting are often found in water less than one meter deep where extreme

water temperature fluctuations often occur. In certain areas of the Port River, thermal effluent from a nearby power station can cause water in the Angus inlet and North arm to approach 30 °C (Jones et al., 1996). The Port River/Barker Inlet region is subjected to extreme weather conditions with summer air temperatures occasionally exceeding 45 °C (National-Climate-Centre, 2008, 2009). Long and short term temperature change resulting from climate warming, seasonal weather and extreme diel fluctuations during heat wave events may all contribute to thermal stress particularly on fish in shallow water environments.

KGW is a typical marine migrant, occupying sheltered waters as juveniles and moving away as adults and was chosen as a representative species to evaluate the thermal stress experienced by fish living in shallow nursery grounds. The level of thermal stress on species in the shallow gulf and nursery areas will have important implications to the overall recruitment success of KGW. We hypothesise that KGW tolerance to high temperatures reduces as fish grow and should correspond with the migratory pattern from shallow nursery water to deep waters after fish reach 2-3 year old. The study of heat shock protein response in KGW will provide insight into thermal tolerance, migration, recruitment success and behaviour of other relevant estuarine opportunist species facing temperature challenge. The aim of this study was to assess if heat shock proteins from KGW red blood cells would respond to changing environmental water temperatures. As KGW migrate away from nursery ground to deeper water at around 3 years, we also tested if thermal tolerance was reduced with fish age and if this coincided with migration away from nursery areas.

2.4 Materials and methods

2.4.1 Acute heat shock treatments

Heat shock treatments and blood sampling techniques were approved by, and implemented in accordance with the requirements set out by the Flinders University Animal Welfare Committee under permit number E218. All fish were acclimatised in communal holding tanks at 18 °C for 2 months. Prior to heat shock treatments, the fish were moved to individual holding containers and acclimatised for 24 h at 18 °C to the experimental conditions. The heat shock was performed by raising the temperature from 18 ^{o}C to 24, 26, 28, or 30 °C for 2 h, maintaining the target temperature for 2 h, and then gradually returning the water to 18 ^{o}C over a 2h period. Fish were then allowed 2 h to recover before taking blood samples. Each treatment temperature and age group was replicated with four separate fish (1 yr, 17.4 g ± 4 SD, and 3 yr, 124.5 g ± 27.1 SD). Control samples were taken 24 h after fish were placed into individual tanks at 18 °C without heat shock.

2.4.2 Extended heat shock

Young whiting usually inhabit natural waters subjected to extended periods of increased temperature. Therefore, only 1 yr (15.5 g ± 6.5 SD) fish were used to assess the impact of extended heat shock. As with acute shock treatment, whiting were held at 18 °C in individual tanks and the temperature was slowly raised from 18 ^{o}C to 24, 26, and 28 °C at a rate of 1 ^{o}C per hour and then fish were maintained at the treatment temperatures for 168 h. Fish were sampled at 24, 96 and 168 h after continuous exposure to the treatment temperatures. Each treatment temperature was replicated with four separate fish.

2.4.3 Obtaining blood samples

Following heat shock treatments, whiting were anaesthetized in a solution of benzocain (0.135 g/L) until the fish lost equilibrium and was unresponsive to handling. A volume of 0.25 ml of blood was drawn from the caudal vein using a 29 gauge needle (0.33 × 12.7 mm) inserted ventrally through the muscle half way between the anus and the caudal fin. Blood was transferred to 1.5 ml eppendorf tubes and centrifuged for 2 min at 13 000 *g* force and 4 °C to separate blood cells from plasma. Plasma was removed from the blood cells and placed in a separate eppendorf tube and both blood cells and plasma were frozen in liquid nitrogen and stored at -80°C. Fish were recovered in aerated seawater after blood was taken.

2.4.4 Western Blot HSP determination

Approximately 200 µl of blood cells were placed into an eppendorf tube along with 200 µl of radioimmunoprecipitation assay buffer (Sigma-Aldrich St Louis, MO, USA) and vortexed for 30 sec to completely lyse the blood cells. Western blots for HSP determination were conducted as described by Li et al. (2007) Briefly, samples containing 40 µg protein were resolved on a 12% pre-cast SDS-PAGE gel (Bio-Rad Protean TGX, Hercules, CA, USA). Proteins were transferred onto a polyvinylidene difluoride (Amresco E578, Solon, OH, USA) membrane and probed using a monoclonal mouse anti-HSP70 antibody (MA3-006, Affinity Bioreagents, Golden, CO, USA; 1/5000) followed by a polyclonal rabbit anti mouse secondary antibody (Dako, No. P0260, Glostrup, Denmark; 1/1000). SuperSignal West Pico Chemiluminescent substrate reagents (Thermo Scientific, Rockford IL, USA) were added to detect the chemiluminescent signal using a LAS-4000 Fuji doc.

To normalise between blots, a standard using a combination of samples was aliquoted into each separate blot and stored at -80 °C. In addition, blots were probed and corrected for the housekeeping gene actin using a pan-actin antibody (Cell signalling, #4968, Danvers, MA, USA; 1/1000). Analysis of bands was performed with the 1D analyses software package, Quantity one (Bio-Rad version 4.5.2) to measure the band density/mm^2 and molecular weight.

2.4.5 Statistical analysis.

All analyses were conducted with the program SPSS v 18. Data were assessed for normality and where violations were incurred in the extended heat shock data for HSP72, data were log-transformed. For the acute heat shock treatment, two-way ANOVA was utilised to assess for differences in band density between temperature and the two fish age classes. The significance of two-way interactions was first examined before the main effects. When the main effect was significant, the post hoc procedure (Duncan's test) was used for multiple comparisons to explore the temperature effects within each fish age class. In the extended thermal exposure study, SPANOVA was used with temperature as the between subjects variable and the time of post heat shock as the within subjects variable. Fish that died as a result of treatments were excluded from analysis to ensure that all fish in the sample had the same time to synthesise HSP during heat shock. The level of significance was set at $P < 0.05$ for all tests.

2.5 Results

2.5.1 Acute temperature shock.

All 1 yr fish survived at all temperature shocks tested, but 50% of the 3 yr fish in the 30 ºC treatment died after one-hour holding at this temperature. At around 50 min, 3 yr fish began to show increased lethargy and then quickly became moribund as their bodies entered a state of muscular seizure leaving them in an immediate state of rigamortis at the point of death. At all temperatures tested, protein bands at 72 kDa and 69 kDa in mobility were detected (Fig. 2.1A). Band density of HSP72 showed no particular trend in relation to the heat shock temperature and mortality rates. Two-way ANOVA showed that the 72 kDa protein band was not significantly affected by age ($P = 0.607$, Fig. 2.2A) or temperature ($P = 0.811$) and there was no interactive effect between age and temperature ($P = 0.608$). One way ANOVA analyses showed that the band density of 72 kDa at 26 °C was significantly higher in 3 yr old fish than 1 yr old fish ($P = 0.037$). However no difference was observed between any other temperatures ($P \geq 0.345$). Band density at each thermal shock was not significantly different from the 18 ºC control (Fig. 2.2A).

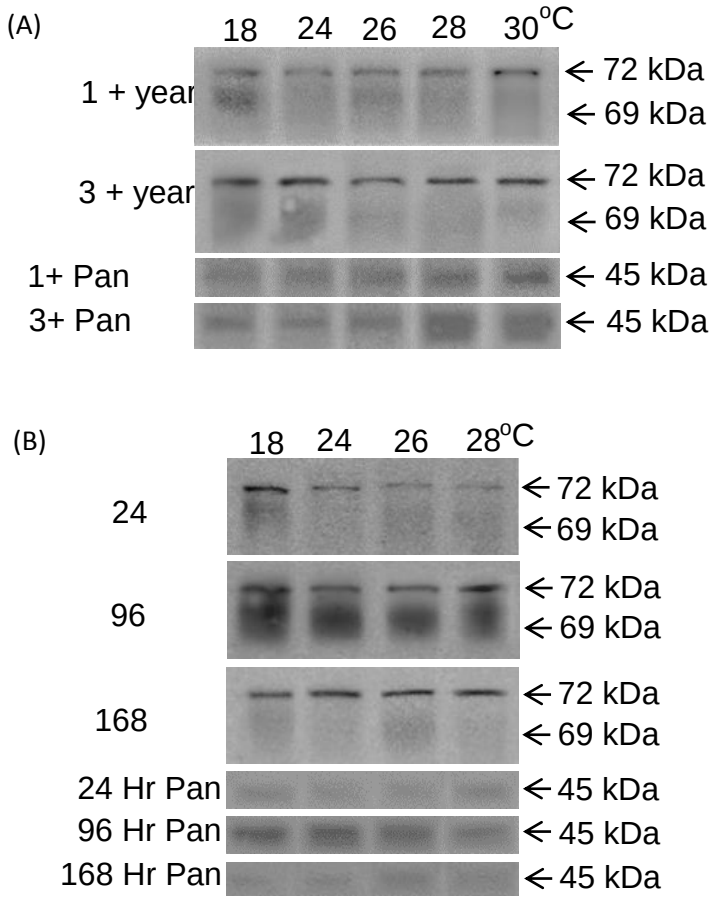

Figure 2.1. Expression of heat shock 70 proteins in King George whiting (KGW). 40 µg of protein from each KGW blood sample was loaded into a 12% SDS-PAGE gel. Representative immunoblots using an anti-HSP70 antibody which detects two bands 72 and 69 kDa in mobility and an anti- pan actin antibody which detects a band at 45kDa in mobility which was used for normalisation. (A) 1 yr and 3 yr old KGW after 18, 24, 26, 28 and 30 ºC acute heat shock, and (B)1 yr old KGW after extended heat shock.

The smaller HSP band was much fainter and not as clearly visualised as the HSP70 band (Fig. 2.1A and B) and it is feasible that this smear represents two forms of HSP69. However greater differences in cellular response were evident for this HSP69 band/s and this band was clearly inducible as its levels increased to a maximum at 96 h and fell back to basal levels after 168 h (Fig. 2.2B). In both age classes, all heat shock treatments elevated the density of HSP69 compared with the 18 ºC control (Fig. 2.2B). The 1 yr old fish showed an ascending trend in HSP69 with increasing temperature, but the 3 yr old fish showed a similar level of HSP69 regardless of shock temperatures. Two-way ANOVA of the HSP69 band showed no interactive effect between age and temperature ($P = 0.456$) whereas there was a significant effect for both age ($P = 0.01$) and temperature ($P \leq 0.001$). For 1 yr fish, all heat shocked temperatures elevated the HSP69 band density significantly above the control ($P = 0.001$), but despite an increasing trend, no significant difference was observed between the four heat shock temperatures ($P \geq 0.15$). Similarly, there was no difference across the 24-30 ºC range in 3 yr fish, but the HSP69 density in all treatment temperatures was significantly higher than the control ($P = 0.001$). Comparing age groups, the HSP69 band density was higher in the control group of 1 yr fish than in 3 yr fish ($P = 0.012$). Similarly, at both 24 and 26 ºC, 1 yr fish had significantly higher HSP69 band density than 3 yr fish ($P = 0.032$ and $P = 0.032$, respectively). Band densities at 28 and 30 ºC were lower in the 3 yr fish, though this result was not significant ($P \geq 0.159$, Fig. 2.3).

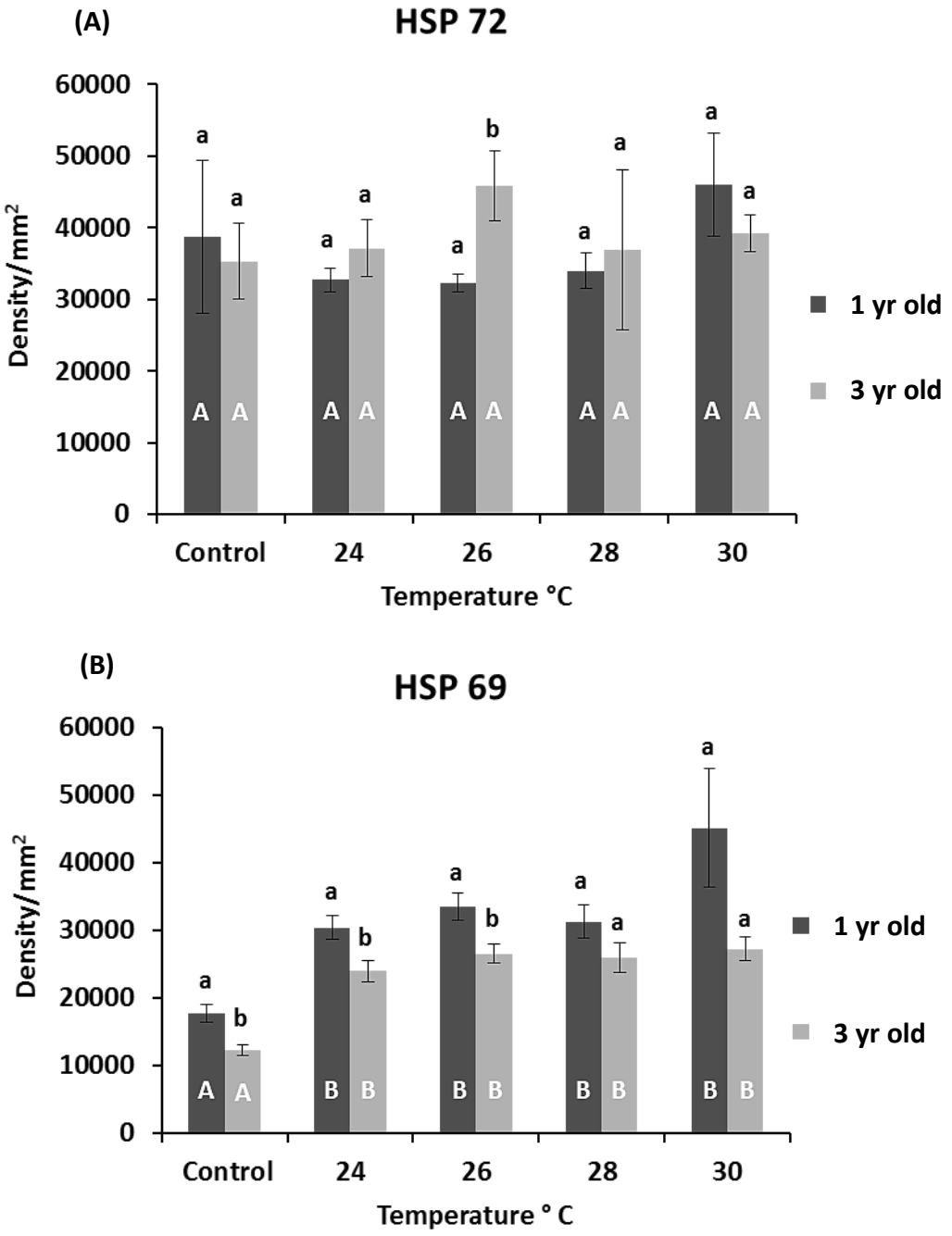

Figure 2.2. Quantification of heat shock protein band density in 1 yr and 3 yr old King George whiting (KGW) after 18, 24, 26, 28 and 30 ºC acute heat shock (mean ± SE). Protein band density was normalised and quantified using Quantity one (Biorad). (A) 72 kDa HSP band density, and (B) 69 kDa HSP band density. Different capital letters represent significant temperature effect ($P < 0.05$) within the same fish age, while different small letters represent significant age effect at a given temperature ($n = 4$).

2.5.2 Extended temperature shock

HSP72 remained similar to the control after 24 h of heat exposure but showed an increase in band density after 96 h (Fig. 2.1B, Fig. 2.3A). One week after heat shock treatment, the density of HSP72 returned to a level similar to 24 h and the control. SPANOVA analysis of HSP72 indicated no significant interaction between time and temperature ($P = 0.959$) and a significant time effect ($P = 0.003$) with band densities of HSP72 at 96 h being significantly higher than the control, 24 and 168 h treatments. There was no statistical difference at any time at 24 °C. HSP72 was significantly different at all time periods at 26 °C ($P \leq 0.001$) while at 28 °C HSP72 was higher at 96 h than at 24 h and 168 h ($P = 0.006$, Fig. 2.4).

HSP69 displayed a similar trend to HSP72 with a peak after 96 h, but HSP69 at the control temperature (18 ºC) had significantly lower band density than at other temperatures ($P = 0.018$). In the HSP69 band density, there was no significant interactive effect ($P = 0.575$), but its density at 96 h was significantly higher than that at 24 and 168 h ($P = 0.02$). At 24 and 96 h, the effect of temperature shocks on HSP69 was not significantly different, but at 168 h, HSP69 at 26 and 28 ºC remained significantly higher than at 24 ºC ($P < 0.002$, Fig. 2.3B).

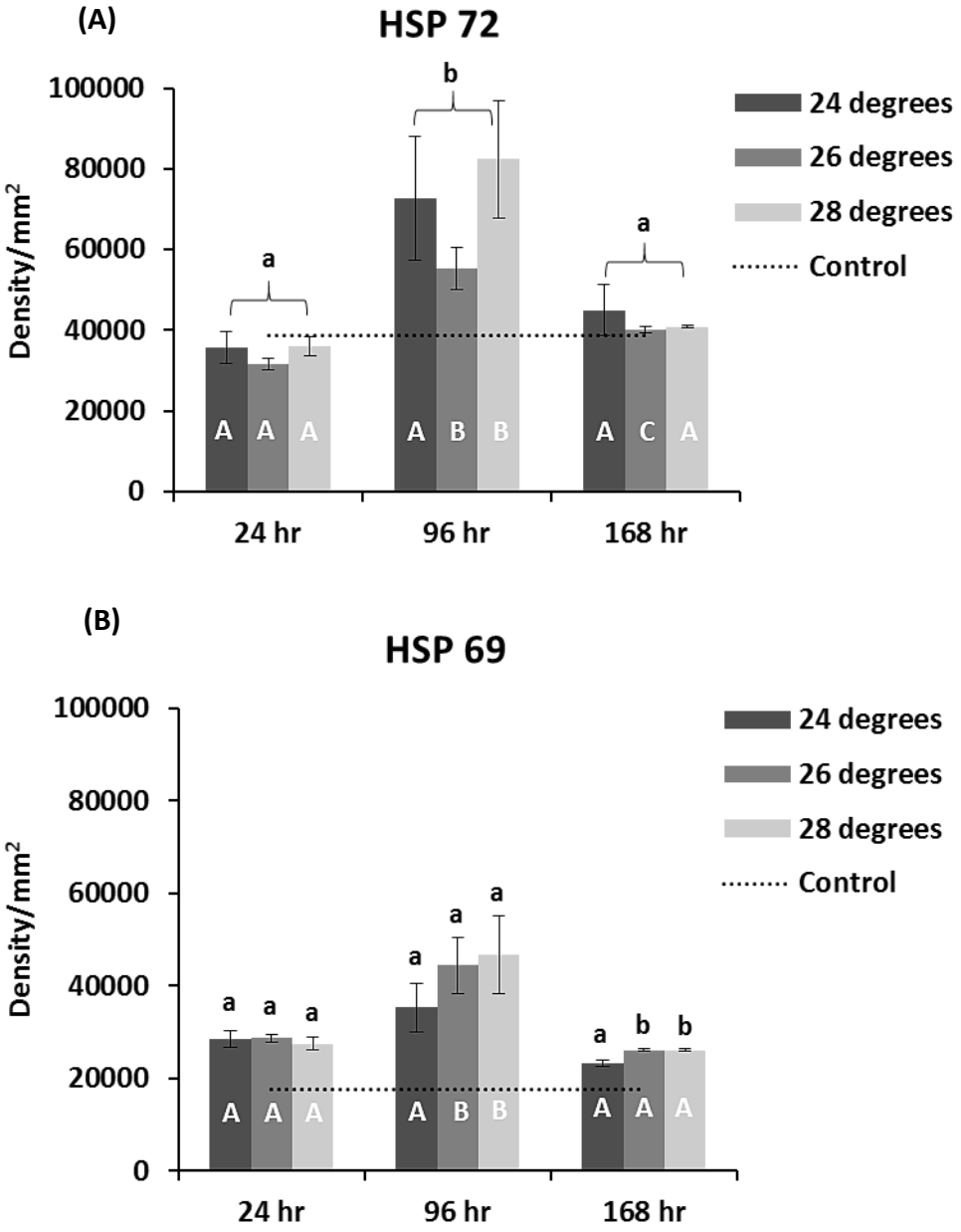

Figure 2.3. Quantification of heat shock protein band density in 1 yr old King George whiting (KGW) after extended heat shock (mean ± SE). Protein band density was normalised and quantified using Quantity one (Biorad). (A) 72 kDa HSP band density, and (B) 69 kDa HSP band density. Different capital letters represent significant time effect ($P < 0.05$) within the same temperature, while different small letters represent significant temperature effect ($P < 0.05$) at each exposure time. The dotted line represents the level of relevant HSPin the control temperature (18 °C) ($n = 4$).

2.6 Discussion

Water temperature plays a critical role in determining distribution and reproductive success of fish populations (Rose, 2005). Juvenile KGW spend up to 3 years in shallow near-shore water before migrating to deeper waters of Gulf St Vincent whereas adults live in a much more stable environment with less variation in water temperature (Fowler et al., 2002). The upper reaches of the Gulf St Vincent do hold KGW of an age and size capable of spawning, which indicates that adequate food supply can meet their foraging requirement. However these fish do not spawn in this region but, instead migrate south to deep reefs with little fluctuation in water temperature. Tagging studies tracking the movement of adult KGW indicate that once in the southern grounds, there is little migration northward towards previously inhabited areas by older fish (Fowler et al., 2002). This suggests that there must be a combination of factors that provide optimal conditions for growth, survival, and reproduction. The induction of heat shock proteins in KGW supports our hypothesis that tolerance to heat shock reduces with fish age and may partially explain the migration route from nursery areas to deep waters.

In this study, two forms of the HSP70 family were expressed and detected after temperature shocks. HSP72 did not significantly change in either juvenile or adult KGW with levels similar to that at the control temperature expressed. From the current work it appears that two proteins around 69 kDa in mobility may be induced by biological stresses such as heat in KGW. In oysters using the same antibody, the HSP69 form was very distinct and often of the same intensity as the HSP72 form (Li et al. 2007). It is possible that during evolution, the two forms of HSP69 evolved in KGW do not share the same degree of sequence identity with the human epitope used to create the antibody in this study. Therefore, this antibody may not have the

same affinity for the inducible HSP form. Nonetheless the HSP69 bands were produced in greater quantities in 1 yr whiting than in 3 yr whiting. Juvenile whiting also exhibited higher basal levels at the control temperature, which may partially explain why older fish migrate away from shallow nursery grounds and remain in deeper water instead of migrating back into the gulfs where temperatures are more prone to fluctuate. Susceptibility to temperature stress is known to increase with age in drosophila (Sorensen and Loeschcke, 2002), mammals (Soti and Csermely, 2003) and fish (Murtha and Keller, 2003). A recent study on rainbow trout reveals that juveniles of this species have higher levels of constitutive HSPs and showed a greater HSP response to temperature shocks than adults (Fowler et al., 2009). The same phenomenon is observed in zebra fish (Murtha and Keller, 2003) and evidence suggests that older blood cells have a reduced ability to induce HSPs compared with younger red blood cells (Lund et al., 2000). While heat shock expression varies between individuals of the same species (Dietz and Somero, 1992; Hofmann and Somero, 1995), this study reveals that young of the year whiting have a better ability to produce HSP69 than 3 yr old fish.

The role of heat shock proteins is to protect cells from damage by environmental stress. Thus low production of HSPs leads to greater susceptibility to cellular damage (Lindquist and Craig, 1998). The response of high heat shock protein production in juvenile whiting indicates that they have been thermally stressed in their nursery environment, which may potentially impact survival and recruitment. The increase in physiological demands due to high temperature may also impact fish foraging and growth via behavioral alterations and place the juveniles at risk of predation. Furthermore, temperature preference in fish often results in movement to more optimal thermal environments (Attrill and Power,

2004). While this may reduce the severity of a temperature shock, it may increase the chance of predation as they are seeking suitable thermal environments to inhabit (Rooker et al., 1998).

In this study, even below the maximum threshold temperatures at 24 °C and 26 °C, the production of heat shock proteins was reduced in 3 yr whiting. This reduced response may be a sign of higher susceptibility to permanent damage if KGW continue to inhabit environments with high temperature variability (Fowler et al., 2009). Further evidence of increased thermal sensitivity in older KGW can be seen with mortality of 3 yr fish observed in the 30 °C temperature shock. This indicates that the critical thermal maximum temperatures of the two age classes are different with greater tolerance to extreme temperatures by younger fish. This study also eliminated differences in thermal tolerance due to acclimatisation as both year classes were laboratory-raised from post larvae and they were only exposed to stable water temperatures. Therefore, the variations in physiological response between different age fish to changing water temperature is unlikely due to previously experienced environments or natural habitat alterations.

When using HSPs as indicators of stress in the field it is vital to establish basal levels of HSP. The study by Fader et al. (1994) indicated that there were seasonal changes in basal levels of HSP in four species of stream fish that inhabit regions of significant seasonal temperature change. This study revealed that the heat shock response in KGW peaked at 96 h post temperature shock, but the level of heat shock proteins reduced to a level similar to the control after 168 h. This indicates that KGW are able to adjust HSP production after a period of acclimatisation. Among aquatic species, the pattern of heat shock protein expression is species-specific. For instance, Fowler et al. (2009) showed that fish age affected the duration of the HSP

response with adult rainbow trout either peaking or plateauing after 8 h whereas juveniles generally kept elevating for 24 h. Werner et al. (2007) found that green sturgeon larvae showed elevated HSP70 for 9 days post heat shock while heat stress resulted in elevated HSP for over 8 weeks in mussel *Mytilus* sp. (Sanders et al., 1992). In tropical fish *Cirrhinus mrigala*, the HSP protein level peaked 6-28 h after heat shock but had declined by 48 h (Das et al., 2005). In contrast to the above temperate species, the cold water species *Gadus morhua* does not respond to heat shock and no significant changes in HSP are observed after temperature changes (Zakhartsev et al., 2005). As the duration of the heat shock response is highly variable between species, the results from this study are important for future research on KGW in the field as they reveal the time lag and acclimatisation period of HSP production after a temperature stress event.

Juvenile KGW were tested from 18, 24, 26, 28 to 30 ^{o}C and they showed tolerance to high temperatures as high as 30 ^{o}C for short periods of time. In aquaculture, the optimal range of temperature for whiting growth is 22-26 ^{o}C (Ham and Hutchinson, 2003) and in the wild, they have shown to avoid temperatures above 26 ^{o}C (Jones et al., 1996). In some instances, expression of the HSP70 family is indicative of thermal injury such as developmental deformity (Werner et al, 2007). Temperature avoidance is common in estuarine species (Childs et al., 2008) and this would suggest that fish can use behavioural change to avoid thermal stress. In this study, the significant increase of HSP69 in 1 year KGW at 26-28 ^{o}C suggests that at this temperature fish are required to protect their cells against protein damage. While it is not clear if this would result in permanent protein change and damage, the physiological response suggests that prolonged exposure may be detrimental.

In addition, the synthesis of proteins is energetically taxing with as much as 40% of all fish oxygen consumption coming from this task (Lyndon et al., 1992). In addition, high temperatures can repress insulin like growth factors (Luckenbach et al., 2007) and result in higher energy activity required for homeostatic maintenance (Wang et al., 2006). Therefore, if a fish is unable to adapt to a temperature stress then these conditions will eventually result in reduced growth, disease resistance and reproductive success (Iwama et al., 1999). Even at 24 °C, there was a significant increase in protein expression in juvenile whiting signifying a significant increase in energy demand for cytoprotection against thermal stress. During the extended period of thermal stress, the increase in HSP69 was greater over time at 26 and 28 °C than at lower temperatures. Similarly, Ham and Hutchinson (2003) reported that the optimal temperature for maximising growth of KGW fingerlings was 22-26 °C, which is consistent with the result of this study. The increased metabolic demand of producing HSPs as well as increases in overall metabolism resulting from increased water temperature will cause the need for greater food consumption. Seagrass nurseries are renowned for their patchy food resources and the increase in foraging behaviour required for growth is likely to expose fish to predation for longer periods further threatening the successful recruitment of post larvae to juveniles and juveniles to adults.

Many species of fish have greater tolerance for increased temperature during their early life (Cook et al., 2006; Wilson and Nagler, 2006), which is often a factor influencing species distribution and habitat selection (Laurel et al., 2007; Morita et al., 2010). In addition, temperature provides important cues for migration (Skov et al., 2010) and reproductive development (Pfafflin and Ziegler, 2006). For estuarine opportunistic fishes like KGW that return to open water for spawning, the reduction

in temperature tolerance that occurs with aging may serve as a cue to initiate migration away from shallow bays and estuaries to deep water where water temperature is more stable.

In summary, understanding the mechanism that fish utilise in response to thermal stress at a molecular level can provide insight into potential constraints to KGW recruitment. In this study, measuring HSP69 in the red blood cells of KGW proved to be a useful, nonlethal tool in assessing thermal stress on fish. Red blood cells were able to produce a measurable heat shock response to the range of temperatures tested and revealed that KGW migrating away from shallow nursery grounds may have reduced capabilities to cope with a sudden temperature rise. The heat shock response was at its peak at 96 h and had reduced after 168 h post heat shock providing baseline information to assess the response of fish to thermal shocks. While current climatic conditions place no direct threat to the survival of juvenile KGW, further increases in water temperature from global warming are likely to impact the growth, behaviour, habitat selection and overall recruitment success of juvenile fish in a nursery ground. This study suggests that the HSP response has a potential application as biomarkers evaluating environment stress on fish. Our understanding on HSP in the cellular stress response should be advanced with further functional and evolutionary genomic research on heat shock protein genes in fish.

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Chapter 3 Growth and physiological parameters of whiting (*Sillaginodes punctata*) in relation to salinity

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3.1 Abstract

This study assessed the impact of salinity on whiting (*Sillaginodes punctata*) in an attempt to understand the mechanisms by which salinity could potentially influence habitat selection and growth of King George whiting in southern Australia. The experiment included whiting of two age classes, young of the year (YOY) and 2^+ yr, at three salinities (30, 40, 50 ppt). YOY whiting showed no significant difference in length or weight gain, specific growth rate, feed intake, food conversion ratio or condition factor when exposed to the three salinities for 72 d. Plasma osmolality of YOY whiting was not significantly different at any salinity, though it was significantly lower than that of 2^+ yr whiting. The 2^+ yr whiting showed significantly higher plasma osmolality than the YOY. Blood plasma potassium and chloride levels of 2^+ yr fish at 50 ppt were significantly higher than those at 30 ppt and 40 ppt. Blood sodium levels at 50 ppt were significantly higher than at 30 ppt but the sodium level at 40 ppt was not different from 30 ppt or 50 ppt. Haematocrit of 2^+ whiting was significantly higher at 30 than at 50 ppt while haematocrit at 40 ppt was not different from 30 or 50 ppt. The 2^+ yr old whiting had a more pronounced increase in plasma osmolality and plasma ions at high salinities, indicating poorer osmoregulatory capacity in older fish. This study provides physiological evidence to partially explain habitat occupancy and growth in relation to salinity of different age groups of whiting in southern Australia.

3.2 Keywords

Growth, Salinity, Osmoregulation, *Sillaginodes puntata,* whiting

3.3 Introduction

King George whiting (*Sillaginodes punctata*) inhabit Australian waters from southern New South Wales to southern Western Australia. Most research on whiting has focused on migratory and recruitment patterns (Hamer and Jenkins 1997; Hyndes et al. 1998; Fowler et al. 2000; Jenkins 2005) habitat use (Connolly 1994; Jenkins and Wheatley 1998), swimming ability (Jenkins and Welsford 2002) and the development of the digestive system (Chen et al. 2003). Studies have shown that the growth of this species is slow taking 3-4 yrs to reach maturity at a size of 30-37 cm (Hyndes et al. 1998; Fowler et al. 2002). Settlement of post larvae occurs in the shallow waters of the Gulf St Vincent and Spencer Gulf, South Australia, and as the fish grow, they gradually move out into the deeper waters of the gulfs and migrate south towards their spawning grounds (Fowler et al. 2002).

Besides ecological and behavioural studies, evidence of environmental factors influencing the physiology of whiting is rare. Fish are often subjected to environmental stresses from changes in temperature, salinity and oxygen concentrations. Prolonged exposure to environmental stressors leads to a tertiary stress response including slow growth, immune malfunction and low reproductive output (Barton 2002).

The Port River and Barker inlet estuary system north of Adelaide is one of the main nursery areas for whiting (Jones 1984). This estuary system receives very little freshwater and is subjected to large seasonal fluctuations in temperature and salinity. Salinity levels in both Gulf St Vincent, and the Barker inlet in summer have

exceeded 40 ppt (Fielder et al. 2007). Jones et al. (1996) reported salinity and temperature fluctuations in the Barker Inlet ranging from 36 ppt and 15 ºC in July, to 41 ppt and 26 ºC in January, while on rare occasions salinity can fall to as low as 30 ppt (Jackson and Jones 1999). Juvenile whiting found in the upper Spencer Gulf have been reported in water up to 48 ppt (Ham and Hutchinson 2002).

Boeuf and Payan (2001) commented that it is unlikely for most marine fish to grow at optimal rates in seawater that exceeds 35 ppt, and Imsland *et al.* (2008) observed that even stenohaline species can benefit from being reared in lower concentrations of saltwater. Salinity is one of the key environmental factors regulating the physiological responses of numerous fish species and the salinity tolerance of fish depends on the environment and developmental stage (Boeuf and Payan 2001). For instance, Californian halibut (*Paralichthys californicus*) exhibit age-dependent tolerance to salinity and inhabit different environments as they grow (Madon 2002). However, little is known on the salinity tolerance of King George whiting, despite its substantial contribution to fisheries in South Australia.

Apart from human influence such as fishing pressure, the impact of changing environments on the whiting fishery has not been paid much attention. In the last decade, South Australia has experienced record extended heat waves, with daytime air temperatures in excess of 45°C and minimum overnight temperatures exceeding 30°C along with long-term meteorological drought (National-Climate-Centre 2008; National-Climate-Centre 2009). Drought induced salinity changes have been shown to affect estuary catch rates (Gillson et al. 2009) and the recent focus on climate change has highlighted issues regarding climate induced changes in fish abundance and distribution (Hughes 2000; Lotze et al. 2006). King George whiting are found in locations with a wide range of salinities and tend to occupy different habitats at

different ages. As the movement of whiting larvae is primarily determined by ocean currents, the ambient salinity at settlement locations should have potential influence on the growth rates and recruitment success of this species. Therefore, the aim of this study was to investigate the impact of salinity on the growth response of juvenile whiting, and to assess the osmoregulatory abilities of young of the year (YOY) and 2^{+} yr old fish. This study will provide insights on the impact of salinity on growth and physiological adaptation of whiting, and improve our understanding of its recruitment in nature in a changing environment.

3.4 Materials and Methods

3.4.1 Fish collection

Post larval whiting were obtained from the Port River in South Australia during October-November (2006 and 2008) using a 5 m beach seine net. The net was hauled by two people across shallow seagrass beds and the whiting were removed and placed in insulated and aerated containers for transport back to the laboratory (~30 km and 40 min). In the laboratory, the juvenile whiting were held in a recirculating aquaculture facility in glass aquaria 30 × 30 × 20 cm with a salinity matching the natural environment at 38-40 ppt and temperature at 18 °C. Initially, whiting were fed a combination of live *Artemia* and diced cockles and were gradually weaned over a 2 month period onto a commercial pellet (Ridley Aquafeed 1-1.8 mm crumble; 54% protein, 10% fat, 2% fibre and 8% ash).

3.4.2 Impact of salinity on YOY whiting

The treatment salinities used in the trial covered the range of salinities in the wild whiting nurseries, 30, 40 and 50 ppt. Each salinity treatment was replicated three times and each replicate contained nine fish with an initial weight of 2.43 g ±

1.1 and length of 6.47 cm ± 1.0 SL (n = 81). Treatment tanks were 30 × 30 × 20 cm glass aquaria and each tank was a closed system with aeration. One third of the tank water was exchanged daily and pre-established biofilters were added to each tank to aid in maintaining water quality. Water quality parameters throughout the experiment were maintained at NH_3-N <0.05 mg/l, NO_2 –N <0.2 mg/l, NO_3 –N <10 mg/l, oxygen 9 mg/l and pH 8. Salinity levels were measured using a salinity refractometer. The growth trial was conducted over 72 d and measurements of fish length and weight were recorded on d 1, 28 and 72. At the completions of the trial, all fish were anaesthetised in benzocaine and weight and length were recorded. At the same time, one third of the fish from each tank were sampled for plasma osmolality measurements via puncture of the caudal vein with a 1-ml 29 gauge needle. Blood was allowed to coagulate on ice before being centrifuged for 3 min at 13,171 × g force. As only a small amount of blood could be taken while still keeping the juveniles alive, total osmolality was measured because all individual ions are included in this parameter. Plasma was analysed using freeze point depression to determine osmolality using a Knauer semi-micro osmometer (K-7400). Growth performance was assessed using weight and length gain, specific growth rate (SGR), condition factor (CF), feed intake and food conversion ratio (FCR).

3.4.3 Salinity effect on 2^+ yr old whiting

To assess the potential age dependant effect, 2^+ yr whiting were analysed for their osmoregulatory capacity. Whiting were held communally in a 600 L tank maintained at 40 ppt then transferred into small holding containers where the fish were acclimatised over a 2 h period to the required salinity. Fish were then transferred into 250-L tanks containing water at 30 ppt, 40 ppt or 50 ppt. Each tank received 10 fish (mean 20.03 ± 9.8 cm SL and 31.56 ± 12.7 g) and each treatment

was replicated four times. Tanks at 30 ppt contained sea water diluted with demineralised water while tanks with 40 or 50 ppt water were made up with evaporated seawater. Fish were left in treatment tanks for 7 d before sampling. Five fish from each tank were captured and non-lethally anaesthetised in a 15 ppm benzocaine solution. Blood (0.1-0.4 ml) was drawn from the caudal vein with a 1-ml 29 gauge needle. The blood was then transferred to 1.5-ml Eppendorf tubes and a portion of the blood was immediately placed into capillary tubes to quantify haematocrit. Haematocrit was measured by centrifuging blood on a micro haematocrit centrifuge for 3 mins at 15,366 × g force. The proportion of red blood cells and plasma were assessed using a sliding haematocrit scale. Remaining blood was placed on ice and allowed to coagulate before being centrifuged for 3 min at 13,171 × g force. Serum was collected and stored at -18 °C until analyses of osmolality, chloride, sodium and potassium were conducted.

Osmolality was determined using freeze point depression with an osmometer (Knauer semi micro K-7400). Plasma chloride levels were determined by coulometry using a chloride analyser (Corning chloride analyser 926). Sodium and potassium were measured using a flame photometer (Eel Corning) at dilution rates of 1:400 and 1:800 respectively to ensure the samples measured between 0 and 1 mM. All measurements were taken in duplicate where enough plasma was available.

3.4.4 Data analysis

All data were analysed using the statistical package SPSS (version 15). Food conversion ratio (FCR) was calculated as: dry food fed (g) / wet weight gain (g); condition factor was calculated as: $W \times 100 / L^3$, where W is the wet weight (g) and L is standard length (cm); and specific growth rate (SGR) was calculated as: ($\ln W_f$ -

lnWi × 100) / t, where, W_f is the final fish weight (g), Wi is the initial fish weight (g) and t is the duration of the experiment in days (d).

Blood physiology was analysed using one-way ANOVA. If statistical differences were detected then data were further analysed using Tukey post hoc tests. Where assumptions of normality were not met, then a Kruskal Wallis non parametric test was performed. Data for sodium levels were transformed with the reflect square root command in order to meet the assumption of normality (Kolmogorov-Smirnov). Two-way ANOVA was used to assess differences in osmolality between year classes of whiting. The level of significant difference was set at $P < 0.05$.

3.5 Results

3.5.1 Response of YOY to salinity level

There was no statistical difference observed for either weight ($P = 0.667$) or length gain ($P = 0.131$) for the whiting reared for 72 d at three salinities although the data did show a decreasing trend with increasing salinity (Table 3.1). Likewise, condition factor remained unchanged throughout the experiment and was not influenced by salinity ($P > 0.335$).

Feed intake was not significantly influenced by salinity ($P = 0.065$). After 72 d FCR was 1.27 in the 30 ppt treatment while 40 and 50 ppt treatments had an FCR of 1.7. Despite this difference, the results were not significant between treatments ($P = 0.057$). No difference was observed from d 0 to 28 ($P = 0.170$) or between 28 and 72 d ($P = 0.171$)

No significant difference was observed between salinity treatments for FCR at either d 28 or d 72 (P = 0.166 and P = 0.243 respectively, Table 3.1). There was also no difference in SGR between the period of 28 and 72 d (P = 0.069).

Table 3.1. Heat shock protein 69 kDa band density of 1^+ yr King George whiting exposed to elevated temperatures over a one week period (168 h) with SE bars. Different capital letters within bars represent significant difference ($P < 0.05$) between temperatures at each time while different small letters above bars represent significant difference between temperatures at each time.

Growth Parameters	30 ppt	40 ppt	50 ppt	P value
Length gain (cm)	1.57 ± 0.06	1.37 ± 0.10	1.22 ± 0.14	0.131
Weight gain (g)	1.74 ± 0.07	1.64 + 0.10	1.59 ± 0.16	0.667
Condition factor	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.880
SGR	0.82 ± 0.05	0.69 ± 0.07	0.72 ± 0.22	0.243
Feed intake	2.65 ± 0.09	2.54 ± 0.09	2.91 ± 0.12	0.065
FCR	1.23 ± 0.07	1.71 ± 0.17	1.72 ± 0.11	0.057

3.5.2 Response of 2^+ yr old whiting to salinity level

Haematocrit levels were significantly different between treatments ranging from 38 % in the 30 ppt treatment to 33 % in the 50 ppt treatment (P = 0.009, Fig. 3.1a). Statistically no difference was observed between the 30 and 40 ppt and 40 and 50 ppt treatments ($P > 0.05$). Potassium levels were significantly increased in whiting at 50 ppt compared to the 30 and 40 ppt salinity treatments (P = 0.018, Fig. 3.1b), while no difference was observed between the 30 and 40 ppt treatments. Chloride levels followed the same trend as potassium with the 50 ppt treatment being significantly higher than the 30 and 40 ppt treatments (P = 0.002, Fig. 3.1c) but no difference was observed between the two lower salinities tested ($P > 0.05$). Plasma sodium also increased with increasing salinity. Although sodium levels were not

significantly different between 30 and 40 ppt or 40 and 50 ppt ($P > 0.05$), there was a significant difference between treatments 30 and 50 ppt ($P = 0.036$, Fig. 3.1d).

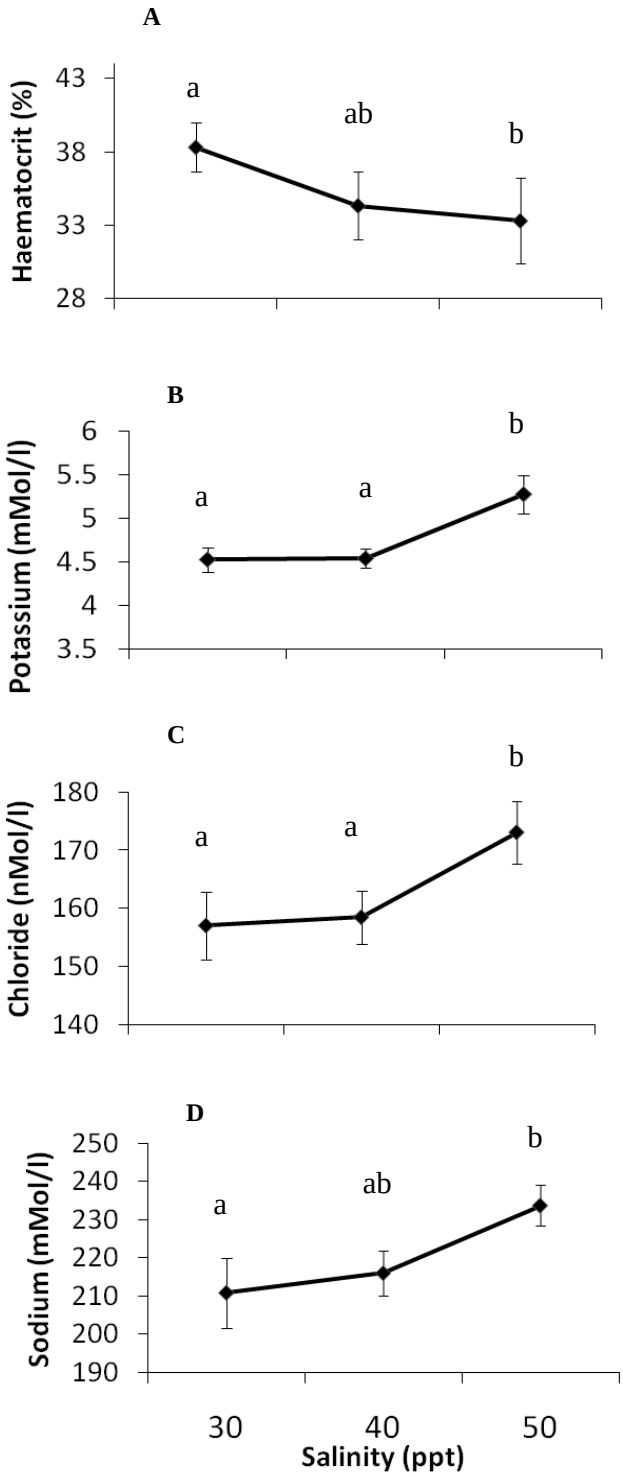

Figure 3.1. Blood physiology of 2^{+} yr old whiting held at three different salinities for 7 d (n = 20 fish per treatment). A) blood haematocrit percentage, B) plasma potassium, C) plasma chloride levels, D) plasma sodium values. Different letters indicate significant differences ($P < 0.05$).

3.5.3 Osmolality comparison between 0^+ and 2^+ yr whiting

Two-way ANOVA indicated no significant interactive effect between treatment and fish age ($P = 0.646$). The main effect for age revealed a significant difference with 2^+ yr old whiting recording significantly higher osmolality values than the YOY whiting (Fig. 3.2; $P = 0.002$). The main effect for salinity treatments also recorded significantly higher osmolality between 50 ppt compared to 30 and 40 ppt ($P = 0.001$). One-way ANOVA of the individual age classes revealed that osmolality levels were significantly different at all three salinities in 2^+ yr whiting ($P = 0.001$), whereas no significant difference was recorded for YOY whiting at any salinity ($P = 0.335$, Fig. 3.2).

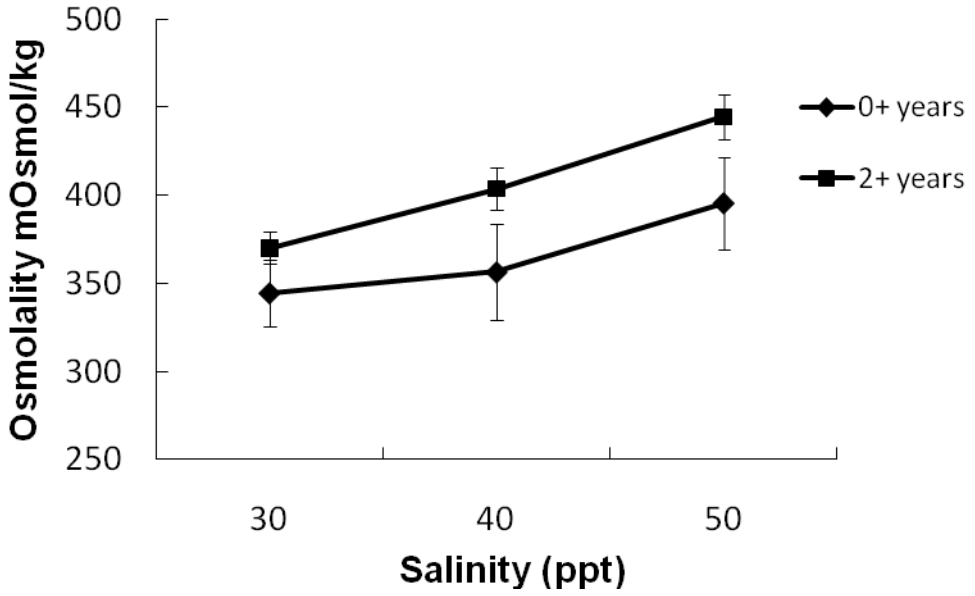

Figure 3.2. Osmolality values for YOY ($n = 9$ fish per treatment) and 2^+ yr old ($n = 20$ fish per treatment) King George whiting held at three different salinities.

3.6 Discussion

3.6.1 YOY juveniles

The impact of salinity on fish growth is not only species specific (Boeuf and Payan 2001), but can also be age-dependent (Madon 2002). In the present study, salinities between 30 and 50 ppt did not affect the growth rate of juvenile whiting. An overall declining trend in body weight and length was observed with salinity elevation, but the differences observed were insignificant. The salinity levels in this study represented a range to be experienced by whiting in the wild. The condition factor of YOY whiting at the completion of the experimental period was almost identical between salinity treatments, indicating that juvenile whiting are well adapted to these salinity levels. The slow growth rate of the whiting may have masked any possible difference from being observed during the 72 d trial whereas in faster growing species such as *Solea sengalensis*, differences in growth were observed after only 32 d (Arjona et al. 2009). Subsequently, an impact of salinity on whiting growth over a longer term warrants further investigation.

Blood osmolality in YOY whiting remained similar among treatments with no significant difference observed. The combination of growth and osmolality results that we reported highlights that juvenile whiting are well adapted to their environment and that additional energy expenditure due to osmoregulation has only marginally negative effects in the short term. There were indications of better growth of juvenile whiting at 30 ppt which is slightly below salinities that whiting would usually experience in the field. Boeuf and Payan (2001) reported that many marine fish species, particularly at the juvenile stage, prefer lower than full strength salinities. For instance, growth performance of gilthead sea bream (*Sparus aurata*)

was optimised at 28 ppt, while the growth of juvenile turbot was maximised at 19 ppt. Even typically stenohaline species like Atlantic halibut (*Hippoglossus hippoglossus*) can benefit from environments closer to isoosmotic conditions (Imsland et al. 2008). However, an exceptional case was reported by Arjona *et al.* (2009) where the tertiary stress response was minimised and growth maximised at 39 ppt in Senegalese sole (*Solea senegalensis*). Our results indicate that the optimal salinity for whiting is closer to 30 than to 50 ppt and that better growth may eventuate at lower salinities.

3.6.2 Osmolality of YOY and 2^+ yr whiting

Blood osmolalities in teleost fishes range from 260 to 400 mOsmol (Jobling 1995). Both YOY and 2^+ yr old whiting appear to be well adapted to a wide range of salinities. Our results showed, however, that there was a significant difference in osmolality values between the two whiting size classes. Osmolality was significantly lower in the YOY whiting compared to 2^+ yr whiting. In addition, osmolality was different at each salinity level in 2^+ yr whiting. However, despite displaying a similar trend, no significant difference in osmolality was observed at any salinity level in YOY whiting. Age dependant changes in osmoregulatory capacity have also been reported in other species such as Californian halibut (*Paralichthys californicus*) where juveniles exhibit increased osmoregulation and consequently occupied nearshore wetlands as opposed to the offshore habitats occupied by older conspecifics (Madon 2002).

The migratory patterns of whiting within the Gulf St Vincent show systematic southward movements, while age structure and tagging studies suggest that it is unlikely that mature whiting travel north once they have reached the southern

spawning grounds (Fowler and March 2000; Fowler et al. 2002). This migration pattern is somewhat matched to the salinity structure of the Gulf St. Vincent which is an inverse estuary. Shallow water, minimal freshwater inflow, high evaporation and a slow flushing rate result in highly saline water (~ 42 ppt) in the upper regions of the gulf which is dispersed down the eastern side of the gulf (Kampf et al. 2009). In contrast to this, there is little seasonal variation in salinity at the mouth of the gulf (~ 36.5 ppt) where adult whiting of Gulf St. Vincent migrate, spawn and ultimately reside (De Silva Samarasinghe and Lennon 1987). As the location of settlement for juvenile King George whiting is primarily determined by ocean currents (Fowler et al. 2000) the further north and east juvenile whiting settle, the higher salinity they are likely to encounter. Fowler and March (2000) surmised that the systematic movement of whiting towards the southern end of the gulf is due to more appropriate habitat requirements for adult fish which do not require the whiting to return up the gulf. It is also possible that the lower salinities in the south of the Gulf St. Vincent are more appropriate to the osmoregulatory capacities of adult whiting and that energy allocated towards reproduction is maximised by the reduction of ion regulation.

The size of adult fish populations is often highly dependent on the successful recruitment of juvenile fish (Myers and Cadigan 1993). In this study, YOY juveniles had a broad range of salinity tolerance, suggesting that settlement and recruitment of juvenile whiting are unlikely to be greatly influenced by salinity levels. The physiological and metabolic demands do not pose a great risk for fish survival and growth when they live in areas with highly saline water (up to 50 ppt). Feed intake and feed conversion ratio which are readily influenced by salinity in many species

(De Boeck et al. 2000; Rubio et al. 2005; Imsland et al. 2007), were not observed to change significantly in whiting.

3.6.3 Response of 2^+ yr whiting to salinity

Sodium, potassium and chloride are essential minerals in the electrolyte and acid base balance of animals (Wilson and Elnaggar 1992). Osmoregulation is determined by the composition and quantity of these ions. Marine teleosts living in hyperosmotic environments are faced with the challenge of maintaining their body osmolality and ionic balance and as a result of osmosis, fish lose water through their gills and skin and are required to drink seawater in order to replace water lost (Moyle and Cech 2004). The 2^+ yr old whiting showed significant increases in plasma ions when exposed to increasing salinity. Chloride and potassium levels were higher in fish at 50 ppt while sodium was significantly higher at 50 ppt than 30 ppt but neither was significantly different from the 40 ppt treatment.

The increasing trend in sodium, chloride, potassium and osmolality with increasing salinity has been observed in a number of different species (Gaumet et al. 1995; Woo and Chung 1995; Morgan and Iwama 1998; Gonzalez et al. 2005; Laiz-Carrion et al. 2005). In fish, increases in the solute contents of blood indicate enhanced osmotic activity (Magnussen et al. 2008). Generally, less energy is required to maintain ionic balance in water closer to isoosmotic levels. Our data showed that there was very little difference in the ionic levels between 30 and 40 ppt. However, at 50 ppt, the increase in ion levels in the blood suggests that 40 ppt is close to the threshold level at which the energy required for osmoregulation significantly increases to influence fish growth and development. The amount of energy required for osmoregulation was not measured in this study but previous

studies indicate that osmoregulation utilises 10-50% of the total energy budget (Boeuf and Payan 2001).

Haematocrit levels are used as an indication of the secondary stress response in fish and the level is altered by stressors including osmotic challenge (Frisch and Anderson 2005). In this study, heamatocrit decreased with increasing salinity and was significantly higher in the 30 ppt treatment than the 50 ppt treatment. At 40 ppt haematocrit was not significantly different from either 30 or 50 ppt. Decreasing haematocrit coinciding with increasing salinity is a trend observed in other studies (Thompson and Withers 1992; De Boeck et al. 2000; Frisch and Anderson 2005). This pattern is believed to be the result of water diffusing into the plasma from surrounding cells due to increased plasma ions and osmolarity. Increases in the stress hormone cortisol can be correlated with decreases in haematocrit, as cortisol is thought to aid with the excretion of blood ions and the retention of water (Redding et al. 1991; Woo and Chung 1995). In general, increases in blood cortisol are correlated with decreases in growth rates (Van Weerd and Komen 1998) and the suppression of reproductive function (Mommsen et al. 1999) further gives indication that salinity levels of 50 ppt may be detrimental to the growth of 2^+ yr whiting.

In conclusion, this species possesses a wide salinity tolerance and has the ability to survive and grow in salinity levels up to 50 ppt. Results from both age classes provide an indication that the energy required for osmoregulation is increased at 50 ppt and that growth may be maximised in salinity levels below 40 ppt. The recruitment success of YOY juvenile and 2^+ yr whiting in the Barker inlet/Port River system is unlikely to be greatly influenced by the salinity fluctuations currently experienced. However, recruitment of juvenile whiting to areas of the Spencer Gulf

where salinities are approaching 50 ppt may have a greater impact on both YOY and 2^{+} yr whiting due to the slightly lower growth and higher plasma osmolality observed. Salinity enhanced plasma osmolality in 2^{+} yr whiting was consistently higher than in YOY whiting which may imply that reduced osmoregulatory abilities in older fish constrain their distribution away from areas with high salinity.

3.7 Acknowledgements

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Chapter 4 Growth, behaviour and colour changes of juvenile King George whiting *Sillaginodes punctata* mediated by light intensities

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4.1 Abstract

Light is an important environmental factor regulating physiological process and ecological activities in fishes. This research aimed to examine the effect of light on the growth, distribution and body colour of juvenile King George whiting *Sillaginodes punctata* when fish were exposed to different light levels under laboratory conditions. In this study, the fish were exposed to three light levels (25, 500, and 1000 lux) in triplicate for two months. Comparisons were made for growth, body colour and behavioural changes between treatments. During the experimental period, there were no statistical differences in specific growth rate, survival, growth efficiency and condition factor between the light levels. Although light did not affect swimming speed or the encounter rates, it did influence group flight activity and fish distribution in the tanks. Whiting in low light preferred to spend more time grouped at the bottom of the tank than at medium (500 lux) or high light (1000 lux). Likewise, after having been exposed to low light, whiting exhibited a brighter colour and had fewer body markings and counter-shading than fish in medium and high light intensities. The differences in fish distribution and body colour between medium and high light intensities were not detected. This study offers a new insight into understanding the impact of light on behaviour and body colour of fish, which may contribute to the spatial distribution, predator avoidance and recruitment of fish in inshore coastal waters.

4.2 Keywords:

King George whiting; fish; habitat; swimming speed; aggression; crypsis; light intensity

4.3 Introduction

The role of light intensity in fish biology has been documented in numerous fish species under experimental conditions (Boeuf & Le Bail 1999; De Robertis et al. 2003) and in nature (Rickel & Genin 2005). Light has been correlated with a variety of responses such as growth (Wallace et al. 1988; Downing & Litvak 1999), survival (Soderberg 1990; Cerqueira & Brugger 2001), swimming activity (Oppedal et al. 2001; Marchesan et al. 2005; Johansson et al. 2006), cannibalism (Appelbaum & Kamler 2000; Han et al. 2005), metabolism (Appelbaum & Kamler 2000), metamorphosis (Puvanendran & Brown 2002), swim bladder inflation (Battaglene et al. 1994), and feeding behaviour (Puvanendran & Brown 1998; Noble et al. 2005).

Light intensity is an important environmental factor associated with changes in fish physiology (Boeuf & Le Bail 1999), skin colour (Yasir & Qin 2009) and even morphology (Pulcini et al. 2008). As a means of reducing detection by predators, many species of animals display some form of camouflage (Stevens & Merilaita 2009a). The term crypsis incorporates several forms of camouflage including countershading, background matching, and disruptive colouration (Stevens & Merilaita 2009a). These colours and patterns may play a dual role in both background matching and disruptive colouration by blending into the environment and creating false edges to obscure the shape of the fish to predators (Ruxton et al. 2004; Rowland 2009; Stevens & Merilaita 2009; Stevens & Merilaita 2009a,b).

Much literature regarding body colouration of fish is associated with fish for

human consumption (Van der Salm et al. 2004; Han et al. 2005) or sale as ornamental fish (Yasir & Qin 2009). However, light intensity is a physical variable that may have impacts on fish body colouration in shallow marine waters, an area that is not well studied in fish biology. Under experimental manipulation, fish exposed to high light intensities exhibit a darkening of the skin (Han et al. 2005; Pavlidis et al. 2008). Fish have the ability to alter their body colour through the aggregation or dispersal of chromatophores within the skin thus altering the light that is absorbed or reflected from the surface of the skin (Fujii 2000). The aggregation or dispersal of chromatophores is highly influenced by light intensity either directly or indirectly via neural and endocrine regulation as a result of visual stimuli (Fujii 2000). There is also evidence that darkening of the dorsal surface is an adaptation against UV radiation as observed in hammerhead sharks (Lowe & GoodmanLowe 1996). Therefore, it is possible that in shallow bodies of water, light induced changes in the skin pigmentation of a fish may alter the effectiveness of its cryptic colouration making it either better camouflaged or more obvious to predators. In a complex ecosystem, fish play a dual role of being a predator and a potential prey item for a larger fish. Therefore, the ability of marine fish to match their body colour to the ambient habitat is advantageous towards their overall fitness (Ruxton et al. 2004). The examination of skin colour adaptation to light intensity is important in understanding the recruitment of fish in shallow estuaries where refuges are not abundantly available.

Visual interactions with the environment are light-dependent and can influence fish behaviour. The diel variation in light levels significantly regulates feeding and predator avoidance behaviours in most teleosts as well as mating,

recognition and interactions between conspecifics (Sloman et al. 2006). For instance, antagonistic behaviours vary depending on light intensity in some fish such as white seabream *Diplodus sargus* (Castro & Caballero 2004) and African catfish *Clarias gariepinus* (Britz & Pienaar 1992) in captivity. Distribution and swimming behaviour of fish can also be influenced by light intensity (Oppedal et al. 2001; Marchesan et al. 2005; Johansson et al. 2006), as can feeding success (McMahon & Holanov 1995) and schooling behaviour (Torisawa et al. 2007).

The King George whiting *Sillaginodes punctata* is one of the most important recreational and commercial fish species in southern Australia. Larvae are transported via ocean currents into shallow inshore waters where they settle onto intertidal seagrass beds interspersed with a sand and mud seafloor. Seagrass beds typically provide juvenile whiting with protection from predation as well as being the habitat for numerous prey items. Research on this species has focused on migratory patterns (Fowler et al. 2000; Fowler et al. 2002), larval recruitment and settlement (Jenkins & May 1994; Hamer & Jenkins 1997; Moran et al. 2004), and habitat usage (Connolly 1994; Jenkins & Wheatley 1998). However, very little is known about the response of this species to light level manipulation. Like many fishes, King George whiting exhibit countershading, with a light brown upper body covered with distinctive brown spots. The lower half of the body is silver and the fins are slightly greenish brown (Gommon et al. 1994). Young King George whiting are considered to be relatively poor swimmers (Jenkins & Welsford 2002) and may rely on their ability to remain camouflaged in order to avoid predation.

The aim of this study was to understand the impact of light on feeding, growth and behaviour of shallow water fishes using juvenile King George whiting as

a representative species. The design of this study allowed us to assess body colour change and adaptation to light variations under an experimental condition. The results will increase our understanding of the distribution of shallow water fish and its cryptic adaption to environmental changes. The results may reveal the role of light as a potential factor influencing recruitment of marine fish larvae in inshore coastal waters.

4.4 Materials and methods

4.4.1 Experimental fish

Juvenile King George whiting were collected from King's and Barry's beaches in the Barker Inlet/Port River estuary, Adelaide, South Australia. A small beach seine net (5 m long, 1.5 m high and a 1 mm mesh) was manually dragged through shallow intertidal waters consisting of a mud bottom interspersed with seagrass beds *Zostera* sp. Several net hauls were made during the period one hour either side of high tide. The size of fish caught ranged from 30 to 50 mm. In the laboratory juvenile King George whiting were initially fed with a combination of *Artemia* nauplii and diced cockles *Donax deltoides* before being weaned onto a 500-800 µm formulated feed. At the end of the weaning period, the experimental fish reached an average standard length of 65 mm and were fed on a pellet diet of 0.8-1.2 mm (Primo Aquaculture NRD 5-8 diet, protein 57%, lipids 9%, ash 13%, moisture 7%, fibre 1.9%).

4.4.2 Experimental design

All whiting were placed in a holding tank, individually netted and anesthetised in a 15 ppm benzocaine solution. The fish were then weighed to the

nearest 0.001 g and measured to the nearest 0.1 mm and assigned to one of the three light levels: high (1000 lux), medium (500 lux), and low (25 lux). Each treatment consisted of three replicates with 19 whiting per tank (2.4 ± 0.19 g SD, 65.7 ± 2.3 mm SD). No statistical difference in the size of fish between treatments was evident at the beginning of the experiment ($P > 0.514$).

The aquaria were 36 L (40 × 30 × 30 cm) and illuminated by fluorescent lights (Osram Lumilux plus 18W/11-860 daylight). Each tank was connected to a recirculating system with a water exchange rate of 1 L/min. Sheets of thick black plastic were used to block any light from outside sources as well as to separate tanks of different light intensities. Light intensity was measured with a light meter at 3 different points on each tank and averaged to obtain the lux values. The different light intensities were achieved through altering the thickness of a neutral shade cloth covering the fluorescent lights. The experiment was conducted over 8 weeks and the weights and lengths of all fish were measured at the beginning, midpoint and end. Fish were fed twice per day (0900 -1600 h) to a point when fish stopped feeding and the amount of food applied to each tank was recorded.

4.4.3 Experimental conditions

Water quality was monitored daily for temperature and salinity and weekly measurements were taken for ammonia, pH and oxygen. The experimental salinity was maintained at a level similar to the field (38.9 ppt ± 0.99 SD). Temperature was maintained at 23.4 ± 1.8 ºC (SD) as suggested by Ham and Hutchinson (2002) and the light cycle was maintained at 12 h light and 12 h dark. Ammonia, oxygen and pH were maintained at <0.25 ppm, 7.8 ± 3.7 (SD) mg/L and 7.50 ± 0.08 (SD),

respectively.

4.4.4 Fish colour analysis

At the completion of the experiment, body colour was analysed using the methods developed by Villafuerte and Negro (1998) and as adapted by Yasir and Qin (2009) using the RGB and HSB values of each pixel of a digital image. Seven fish from each aquarium (21 fish per treatment) were chosen at random for colour analysis. To reduce the effects of stress, fish were quickly placed into a non lethal dose of benzocaine and lightly anaesthetised. This process took less than 30 seconds from placing the fish in the anaesthetic and taking the photograph. Fish were then placed on a white background below four mounted natural white colour bulbs (NEC 18 watts) on a glass plate beside an identification tag for the fish and a colour reference card (Gretag Macbeth colorchecker, Fig. 4.1). The digital images were taken with a Canon Eos 20D digital SLR camera (EFS 15-55 mm). Images were then transferred to Adobe Photoshop (version 7.0.1) for analysis. The spectrum of the lighting used in the experiments was measured using a fibre optic spectrometer (Ocean optics USB 2000+UV-VIS). Results from the spectrometer revealed wavelengths of the lighting ranging from 405 nm, to slightly into the infrared wavelengths (710 nm). Major peaks in intensity were observed at 435.6 nm (violet), 542-546 nm (green), and 610 nm (orange) (Fig. 4.2).

Figure 4.1. Sample digital image used for colour analysis showing colour squares for checking colour between photos, fish identification tag and placement of fish

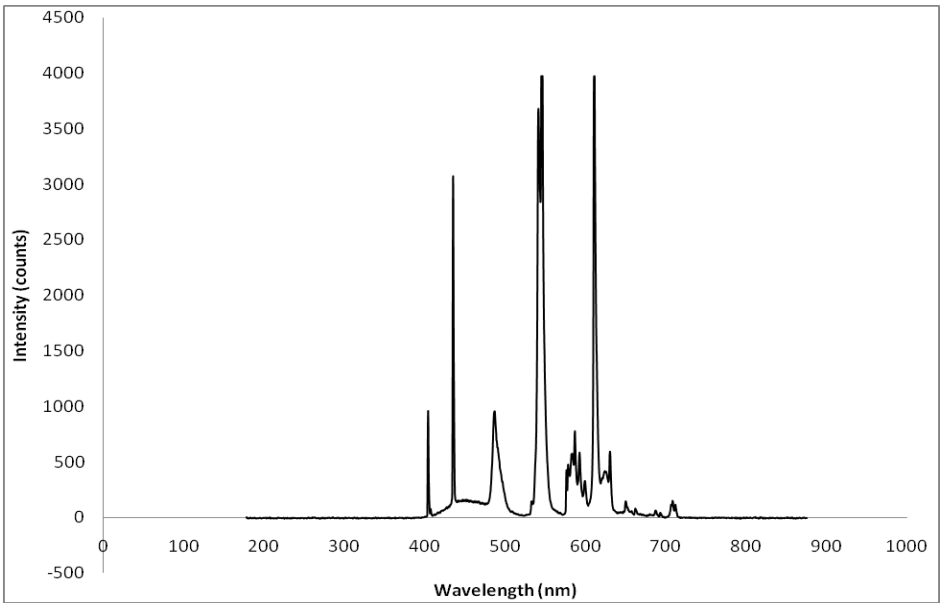


Figure 4.2. Wavelength spectrum of the light source used throughout the experiment.

Each fish was analysed for spot colour (3 spots per fish), dorsal colour above the lateral line and ventral colour below the lateral line. Fins and the fish head were excluded from analysis. In Photoshop the three sections were isolated and the colour pixels were quantified by the program developed by Villafuerte and Negro (1998). In addition, 500 pixels from the top left corner of the dark and light skin colour squares of the colour checker were also selected for comparison of colours between photos. This program then converted the image into red, green and blue values for each pixel. These values were subsequently transferred into the Microsoft Excel program and converted to hue, saturation and brightness values using the algorithms of Gardner (2007).

Hue

(IF (Min = Max, 0,

IF (R = Max, (G - B) / (Max - Min),

IF (G = Max, 2 + (B-R) / (Max-Min), 4 + (R-G) / (Max - Min))))*60°

Saturation

(IF (Max = 0, 0, (Max –Min) / Max)

Brightness

(Max / 255)

where ‘max’ and ‘min’ represent the largest and smallest red (R), green (G) and blue (B) values obtained for each data set.

4.4.5 Behavioural analysis

Fish behaviour was recorded using a digital video recorder mounted on a tripod. The camera was mounted in front of the tank and recorded fish movement. Each treatment was recorded for a period of 5 min and this was replicated six times both 1 h pre and post feeding for each of the three treatments. Swimming behaviour was assessed by randomly selecting six fish per treatment and following each fish for 4 × 60 sec intervals. Within each 60 sec intervals, the number of times the fish changed direction (turns) or swam with a sudden burst of speed was recorded. This process was repeated for each treatment pre and post feeding. The whole tank was also watched and recorded for the flight response (characterised by a group of three or more fish fleeing in unison), and aggressive encounters over a 5 min period. This was replicated 6 times per treatment pre and post feeding.

Fish distribution was also examined using the same video footage. A simple method was used where the tank was divided into four quarters (TL = top left, TR = top right, BL= bottom left and BR = bottom right, left and right side were used to ensure that the water inlet did not influence distribution) and each quarter was given the value of 25 (whole tank = 100). For each replicate recording period, an estimate was made of the fish distribution at one minute intervals. The position of all fish was observed and given a value depending on which quadrants of the tank the fish occupied. For example if all fish were swimming on the bottom of the tank then BR and BL were both given the value of 25 while TR and TL were given the value of 0. This value would indicate the position that the fish was in the tank and how much of the tank was being utilised. If only a portion of a quadrant was occupied, then aquaria quadrants with fish occupying only part of a space were allocated a value of

12.5. If fish occupied the whole tank then each quarter was allocated a value of 25. Average occupancy percentages for each treatment could then be calculated as a percentage by adding the total scores recorded for each quadrant and dividing it by the maximum score possible and multiplying by 100.

4.4.6 Data analysis

Results of growth performance and body colour were analysed using SPSS (version 15) and data were analysed with one way ANOVA. Where violations of the assumptions of ANOVA occurred, the Kruskal-Wallis non-parametric test was used. If the Kruskal-Wallis test indicated a significant difference, then a Mann-Whitney U test was performed between pairs to find the values that were significantly different. Two-way ANOVA was conducted in order to assess whether the state of satiation had any influence on fish behaviour between the three light treatments. Growth efficiency (*GE*) was calculated as: *GE* = fish wet weight gain/dry food consumed , where the weight unit was in grams; condition factor (*K*) was calculated as: $K = W \times 100/ L^3$, where *W* is the wet weight (g) and *L* is standard length (cm), and specific growth rate (*SGR*) was calculated as: $SGR = (\ln Wf - \ln Wi \times 100) / t$, where, *Wf* is the final weight of the fish (g), *Wi* is the initial weight of the fish (g) and *t* is the duration of the experiment in days.

4.5 Results

4.5.1 Growth and swimming behaviour

Light intensity had no significant impact on growth performance, growth efficiency or survival of the juvenile whiting ($P > 0.105$, Table 4.1). Light intensity had no influence on the amount of swimming activity according to turning frequency

with no significant difference observed between light levels (P = 0.113, Table 4.2). Turning frequency was also not affected by the state of satiation (pre feed or post feed) and there was no interactive effect between the state of satiation and light intensity (P = 0.294 and P = 0.946 respectively, Table 4.2). There was a trend for the number of aggressive interactions to increase with increasing light, though the results were not statistically different (P = 0.076, Table 4.2). A significant difference was observed in group flight activity with the 1000 and 25 lux treatments exhibiting more of this behaviour than the 500 lux treatment (P = 0.007, Table 4.2). This difference was not related to the state of satiation (P = 0.71).

Table 4.1. Growth and survival (mean ± SEM) of King George whiting held at three light intensities for 2 months. SGR = specific growth rate.

Light intensity (lux)	Final weight (g)	Final length (mm)	SGR (% day^{-1})	Survival (%)	Condition factor	Growth efficiency
1000	5.83 ± 0.23	88.45 ± 1.45	1.57 ± 0.07	98.3 ± 1.67	0.81 ± 0.01	1.01 ± 0.02
500	5.95 ± 0.17	88.57 ± 0.45	1.58 ± 0.02	94.7 ± 3.18	0.81 ± 0.01	1.04 ± 0.03
25	6.05 ± 0.24	88.69 ± 1.04	1.70 ± 0.04	93.0 ± 2.00	0.82 ± 0.01	1.09 ± 0.03

Total tank utilisation was significantly different between light intensities. Fish in the 1000 lux treatment utilised a larger percentage of the aquarium volume than both the 25 and 500 lux treatments ($P = 0.008$, Table 4.3). Further analysis revealed that fish in the 25 lux treatment were utilising the bottom half of the tank and occupying significantly less space in the top half of the tank. There was no significant difference between treatments for occupancy of the bottom left or bottom right quadrants ($P > 0.11$), whereas significant differences were observed for fish occupancy in both the top left and top right quadrants ($P = 0.024$ and $P = 0.001$, respectively, Table 4.3).

Table 4.2. Swimming behaviour of King George whiting exposed to different light intensities.

Swimming behaviour	1000 lux	500 lux	25 lux	*P* value
Swimming manoeuvres (turns/min)	12.67 ± 2.7	12.5 ± 2.9	11.61 ± 2.0	0.113
Swimming rapid burst/5min	0.67 ± 1.06	0.46 ± 0.7	0.32 ± 0.67	0.221
Encounters/5min	1.00 ± 0.95	0.75 ± 0.8	0.27 ± 0.65	0.076
Group flight/5min	1.00 ± 0.85	0.08 ± 0.29	0.82 ± 0.27	0.007
Tank usage (%)	82.5 ± 17.1	64.17 ±15.0	62.73 ± 13.5	0.008

Table 4.3. Swimming behaviour of King George whiting exposed to different light intensities.

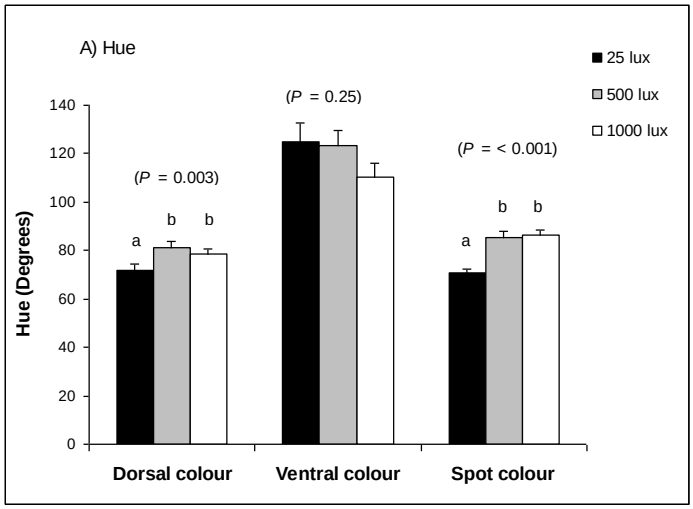
Fish distribution	1000 (Lux)	500 (Lux)	25 (Lux)	*P* value
Top Left	87.5 ± 23.4	70.8 ± 38.0	57.3 ± 28.3	0.024
Top right	90.0 ± 23.4	57.5 ± 34.7	35.5 ± 29.3	0.001
Bottom left	75.0 ± 33.2	70.8 ± 26.1	90.0 ± 18.4	0.117
Bottom right	77.5 ± 29.3	57.5 ± 35.2	68.2 ± 34.9	0.298

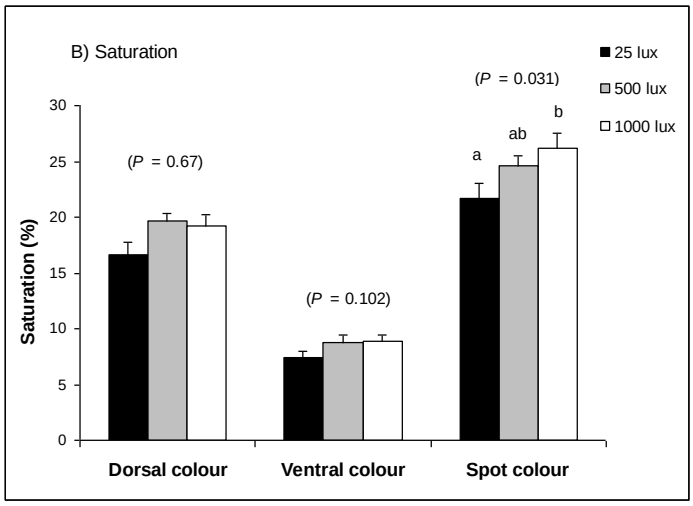
4.5.2 Body colour

Significant differences were observed between treatments in body colour. Hue values were significantly different in the 25 lux treatment than in 500 and 1000 lux treatments for both dorsal colour and spot colour ($P < 0.003$). No significant difference was observed for hue values of ventral colour ($P \geq 0.05$, Fig. 4.3A).

No significant difference between treatments was observed for colour saturation values of dorsal colour or ventral colour ($P > 0.05$). Spot colour saturation values were significantly different between the 25 lux treatment and the 1000 lux treatment ($P = 0.031$), but those in the 500 lux treatment were not different from either 1000 or 25 lux treatment (Fig. 4.3B).

The pigmentation of the fish dorsal and ventral sections and spots on the fish body at the 25 lux treatment were brighter than the other two light treatments ($P < 0.001$; Fig. 4.3C). Brightness values revealed the typical pattern for countershading in fish with the brightest surface being the ventral side. Less countershading was observed in the 25 lux treatment with only a 7% difference in brightness values between dorsal and ventral sides while the 500 and 1000 lux treatments had a 9-12% difference respectively (Fig. 4.3C).





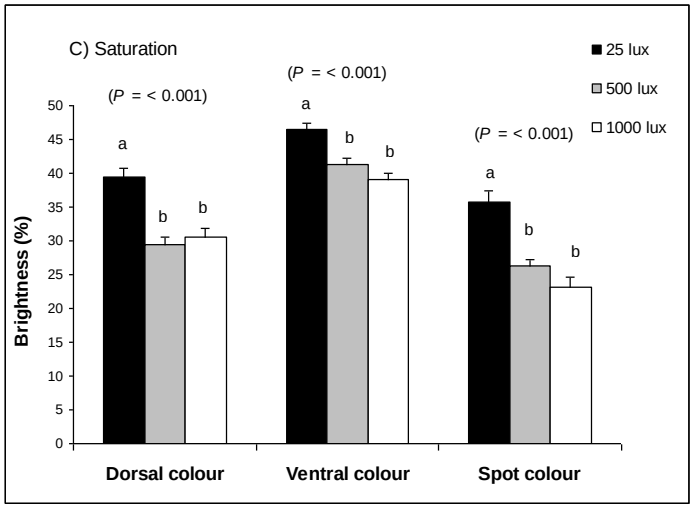


Figure 4.3. Mean colour A) hue (degrees), B) saturation (%) and C) brightness (%) ± standard error for body colour of juvenile whiting reared under different light intensities. Different letters indicate significant differences between treatments.

4.6 Discussion

4.6.1 Growth performance

There was no noticeable impact of light intensity on the growth of juvenile whiting during the 2-month experiment. This is consistent with studies of light effect on the growth of Atlantic salmon *Salmo salar* L where no influence on growth was recorded between 715 and 27 335 lux, and European sea bass (*Dicentrarchus labrax*) where growth was unaffected when reared under total darkness compared to a 12 L-12 D lighting regime (Stefansson et al. 1993; Papoutsoglou et al. 2005). Other studies have also shown that the response of fish to light intensity varies greatly between species and the stage of fish development. Light intensity usually has a stronger impact on larval fish (Boeuf & Le Bail 1999), although its influence on growth and foraging is also recorded in juveniles (Wallace et al. 1988; Han et al. 2005) and adult fish (Vasek & Kubecka 2004).

In our study, most parameters of fish performance were slightly improved under low light conditions. However, none of these differences were statistically significant. In comparison, the growth of juvenile Chinese longsnout catfish (*Leiocassis longirostris* Gunther) tested between 5 and 434 lux was reduced at both the minimum and maximum intensities whereas higher weights were attained at light intensities ranging from 74 to 312 lux (Han et al. 2005). Growth of Arctic charr juveniles was enhanced at relatively low light intensities of 50 lux when compared to other intensities up to 700 lux (Wallace et al. 1988).

4.6.2 Swimming behaviour

Fish swimming behaviour in the 1000 lux treatment was different from that in other light treatments and exhibited greater spatial distribution throughout the aquaria. This result differs from other studies that have reported wider fish dispersal at low light intensities (Monk et al. 2006; Torisawa et al. 2007). Fish in the 25 lux treatment tended to swim lower in the water column occupying the bottom half of the tank. Periods of reduced light intensity such as dawn or dusk are often peak feeding times for piscivorous fish and as light intensity decreases, a fish's ability to detect predators also decreases (McFarland & Wahl 1996). Compaction of fish shoals is advantageous as it can enable the rapid communication required for synchronised movement in order to reduce the success rate of predator attacks (Pitcher 1993). Therefore, the increased schooling behaviour and lower distribution of whiting in the tanks with low light intensity may have been due to a heightened awareness to potential predation. Alternatively, King George whiting juveniles forage less actively at night (Connolly 1995) and were observed to cease swimming and settle on the bottom of the tank under total darkness. The lower fish dispersal in the 25 lux treatment may have been related to this behaviour, although feed intake and growth were not affected, nor was swimming activity (quantified by turning frequency). In the wild, King George whiting juveniles are found in shallow seagrass beds interspersed with muddy or sandy patches (Connolly 1994; Fowler et al. 2002). While seagrass beds may provide some shade for these animals, water depths of less than 1 m would mean that this species is well adapted to coping with high environmental light intensities as opposed to species that show avoidance to high light (Johansson et al.

2006).

In some instances, high light intensities can result in increased swimming activity which can then lead to an increase in aggressive encounters due to more regular interaction (Almazan-Rueda et al. 2004). In this study, there was a slight decreasing trend present as light intensity decreased. However, no mortalities could be attributed to aggressive interactions. Whiting are a schooling fish and are not typically aggressive although, aggressive interactions did occur and were typically characterised by ramming or chasing.

The frequency of group flight activity was highest in the 1000 lux and the 25 lux treatments. In the 25 lux treatment this behaviour may be explained by reduced visibility and concentration of fish towards the bottom of the tanks. No obvious explanation was evident for flightiness in the 1000 lux treatment whereas very little flight activity occurred at 500 lux.

4.6.3 Body colour

Skin colour can be influenced by numerous factors including background colour, light intensity, light spectrum and temperature (Pavlidis et al. 2008). The colouration of King George whiting could allow for good camouflage while foraging over bare sand or sheltering in patchy seagrass beds. A less reflective dorsal surface may reduce predation from birds while the characteristic spots may serve to both confuse the shape of the whiting to predatory fish as well as aiding in blending into a sandy background. Many fish species living in close proximity to the water's surface have colour vision (Bowmaker 1995) and there is evidence that prey crypsis can increase the search time of

predators (Rowland et al. 2007; Johnsson & Kjallman-Eriksson 2008). Therefore, cryptic body colouration may be an important means of predator avoidance for King George whiting juveniles. Light intensity appears to play an important role in maintaining the cryptic body patterns and colouration in this species inhabiting shallow water. Fish were significantly brighter at 25 lux and hue and colour saturation levels were lower than fish at higher light levels. Many marine animals can mobilise pigment in response to changes in habitat type or structure. As the background for each treatment in this study was identical, the changes in body colour were mediated by light intensity. This was particularly obvious for spot colour and dorsal colour. Brightness values between dorsal and ventral surfaces presented the greatest difference in the 500 and 1000 lux treatments whereas fish reared at 25 lux exhibited less countershading and a more reflective silver colour. This increase in brightness with lower light intensities is consistent with observations in other species such as Chinese catfish (*Leiocassis longirostris*) (Han et al. 2005), false clownfish (*Amphiprion ocellaris*) (Yasir & Qin 2009), and Australian snapper (*Pagrus auratus*) (Booth et al. 2004).

In a recent study, the method of anaesthesia was found to affect fish colour before and post application of anaesthetic (Gray et al. 2011). In the present study, to avoid any possible impact of anaesthetics on fish colour property, a range of anaesthetic doses was carefully tested (90, 120, 150 and 180 µg/L). The lowest concentration (i.e., 90 µg/L benzocaine solution) was optimal in minimising any fish colour change by anaesthesia and allowed for a high recovery rate. Prior to administering the anaesthetic, visual observations showed that the fish were brighter in the low light tanks than the medium and high light tanks. The result of image analysis confirmed the visual observation. In

addition, all fish were treated equally in the process of anaesthetising to the photograph being taken. If any colour change occurred through this process it would have equally applied to all the treatments. Therefore, it is unlikely that the procedure of anaesthetic would impact the measurement of fish colour in this study.

Fish alter skin pigmentation by dispersing or concentrating melanin pigments in the skin (Rotllant et al. 2003). Wavelengths in the UVB (280-320 nm), UVA (320-400 nm) ranges and beyond (436 nm) have been observed to cause melanomas in some fish species (Ahmed et al. 1993; Setlow et al. 1993). The darkening of the dorsal surface from increases in melanin could be a photoprotective response to prevent epidermal tissue damage from ultraviolet (UV) light (Lowe & GoodmanLowe 1996). Therefore, wavelengths of 404.28 nm and 435.6 nm emitted from the lights in this study may have been responsible for the dispersal of pigment in the dermal malanophores and hence darker skin colouration seen in whiting exposed to the high light treatments.

Juvenile King George whiting are not considered to be strong swimmers (Jenkins & Welsford 2002) and are easily captured by slow moving seine nets. It is possible that these fish rely heavily on their ability to remain camouflaged in order to avoid predation. In this study, light intensity was responsible for increasing or decreasing the cryptic patterns of King George whiting. Therefore, occupying habitats subjected to bright illumination may be an advantageous strategy used to ensure cryptic colouration and predator avoidance.

4.7 Acknowledgements

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Chapter 5 Food competition and resource partitioning of recruiting juvenile fish with permanent residents in a seagrass nursery habitat

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5.1 Abstract

Recruiting King George whiting *Sillaginodes punctata* were studied to assess the potential for food competition with permanent resident fish species in a nursery habitat. Marine migrant post larval *S. punctata* <60 mm consumed primarily harpacticoid copepods and had high (>0.6) diet overlap with permanent residents *Favonigobius leteralis* and *Stigmatopora nigra*. Food electivity index indicates that *S. punctata* juveniles preferred harpacticoid copepods and amphipods, while juvenile *Heteroclinus adelaide* and *Gymnapistes marmoratus* migrating to the nursery habitat targeted larger prey such amphipods. Preference for larger prey by *H. adelaide* and *G. marmoratus* species coupled with differences in the state of digestion of prey within the stomach of these species was due to mouth size and feeding habits resulting in different food preference compared to *S. punctata*. The *S. punctata* showed an ontogenetic shift in diet with early settlers >60 mm consuming less copepods and more amphipods while previous year recruits >120 mm consumed polychaete worms. This study indicates that competition for food resources between recruiting *S. punctata* juveniles and permanent resident juveniles is likely reduced through differences in temporal and spatial feeding behaviours, mouth morphology, and ontogenetic shift in prey consumption.

***5.2 Keywords*:** competition, resource partitioning, King George whiting, diet, seagrass, estuary

5.3 Introduction

Seagrass habitats in estuaries form a highly productive ecosystem and are important nursery sites for many fish species (Able, 2005; Akin & Winemiller 2006). In southern Australia, seagrass beds can hold 40 times more benthic invertebrates than surrounding bare sand habitats (Bologna & Heck 2002). However, these habitats face considerable stress from climate change and anthropogenic activities such as urban development, eutrophication, pollution and recreational use (Bologna & Heck 2002, Bintz et al., 2003; Oviatt 2004). The loss of seagrass beds will impact both food availability for fish species associated with this habitat and biodiversity in the coastal environment (Heck et al., 2003). Understanding environmental factors and their influence on fish distribution and abundance is important to effectively manage fisheries and protect aquatic resources (Moyle & Cech 2004).

Competitive interactions between teleosts can play an important role in habitat selection (Munday et al., 2001), foraging behaviour (Mookerji et al. 2004), growth, and survival (Polivka, 2005) and exerts influence on recruitment to spawning stock. Food competition among fish species is generally greatest during early life stages and declines as fish grow (Ross, 1986). The increased competition is due to the influx of marine migrants and marine stragglers competing with permanent residents for resources (Najjar et al., 2000; Elliot et al., 2007). It is well accepted that the success of one species can displace or outcompete another species if they cannot successfully share and partition resources (McDonald et al., 2001; Marks et al., 2011). In an inshore or estuarine environment, the success of permanent residents has the potential to impact on the success of arriving

migrants via direct competition, whereas conversely, the successful recruitment of a migrant may place pressure on resources for permanent residents. Loss of suitable habitat may exacerbate competition among species as marine migrants compete for resources with permanent residents, while low recruitment of a species due to overfishing can allow for the increase in abundance of less targeted fish species that share a similar diet (Hobday et al., 1999).

The Barker Inlet is the largest estuary in South Australia and serves as a nursery ground for both post larvae and juveniles of the commercially important King George whiting *Sillaginodes punctata*. This nursery system provides beds of *Zostera* sp. and *Heterozostera* sp. seagrasses that are vital for the recruitment success of *S. punctata* post larvae. However, susceptibility to global warming and anthropogenic activities such as storm water runoff and treated effluent has resulted in considerable changes to habitat structured and distribution in this region. (Petrusevics 1993; Turner et al., 2004; Tanner 2005,). In South Australia, *S. punctata* spawn in the deep offshore waters off Kangaroo Island where ocean currents transport larvae northwards into the shallow waters of Gulf St Vincent. Throughout winter and autumn (May-November), *S. punctata* larvae recruit into the shallow seagrass beds in protected bays. The juvenile fish develop in these nursery areas for 1 - 2 years where they primarily feed on copepods, amphipods and polychaete worms (Connolly 1995) before moving out into the open waters of the gulfs. At approximately three years of age *S. punctata* begin their migration back towards the spawning grounds (Fowler et al. 2002). The recruitment success of *S. punctata* post larvae that settle into seagrass beds likely drives the population dynamics of adult fish (see e.g., Myers & Cadigan 1993). Despite competition links to survival of early life

stages, no study on *S. punctata* has evaluated the potential for trophic interactions with other seagrass dwelling fishes, or how *S. punctata* recruitment might be impacted by habitat loss and competition for resources.

Within a nursery, there is a dynamic use of prey resources from different age classes and species, leading to complex trophic interactions. The objective of this study was to use juvenile *S. punctata* of various ages throughout their temporary residency as a representative species to assess the potential food competition between recruiting fish and permanent resident fish over the period of ontogenetic diet shift. This study tests the hypothesis that permanent resident fishes compete for food with new settlers. The results would provide insight into the understanding of possible exclusion of recruiting fish by permanent dwelling fishes in the same nursery ground within a seagrass habitat.

5.4 Materials and Methods

5.4.1 Water quality parameters and fish collection

Monthly sampling for post larvae of *Sillaginodes punctata* and other resident fish was undertaken for a period of six months between June and November 2007. The sampling area was at King's Beach, located at the northern end of the Barker Inlet (Fig. 5.1), which historically has provided the largest catches of juvenile *S. punctata* (Jackson & Jones 1999). Sampling was conducted using a 5-m beach seine net with a 1-mm mesh. Three replicate hauls were conducted each month with a one-hour effort at either side of a low tide between 1000 h and 1200 h. For each haul, the net was towed between two people over a 20-m stretch of patchy *Zostera* sp. and *Heterozostera* sp. seagrass beds

perpendicular to the beach. After completing each haul, the net was redirected towards the beach, pursed and lifted onto the beach. All fish were removed from the net and placed into a lethal dose of benzocaine prior to preservation in 10% buffered formalin., In order to compare diets of post larvae with larger-sized *S. punctata*, a 60-m seine net with 2-m mesh wings and a bag mesh of 0.5 cm was deployed in January 2008 at the same location to capture early settlers (61-120 mm) and previous year recruits (>120 mm) for dietary comparisons. The sizes of *S. punctata* used in this study were well below the size at maturity (30 cm) around 3 years old (Fowler et al. 2000). Temperature and salinity were measured in triplicate upon arrival at the study site in each month prior to fish and zooplankton sampling (Table 5.1).

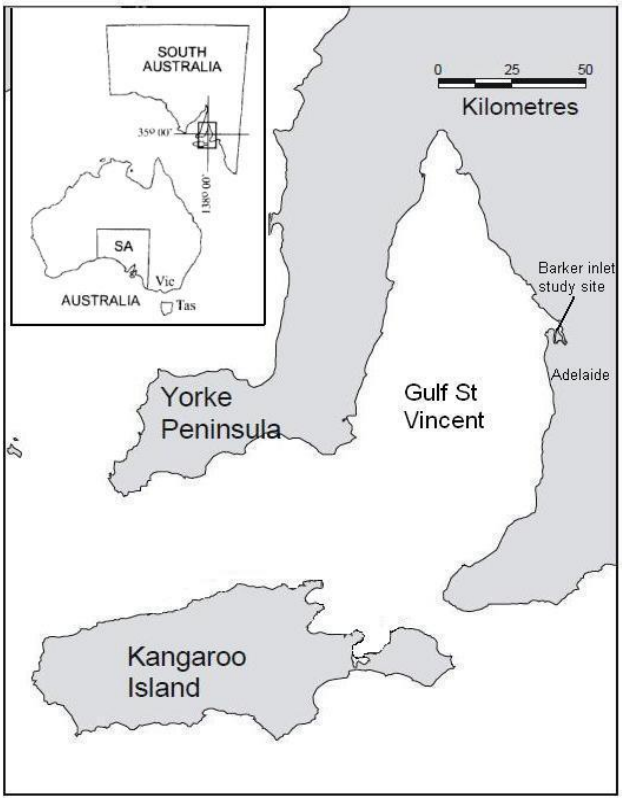

Figure 5.1. Location of study site (King's Beach, Barker Inlet) in relation to South Australian coastline (adapted from Cann *et al.* 2009).

5.4.2 Collection and treatment of pelagic and benthic food sources

Pelagic zooplankton samples were collected monthly by towing a plankton net (30-cm open diameter, 100-μm mesh) fitted with a flow meter over the seagrass where fish were collected. The water level was different at each sampling month so the zooplankton net was towed just above the level of the seagrass on all occasions. Each tow covered a length of 60 m and was replicated three times. The zooplankton samples were then transferred to storage containers and preserved in 3-5% neutrally buffered formalin. An exact volume (2.5% of the total) was subsampled in triplicate after thoroughly mixing the sample and then pipetting each subsample into a Sedgewick-Rafter counting cell under a microscope at 40 -100 × magnification.

To collect sediment samples of benthic fauna (e.g., copepod, amphipod, decapod, polychaete, nematode and molluscan), two replicated core samples were monthly taken at each of the three net haul sites, yielding a total of six cores per month. To collect samples, a 10.5 cm diameter piece of PVC pipe with a 0.25-mm mesh covering the top was pushed 5 mm into the sediment. A steel plate was then forced under the pipe and the corer was inverted to capture the sediment as described in Jenkins & Hamer (2001). Most of the water was drained through the mesh and the sediment was placed into containers for processing in the laboratory. To facilitate sorting organisms from the sediment, all sediment samples were sieved through four mesh sizes: 2 mm, 1 mm, 0.5 mm, 0.25 mm and the infauna and epifauna from each core were collected. Samples were placed into a counting wheel, which held 5-10 mL samples in a rotating circular trough containing a divider to mark the start and finish point. Infauna and epifauna were counted and

identified on a dissecting microscope at 40× magnification. For the small mesh sizes where sediment remained, the samples were preserved in a formalin sucrose solution and stained with rose Bengal during counting and identification. Infauna and epifauna were taxonomically identified to the level of order.

5.4.3 Fish dietary analysis

In the laboratory, all fish captured in the net tows were identified and measured to the nearest millimetre using standard length (tip of the snout to caudal peduncle). Fish stomachs were dissected by removing the outer flesh and ribcage from one side of the fish and removing the stomach and intestine. Only prey items within the oesophagus and stomach were analysed by removing the intestine from the posterior of the stomach with a scalpel. The stomachs were then placed in a drop of water and an incision was made so that the contents could be flushed and teased out into the water. The water containing prey items was then drawn through a pipette and placed onto a Sedgewick counting chamber for identification and enumeration. The only exception to this was for pipefish which have no distinct stomach and consequently, the entire digestive tract was used. Depending on the total number of fish collected, up to 20 stomachs of each fish species were analysed for diet comparison. Percentage of prey occurrence in stomachs was calculated as the percent of a prey found in all stomachs of a particular species. If for example, a prey is found 10 times in 20 stomachs, the prey occurrence is 50% (10/20) for that species. Mouth gape for each fish was recorded by placing the fish under a microscope fitted with a digital camera (Moticam 2500), extending the mouth with a pair of forceps and taking a digital image. The Motic image program (Motic image plus 2.0)

was then used to measure the mouth gape to the nearest 0.1 mm using a subsample of 10 fish per species.

Prey types identified in the fishes' diets were used in calculating dietary characteristics. These prey types included copepods (cyclopoid, calanoid, harpacticoid porcelid harpacticoid, and nauplii), amphipods (gammarids and caprellids), tanaids, mysid shrimp, cumaceans, penaeid shrimp, polychaete worms, barnacle nauplii and plant material. Porcelid harpacticoids and caprellid amphipods were placed separately to other harpacticoids and gammarus amphipods due to the differences in body morphology.

Food preference

The Manly/Chesson index (Chesson 1983) was used to determine prey preference of the different fish species.

$$\alpha_i = \frac{r_i / n_i}{\sum_{i=1}^{m} r_i / n_i}, = 1..., m$$

where α_i is the preference for prey type *i* ranging from 0 to 1, n_i is the proportion of prey type *i* in the environment, r_i is the proportion of *i* in the fish stomach, and *m* is the number of prey types in the environment.

To obtain a better comparison, dietary preference was further calculated as an electivity index **(ε):**

$$\varepsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}, i = ..., m$$

where the α_i is the preference value and *m* is the number of prey types. The values (ε_i) range from -1 to +1, where -1 indicates the absence of prey in stomachs and therefore suggests prey avoidance. Conversely, positive values suggest active selection of prey types. Results of zero indicate that there is no or little selection and that feeding is at random.

Dietary overlap

Diet overlap was calculated using Schoener's overlap index (Schoener 1970).

$$\alpha = 1 - 0.5\left(\sum_{i=1}^{n} \left| P_{ij} - P_{ik} \right|\right)$$

The index determines overlap (α), where P_{ij} = the proportion of the $_i$th resource (prey category) used by species $_j$, and P_{ik} = the proportion of the $_i$th resource used by species *k.* Overlap index values range from 0 (no overlap) to 1.0 (complete overlap), Values 0 – 0.29 indicate low overlap, 0.3 – 0.59 indicate moderate, and ≥0.6 indicate high dietary overlap between the two fish species being compared (Langton 1982).

Feeding strategy

To determine if fish fed as specialists or generalists, Levins index (Krebs 1999) niche breadth was utilised:

$$B = \left[\sum_{i=1}^{n} P_i^2 \right]^{-1}$$

where P_i is the proportion of the $_i$th prey in the diet of a fish species and the n is the number of prey groups. The average percentage of prey numbers (n) was used as the proportion (n%). Low values (B) indicate specialists and high values indicate generalists (Fjosne & Gjosaeter 1996). A standardised measure of this niche breadth B_A is calculated as:

$$B_A = \frac{B-1}{n-1}$$

Where, the value of B_A ranged from 0 (specialist) to 1 (generalist).

Statistical analysis and calculation

Due to data availability, the abundance of selected fish species (i.e., *S. punctata* <60 mm, *Stigmatopora nigra*, *F. lateralis*, *Gymnapistes marmoratus*, and *Heteroclinus adelaide*) and prey types (i.e., harpacticoid copepods and amphipods) were used in correlation analysis. Spearman's correlation analyses were used to determine the relationship between prey abundance and fish abundance using SPSS (version 18, IBM Corporation).

5.5 Results

During the study period from a cold and wet season (June) to hot and dry season (November), water temperature varied from 11.1 ºC to 20.8 ºC. On the other hand, only a small increase in salinity was recorded from 38 ppt to 40.5 ppt (Table 5.1). However, in this study, there was no significant correlation between total number of fish collected and temperature or salinity (r = 0.43, P = 0.40; r = 0.78, P = 0.07, respectively).

Table 5.1. Monthly variations of water temperature, salinity and fish abundance with the same fishing effort (60 m trawling distance) at King's Beach, Barker Inlet, South Australia.

Month	Temperature (°C)	Salinity (ppt)	Fish (*n*)
Jun	11.7	38.0	155
Jul	11.1	38.5	28
Aug	12.2	38.8	168
Sep	20.8	38.8	51
Oct	17.9	40.0	176
Nov	20.4	40.5	244

In benthic samples, prey species were dominated by harpacticoid copepods (3518.3) followed by gammarid amphipods (982.3) and errant polychaete worms (802.0) as quantified by number per core area (i.e., 86.6 cm^2) (Table 5.2). Over the sampling period, total abundance of harpacticoid copepods peaked in July (1640.7) and was lowest in October (62.8) (Table 5.2). In comparison, the maximal abundance of gammarid amphipod occurred in September (285.7), but its lowest abundance was found in November (13.2). Errant polychaete abundance first peaked in June (161.0) before declining to 73.0 in July. A second peak of this species occurred in August (182.2) and then declined to the lowest abundance in November (9.5).

Table 5.2. The abundance of benthic infauna and epifauna (mean ± SD, *n* = 6) included in a sediment core area (86.6 cm^2) at King's Beach, Barker Inlet, South Australia (2007).

	Jun	Jul	Aug	Sep	Oct	Nov	Total
Amphipoda							
Caprellid amphipod	0.2 ± 0.2	2.8 ± 2.8	4.3 ± 2.6	0	0.7 ± 0.3	0	**13.9**
Gammarid amphipod	18.5 ± 7.3	152.0 ± 25.8	61.5 ± 38.6	285.7 ± 64.8	79.5 ± 32.3	13.2 ± 2.8	**982.0**
Copepoda							
Calanoid copepod	0	0	0	0	0	0	**0**
Cyclopoid copepod	12.8 ± 7.1	0	5.7 ± 4.4	0	0	0	**30**
Harpacticoid copepod	228.5 ± 42.4	1640.7 ± 148.2	880.5 ± 155.0	161.2 ± 56.5	62.8 ± 12.8	119.2 ± 10.5	**3518.3**
Porcelid harpacticoid	0	0	2.2 ± 2.2	0.3 ± 0.3	0.5 ± 0.5	0	**6.0**
Cumacea							
Cumacean	0	2.2 ± 2.2	2.7 ± 2.3	0	0	0	**9.4**
Decapoda							
Penaeid prawn	0.3 ± 0.2	0	0	0.2 ± 0.2	0	0.2 ± 0.2	**1.1**
Mysida							
Mysid shrimp	0	0	0	0	0.2 ± 0.2	0	**0.4**
Tanaidacea							
Tanaid	0	0	4.3 ± 2.2	0.2 ± 0.2	4.3 ± 2.3	0.2 ± 0.2	**13.9**
Mollusca							
Molluscan veliger	0	0	0	0	1.5 ± 1.0	0	**2.5**
Nematoda							
Nematode	109.3 ± 32.8	0	13.0 ± 8.6	3.5 ± 1.8	0.8 ± 0.7	5.7 ± 1.4	**177.6**
Polychaeta							
Errant polychaete	161.0 ± 48.6	73.0 ± 11.4	182.2 ± 48.9	134.2 ± 31.6	62.5 ± 34.8	9.5 ± 1.7	**802.0**
Sedentary polychaete	20.3 ± 6.7	18.7 ± 4.2	31.0 ± 17.2	5.5 ± 2.1	20.3 ± 5.8	34.5 ± 3.3	**169.8**
Total	**551.0**	**2084.0**	**1669.4**	**590.8**	**323.8**	**202.6**	**5421.6**

In pelagic samples, harpacticoid copepods (7888.8 N m^{-3}) and copepod nauplii (4433.8 N m^{-3}) were the most common pelagic prey available to fish living in the seagrass beds (Table 5.3). Cyclopoid copepods (2111.3 N m^{-3}) and polychaete larvae (1644.8 N m^{-3}) were the next abundant although polychaete larvae did not appear in the stomach contents of any fish species. After reaching the first peak (1986.6 N m^{-3}) in September, harpacticoid copepods reached the maximum in November (3017). Copepod nauplii showed a similar pattern to harpacticoid copepods, but its lowest value occurred in June (203.6 N m^{-3}). Similar to harpacticoid copepods, polychaete larvae showed two peaks with the first one in August (783.7 N m^{-3}) and the second occurring in November (353.9 N m^{-3}). Noticeably, mollusc veliger larvae were only found in October and absent in other months.

In general, there was a negative trend between prey density and fish population with the strength of the relationships ranging from weak ($r \leq 0.3$) to strong ($r \geq 0.5$). Although most comparisons for individual fish had no significant correlations, a significant correlation was found between total fish abundance and total benthic crustaceans ($r = 0.83$, $P = 0.04$, $n = 6$). No significant correlations were observed between prey density and water temperature or salinity. There was negative correlation between benthic prey abundance and temperature ($r = -0.72$) and salinity ($r = -0.53$) while there was positive correlation between pelagic prey and temperature ($r = 0.62$) and salinity ($r = 0.57$). For total prey density, positive correlations were observed ($r = 0.40$ and $r = 0.42$ for temperature and salinity respectively).

Table 5.3. Abundances of pelagic zooplankton (N m^{-3}, mean ± SD) collected with a zooplankton trawl net at King's Beach, Barker Inlet, South Australia (2007).

	Jun	Jul	Aug	Sep	Oct	Nov	Total
Amphipoda							
Gammarid amphipod	0	3.6 ± 2.3	2.6 ± 2.6	47.2 ± 15.7	1.6 ± 1.6	0	**55.0**
Copepoda							
Calanoid copepod	16.5 ± 7.2	25.7 ± 14.8	15.7 ± 6.8	293.6 ± 109.5	7.8 ± 1.5	332.9 ± 50.6	**692.2**
Copepod nauplii	203.6 ± 121.6	258.5 ± 28.1	733 ± 170.7	870.2 ± 299.0	237.5 ± 29.1	2131.0 ± 461.5	**4433.8**
Cyclopoid copepod	130.5 ± 91.6	92.8 ± 25.6	562.2 ± 164.7	256.9 ± 29.2	51.9 ± 16.6	1017 ± 112.1	**2111.3**
Harpacticoid copepod	597.6 ± 215. 7	476.4 ± 145.4	1425.9 ± 282.1	1986.6 ± 391.4	385.3 ± 55	3017 ± 207.1	**7888.8**
Mollusca							
Molluscan veliger	0	0	0	0	196.6 ± 47.6	0	**196.6**
Polychaeta							
Errant polychaete	0	10.5 ± 6.9	5.2 ± 5.2	10.5 ± 5.2	4.7 ± 2.7	5.2 ± 2.6	**36.1**
Polychaete larvae	80.2 ± 59.2	61.9 ± 19.1	783.7 ± 129.1	340.7 ± 51.7	23.6 ± 5.4	353.9 ± 136.4	**1644.8**
Total	**1028.4**	**929.5**	**3528.3**	**3805.7**	**909.0**	**6857.7**	**17058.6**

5.5.1 Fish species composition

Over the 6 months, 13 fish species were sampled from the seagrass beds (Table 5.4). The family of greatest abundance was the Gobiidae (62%) with *Favonigobius lateralis* being the most abundant, accounting for 57% of all fish caught. The second most common family was the Syngnathidae, with three species providing 15% of the total catch, of which *S. nigra* was the most common at 14%. Both *F. lateralis* and *S. nigra* were the only species observed in all months and were the main permanent residents in this system. The greatest influx of fish to the region occurred in October and November with the third (Clinidae, *Heteroclinus adelaide* 8.4%), fourth (Sillaginidae *S. punctata* 4.1%) and fifth (Scorpaenidae *Gymnapistes marmoratus* 2%) most abundant families recruiting throughout this period (Table 5.4).

5.5.2 Diet composition

Diet varied between *S. punctata* of different size classes with the 20 - 60 mm class consuming primarily harpacticoid copepods (86%) and small gammarus amphipods (9%, Fig. 5.2). Despite the low quantity of gammarus amphipods consumed, this prey item was consumed regularly with 80% of *S. punctata* stomachs making it a very important food source (Table 5.5). The remainder of the diet consisted of calanoid and cyclopoid copepods, and the occasional worm or crustacean (mysid shrimp). The *S. punctata* in the 61 - 120 mm class consumed primarily amphipods (84%) with worms and other crustaceans making up the remainder at low frequencies (Table 5.5). Stomachs of the largest *S. punctata* size class (>120 mm) contained only polychaete worms (Fig. 5.2).

Table 5.4. Monthly catch summary of fish species collected at King's Beach, Barker Inlet, South Australia from June to November 2007.

Species	Jun	Jul	Aug	Sep	Oct	Nov	**Total**
Atherinidae							
Atherinosoma microstoma	4	0	2	2	12	19	**39**
Clinidae							
Heteroclinus adelaide (adult)	1	0	0	0	0	0	**1**
Heteroclinus adelaide (juvenile)	0	0	3	4	17	44	**68**
Engraulididae							
Sprattus novaehollandiae	0	0	0	0	0	4	**4**
Gobiidae							
Arenigobius bifrenatus	1	0	1	1	2	8	**13**
Bathygobius kreffti	0	0	0	0	0	9	**9**
Favonigobius lateralis	140	15	77	28	108	101	**469**
Gobiopterus semivestitus	1	0	1	0	1	0	**3**
Unidentified	0	0	0	0	4	13	**17**
Monacanthidae							
Meuschenia freycineti	1	1	1	0	0	0	**3**
Mugilidae							
Aldrichetta forsteri	0	0	5	0	0	0	**5**
Odacidae							
Neoodax balteatus	1	1	1	0	0	0	**3**
Pleuronectidae							
Rhombosolea tapirina	0	1	1	0	0	0	**2**
Scorpaenidae							
Gymnapistes marmoratus (adult)	0	0	1	0	0	0	**1**
Gymnapistes marmoratus (juvenile)	0	0	0	0	1	15	**16**
Sillaginidae							
Sillaginodes punctata	3	3	0	10	15	3	**34**
Syngnathidae							
Stigmatopora nigra	3	4	73	6	11	18	**115**
Vanacampus vercoi	0	0	0	0	3	5	**8**
Kaupus costatus	0	0	0	0	2	0	**2**
Terapontidae							
Pelates octolineatus	0	3	1	0	0	0	**4**
Tetraodontidae							
Contusus brevicaudus	0	0	1	0	1	0	**1**
Tetractenos glaber	0	0	0	0	0	5	**5**
Total	**155**	**28**	**168**	**51**	**176**	**244**	**822**

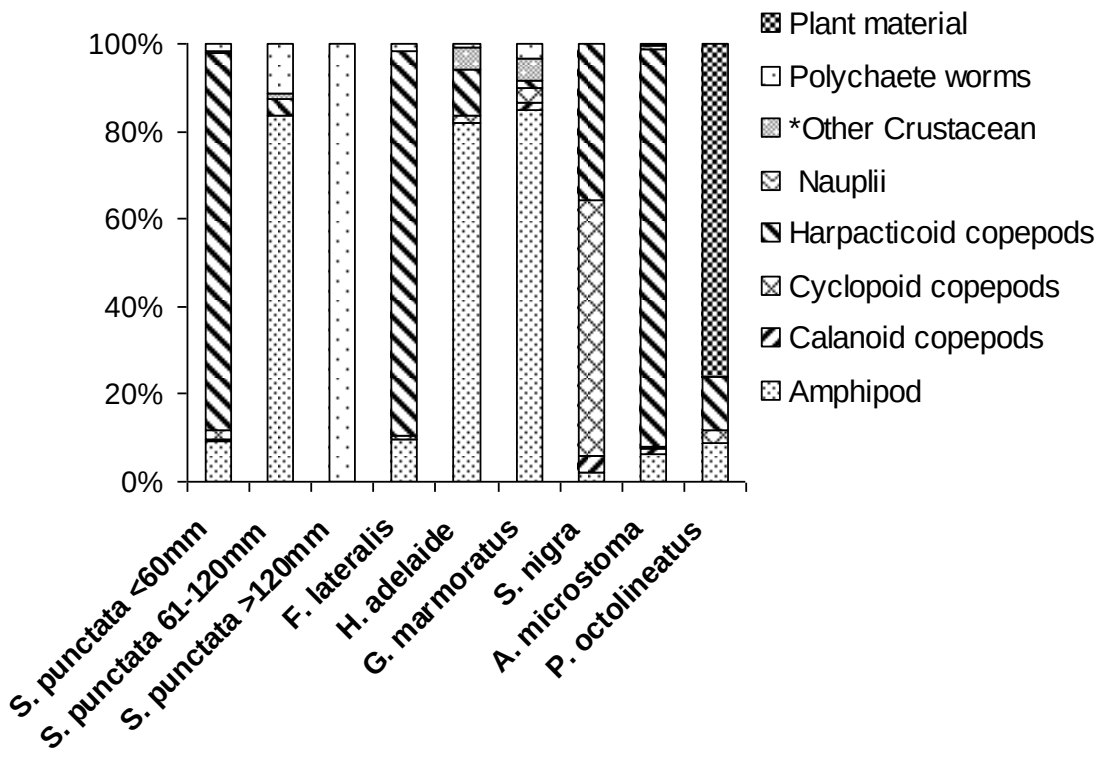

Figure 5.2. Individual prey types (%N) in the diet of the most common fish species captured in a seagrass bed in the Barker inlet from June 2007 to January 2008. *Other crustaceans include cumacean, tanaids and penaeid prawns grouped together.

The stomachs of *F. lateralis* contained a large proportion of harpacticoid copepods (88%) with a small percentage of the diets made up of amphipods (10%) and polychaete worms (2%, Fig. 5.2) although amphipods were a frequently consumed prey item being present in 40% of stomachs (Table 5.5). The juvenile *G. marmoratus* and *H. adelaide* had similar diets which consisted mostly of crustaceans including amphipods (83%), tanaids, penaeid and mysid shrimp (<10%). A small percentage of their diets consisted of worms (<2%) and copepods (7% *G. marmoratus* and 11% *H. adelaide*, Fig. 5.2) and only occurred in a few of the stomachs (Table 5.5). The *S. nigra* primarily consumed copepods (59% cyclopoids, 35% harpacticoids, and 4% calanoids) with some

incidents of small crustacean nauplii (0.7%, Fig. 5.2) although the frequency of most prey items consumed was greater than 50% suggesting broad feeding habits (Table 5.5). The *P. octolineatus* contained a mix of copepods (15%) and amphipods (9%) but stomach contents mainly consisted of plant material (73%, Fig. 5.2). The *A. microstoma* diets also consisted of a mix of calanoid, cyclopoid and harpacticoid copepods (93%) which were the most frequently consumed item (Table 5.5).

5.5.3 Niche breadth, prey preference and diet overlap

According to the preference index, *S. punctata* less than 60 mm and *F. lateralis* shared similar feeding behaviour showing active preference for harpacticoid copepods (ε_i = 0.5) and neutral selection for amphipods (ε_i = 0.05, -0.24, respectively). Food preference of both *G. marmoratus* and *H. adelaide* was positive for amphipods ($\varepsilon_i > 0.5$). With the exception of *S. nigra*, all fish species showed active avoidance of calanoid and cyclopoid copepods. All fish species exhibited selection against polychaete worms and large crustaceans while *G. marmoratus* and *H. adelaide* were the only two fish species that selected against harpacticoid copepods (Table 5.6). Niche breadth was quite low for all species with the highest value observed in *S .nigra* (B_A = 0.09, Table 5.6). The *G. marmoratus* and *H. adelaide* with their similar diets had a slightly lower niche breadth (B_A = 0.04) while the largest class of *S. punctata* showed no dietary breadth consuming only one prey type (Table 5.6).

Table 5.5. The occurrence (%) of each prey in the stomach of fishes collected at King's Beach, Barker Inlet, South Australia from June 2007 to January 2008.

	S. punctata 20-60 mm	*S. punctata* 61-120 mm	*S. punctata* >120 mm	*F. lateralis*	*H. adelaide*	*G. marmoratus*	*S. nigra*	*A. microstoma*	*P. octolineatus*
Fish sample size (*n*)	20	20	11	20	20	20	20	20	20
Amphipoda									
Gammarid amphipod	80	76	0	40	100	75	55	50	0
Caprellid amphipod	0	0	0	0	5	0	0	5	5
Copepoda									
Cyclopoid copepod	25	0	0	10	10	10	65	15	15
Calanoid copepod	15	0	0	0	0	5	60	5	0
Harpacticoid copepod	80	14	0	90	0	5	80	80	30
Porcelid harpacticoid	20	0	0	0	40	0	15	5	0
Copepod nauplii	5	0	0	0	0	0	0	15	0
Cirripedia									
Barnacle nauplii	0	0	0	0	0	0	5	10	0
Cumacea									
Cumacean	0	0	0	0	5	0	0	5	0
Decapoda									
Penaeid prawn	0	5	0	0	10	10	0	5	0
Mysida									
Mysid shrimp	5	0	0	0	10	5	0	5	0
Tanaidacea									
Tanaid	0	0	0	0	5	0	0	0	0
Polychaeta									
Polychaete worm	15	38	90	10	0	5	0	10	0
Plant material	0	0	0	0	0	0	0	0	80

Table 5.6. The range of fish length and mouth gape, mean stomach fullness rank and electivity index of fish caught between June 2007 – January 2008. Stomach fullness ranged from 0 (empty) to 10 (full). Niche breadth index values range from 0 (specialist) to 1 (generalist) in feeding. The food electivity (εi) ranges from -1 (avoidance) to +1 (positive selection). Cal: calanoid, Cyc: cyclopoid, Har: harpacticoid, Nau: Nauplii, Amp: amphipod, Cru: other crustaceans, Wor: polychaete worms.

Fish species	Length (mm)	Mouth gape (mm)	Stomach fullness	Niche breadth	Electivity (εi)						
					Cal	Cyc	Harp	Nau	Amp	Cru	Wor
Atherinidae											
Atherinosoma microstoma	31 – 62	1.5 – 4.3	7 ± 1.8	0.02	-0.78	-0.90	0	-0.85	-0.04	-0.89	-0.86
Clinidae											
Heteroclinus Adelaide	23 – 46	1.8 – 4.5	7 ± 1.2	0.04	-1.00	-1.00	-0.69	-1.00	0.77	-0.51	-1.00
Gobiidae											
Favonigobius lateralis	27 – 45	2.0 – 3.9	4 ± 3.0	0.02	-1.00	-0.91	0.54	-1.00	-0.24	-1.00	-0.9
Scorpaenidae											
Gymnapistes marmoratus	18 – 26	2.5 – 4.4	4 ± 2.6	0.04	-0.87	-0.85	-0.97	-1.00	0.52	-0.35	-0.98
Sillaginidae											
Sillaginodes punctata (20-60 mm)	17 – 45	0.8 – 2.8	5 ± 2.4	0.03	-0.72	-0.75	0.50	-1.00	0.05	-0.9	-0.76
S. punctata (61-120 mm)	84 - 112	3.0 – 6.0	5 ± 2.2	0.03	-1.00	-1.00	-0.89	-1.00	0.57	-1.00	-0.34
S. punctata (>120 mm)	177 - 203	6.0 – 9.0	7 ± 1.8	0	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	1.00
Syngnathidae											
Stigmatopora nigra	67 – 100	0.7 – 1.1	6 ± 3.5	0.09	-0.22	0.17	0.03	-0.97	-0.78	-1.00	-1.00

The juvenile *S. punctata* (<60 mm) had high dietary overlap ($\alpha \geq 0.6$) with *F. lateralis* and *S. nigra* while moderate ($\alpha = 0.3 - 0.59$) overlap was observed with *P. octolineatus* (Table 5.7). Whiting <60mm exhibited low ($\alpha < 0.029$) overlap with *H. adelaide*, *G. marmoratus*, *A. microstoma* and also with the larger size of *S. punctata* (61-120 mm) and high dietary overlap with *H. adelaide* and *G. marmoratus*, moderate overlap with *A. microstoma* and low overlap with all other species and *S. punctata* size classes. The *S. punctata* >120 mm had very little overlap with other species or other size classes of *S. punctata* (Table 5.7).

Table 5.7. Dietary overlap of King George whiting (*Sillaginodes punctata*) at three size classes with other commonly occurring fishes in Barker inlet from June 2007 to January 2008. Values 0–0.29 indicate low; 0.3–0.59 moderate; and ≥0.6 high dietary overlap. The values (>0.6) are bolded to show the trend of high dietary overlap.

	S. punctata (20-60 mm)	*S. punctata* (61- 120 mm)	*S. punctata* (>120 mm)
Atherinidae			
Atherinosoma microstoma	0.075	0.489	0.003
Clinidae			
Heteroclinus adelaide	0.1	**0.870**	0.001
Gobiidae			
Favonigobius lateralis	**0.965**	0.095	0.013
Scorpaenidae			
Gymnapistes marmoratus	0.17	**0.885**	0.031
Sillaginidae			
S. punctata (20-60 mm)	-	0.1	0.019
S. punctata (61-120 mm)	0.235	-	0.115
S. punctata (>120 mm)	0.019	0.115	-
Syngnathidae			
Stigmatopora nigra	**0.928**	0.055	0
Terapontidae			
Pelates octolineatus	0.4	0.13	0

5.6 Discussion

5.6.1 Fish species composition and recruitment

The Barker Inlet is an estuary system lying within the Gulf St Vincent which is known as an inverse estuary due to high evaporation and low freshwater inflow. This resulted in the salinity level recorded in the estuary being higher than normal ocean salinities. Despite the high salinity and susceptibility to the impact of hot weather, the fish species composition in Barker Inlet is similar to that reported in other studies of seagrass areas in temperate Australia (Crinall & Hindell 2004; Smith et al., 2008a). In the Barker Inlet habitat, fish abundance was low and the seagrass beds at the sample site were covered by a thick coating of sediment which probably devalued the habitat quality. Habitat quality is often related to habitat selection, fish growth and survival (Nemerson & Able 2004). The high sedimentation on seagrass may be related to the overall low fish abundance and low recruitment of *S. punctata* into this historically productive nursery. In a 10-year study, Jackson & Jones (1999) provided a thorough historical analysis of fish assemblages in the Port River and specifically at King's Beach where this study was conducted. King's Beach previously had a large population of *S. punctata* and was the most abundant species at this location, approximately 2.8 times higher than the next most abundant family (Mugilidae). In this study, *S. punctata* were only ranked the fifth behind Gobiidae, Syngnathidae, Clinidae and Atherinidae. Fish assemblages appear to have changed considerably between the two studies especially in regards to the number of *S. punctata*.

The timing of benthic settlement can be important where resources are limited because exploitative competition may lead to individuals depleting a food supply before the arrival of a competitor (Ward et al., 2006). Differences in recruitment timing can also be important by increasing niche separation between species (Golani, 1994). In this study, *S. punctata* were steadily recruiting to the seagrass beds in small numbers from June to November. The arrival of juveniles of *H. adelaide*, *G. marmoratus* and *F. lateralis* occurred later in the sampling period from June to November generally peaking in abundance in November. The early arrival of *S. punctata* to the seagrass may have an advantage for recruiting success as we observed greater abundance of this species in October and November allowing early recruiters to have fewer species and conspecifics to compete for food resources with permanent resident fish.

5.6.2 Feeding interactions and assessment of competition

Copepods and amphipods are the most highly consumed prey items by numerous larval fish species in temperate waters (Whitfield, 1985; Schlacher & Wooldridge 1996). While there are similarities in the size of fish species, mouth morphology appears to be important in separating diet selection as is seen in other species occupying the same habitat (Hyndes et al., 1997; Linke et al., 2001). Despite the similar range of body size, *S. punctata* and *F. lateralis* had a much smaller mouth gape and selected for small prey such as copepods. On the other hand, *H. adelaide* and *G. marmoratus* had relatively larger mouth gapes and actively selected for the larger amphipod prey. This morphological characteristic separated the diet of post larval *S. punctata* <60 mm from other species and could possibly reduce food competition.

The *F. lateralis* shared a diet consisting mainly of harpacticoid copepods with *S. punctata* <60 mm, as indicated by the high overlap index close to 1 (i.e., complete diet overlap). Smith et al., (2008b) observed that most fish favoured the seaward side of the seagrass, whereas gobies *Nesogobius* sp. favoured the shoreward side of seagrass beds, suggesting spatial segregation with other seagrass-associated species. In this study, *S. punctata* were located primarily over seagrass beds while *F. lateralis* occupied areas on the shoreward side of the seagrass beds in shallower water. Therefore, despite the high food overlap, it is likely that these species are spatially separated to some extent. The sea weed *Ulva australis* is often present at King's Beach (Jackson & Jones 1999) and we found that *F. lateralis* often used this weed as shelter on the sand between the shore and the seagrass beds, whereas most other fish species were located in the seagrass beds. The difference in habitat selection along with preference for the most abundant prey species (harpacticoid copepods) may diminish food competition between permanent resident *F. lateralis* despite its high overlap with recruiting *S. punctata* <60 mm (Cabral et al., 2007). The increased shelter of *Ulva australis* for *F. lateralis* may explain the high abundance of this species despite the poor state of the seagrass beds. The preference for *Ulva australis* by *F. lateralis* may become advantageous allowing them to thrive in a sub-optimal seagrass habitat. Given the similarity in diets between *F. lateralis* and *S. punctata*, loss of seagrass beds will likely reduce the habitat separation as *S. punctata* seek other forms of shelter potentially contributing to an increase in competition for food resources. Generally, there are positive relationships observed between seagrass biomass and abundance of prey items such as harpacticoids and amphipods (Bologna &Heck 2002; Murphy et al., 2010). Loss of shelter may result in a loss of amphipods and force *H.*

adelaide and *G. marmoratus* to exploit a less favoured prey item increasing the competition between seagrass associated fish.

The stomachs of *S. nigra* usually contain small amphipods and harpacticoid copepods (Howard & Koehn 1985). The *S. nigra* exhibited neutral selection for all families of copepods suggesting a diverse and generalised feeding habit. These data agree with Howard & Koehn (1985) who observed that *S. nigra* feed on pelagic or epibenthic prey on the eelgrass. In contrast, the lack of pelagic prey in the diets of *S. punctata* suggests that its feeding area is close to the benthic region, although in shallow environments such as the study site, feeding on pelagic prey items can be done without much change to the fish position in the water column. Mobility is a factor that can potentially reduce competition within an ecosystem (Gibson & Ezzi 1987). The *S. punctata* are not confined to seagrass beds often feeding over bare sand (Jenkins &Wheatley 1998), providing evidence that feeding interactions may also be avoided between recruiting *S. punctata* and resident *S. nigra* via spatial differentiation.

Ontogenetic changes in the diet of *S. punctata* also aided in dietary separation. The *S. punctata* >60 mm consumed a greater proportion of amphipods and fewer copepods. In contrast, *S. punctata* >120 mm had a diet consisting almost entirely of polychaete worms which were not consumed in large amounts by any other species or *S. punctata* of different sizes. Changes in ontogenetic food preference are important in creating greater niche partitioning and reducing competition (Schellekens et al., 2010). The relatively large mouth gape of juvenile *H. adelaide* and *G. marmoratus* indicates that they target larger prey than newly settled *S. punctata* and this would make them more

competitive with early recruited than newly settled *S. punctata*. The ontogenetic shift away from small prey items as *S. punctata* grow also limits the time spend competing for food with small permanent resident species.

Resource partitioning of fish can also be enhanced by temporal differences in the period of peak feeding (Piet & Guruge 1997). In this study, the stomach contents of both *G. marmoratus* and *H. adelaide* were almost fully digested. Scorpaenids feed at both day and night (Hobson, 1974), while weedfish *Cristiceps australis* move to areas of higher food abundance at night (Smith et al., 2008b). In our study, sampling was conducted in the late morning, therefore the highly digested food in the stomachs of *G. marmoratus* and *H. adelaide* were probably ingested during the night. Juvenile *S. punctata* are reported to only feed during the day (Connolly, 1995), therefore competition with *G. marmoratus* or *H. adelaide* might be reduced through feeding at different times. The low abundance of different prey species may contribute to a high degree of dietary overlap between fish species (Moyle & Cech 2004). In this study, niche breadth was low due to the relatively small amount of prey items available at high concentrations. Benthic prey was dominated by harpacticoid copepods and amphipods with only very small amounts of other prey items being recorded. The lack of prey items is reflected in the niche breadth index, suggesting that most of these fish species feed as specialists.

Potential competitors rarely reach the population densities that exceed resource supplies (Moyle & Cech 2004). No significant relationship was observed between total or individual fish population and prey availability. Predation on harpacticoid copepods is not thought to be the principal cause of variations in seasonal abundance of copepod prey

(Gee, 1989). Likewise, predation on meiofauna in general does not significantly reduce prey density due to the rapid reproductive rates of meiofauna (Coull, 1999). Therefore, it is unlikely that this nursery is resource limited in terms of food, despite some significant overlap being observed between a few species. The overall low numbers of fish and the abundance of harpacticoid copepods and amphipods suggest that there should be an adequate supply of food in the system and that competitive exclusion by depletion of food resources is not at a level likely to have significant influence on post larval recruitment or permanent resident populations.

In conclusion, *S. punctata* of different size classes show distinct ontogenetic shifts in diet preference reducing the period of competition for food between age classes of *S. punctata*, and small permanent resident fish. This study supports the hypothesis that species living in close proximity show dietary overlap and food competition, but the main prey types targeted by all fish are abundant and competition for scarce resources is unlikely to affect the recruitment success of recruiting fish in this system. Competition is also currently reduced by differences in mouth morphology between species and by discrepancy in temporal and spatial uses of prey items, allowing a number of species to coexist without reducing resources to a limiting level. However, loss of seagrass habitats is likely to exacerbate the competitive interactions of fish within this nursery through decreased prey abundance and a reduction spatial segregation.

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Chapter 6 General discussion, conclusions and future research

6.1 General discussion

The future of fish stocks not only depends on effective fisheries management, but also on understanding the implications of environmental change and the factors that influence species survival. Gaining broad knowledge of environmental impacts on fish before major stock losses is essential in the overall assessment of constraints to fish recruitment and provides a baseline for future comparisons. Key factors such as temperature, salinity, light intensity and food competition are likely to alter fish physiology and behaviour in response to future changes in environmental conditions. Understanding the environmental impact to a species is important in understanding how these stressors are affecting fish stocks as well as identifying when and how a species may be threatened. The Gulf St Vincent provides an excellent test environment as it is a dynamic system that has been subjected to considerable environmental stress by climatic factors and anthropogenic degradation. Juvenile fish such as King George whiting inhabiting regions subjected to harsh and dynamic conditions can provide pivotal information as to how fish species respond to a rapidly changing environment and allow for the assessment of factors influencing recruitment success.

King George whiting (KGW) showed quite a remarkable tolerance to extremes in abiotic conditions. Juvenile KGW in particular, showed greater resilience than adults to the changes in the environment. This study reveals that the biological adaptability required to thrive in a harsh environment is well developed in juvenile KGW. While the

direct impact of short term changes in abiotic environmental conditions will result in moderate physiological changes to KGW, the long term effects of anthropogenic and climate change on temperature, salinity, light intensity and food availability are likely to have profound influence on marine fishes as climate changes continues to occur in the future.

6.1.1 Temperature

In general, fish exhibit varying degrees of temperature tolerance and adaptation among species. Most organisms exposed to high temperature can produce heat shock proteins (HSP) to protect against the cellular damage caused by thermal stress regardless of inhabiting aquatic or terrestrial habitats . Climate change will not only lead to increases in average temperatures, but also increased frequency of extreme weather events such as heat waves. In this study, the abrupt temperature spikes in shallow coastal water were replicated in laboratory experiments and the heat shock response of KGW was initiated at all temperatures tested from 24 to 30 °C. Despite KGW having a lifespan in excess of 18 years, a reduction in temperature tolerance was observed in young KGW of only 3^{+} yr compared with 1^{+} yr KGW which interestingly coincides with the migratory patterns of this species from shallow warm water to offshore cooler water.

A reduction in the heat shock response is common in older organisms of many species and is likely to affect estuarine opportunists and nearshore fish species. The consequences of increased ocean temperatures include changes to physiology, distribution, timing of spawning and migration patterns (Allison et al. 2009), which in turn, has complicated implications for fisheries and fishery management (Brander 2007).

Should water temperatures increase in areas where populations of maturing fish with a reduced heat shock response inhabit, then this might interrupt with natural migration patterns and result in fish migrating earlier and leaving traditional grounds in search of cooler waters. Mortality of adult 3^+ yr old fish indicates that maturing KGW would be the first to be impacted if they were to remain in shallow habitats, or if the northern section of Gulf St Vincent was to show a permanent temperature increase in regions where 3^+ yr KGW reside. Importantly, this study eliminates the confounding impact of acclimatisation because animals were all held in controlled conditions. The process of acclimatisation can impact a species thermal tolerance (Hofmann and Somero 1995). In the wild, the difference in thermal tolerance in fish of different ages can often be attributed to acclimatisation as adults and juveniles occupy different habitats subjected to different environmental conditions. However this can be ruled out in this study as the adult KGW were reared from post larvae in the laboratory under the same stable conditions as the 1^+ yr KGW indicating that the differences observed were likely mainly due to the age of the fish.

When 1^+ yr KGW were exposed to increased temperature over a longer period of time (up to 1 week), results indicated that production of HSP was at its highest after 96 h. The duration of HSP production varies between species so this information provides valuable evidence on how KGW cope with temperature changes and may also be applied to other fish species living in fluctuating environments. Furthermore, the level of HSP production was elevated above the control temperature (18° C) even under the lowest temperature stress tested (24 °C) in both age classes. This information together with previous findings on the growth (Ham and Hutchinson 1997) and distribution (Jones et al.

1996) of KGW suggests that water temperatures around 26 °C may be near the upper optimum for this species. As the processes involved in synthesizing HSPs are energetically costly (Lyndon et al. 1992), increases in the frequency of heat waves may have negative effects on other processes such as growth and immune response in the long term (Iwama et al. 1999). It is a natural response for fish species to show active avoidance of unfavourable water temperatures (Coutant 1977). In shallow water environments, rapid warming during heat waves may indirectly result in lower recruitment success as juvenile fish leave their normal habitats temporarily in search of more suitable water temperatures exposing them to greater risk of predation. In the Barker Inlet region where the juveniles for this study were collected, cooler water can be found along channel drop-offs where there is deeper water and greater tidal movement. This sort of temporary migration would expose KGW to a number of predatory fish species in the region including *Acanthopagrus butcheri*, *Sphyraena novaehollandiae*, *Arripis truttacea*, *Arripis georgianus.*

6.1.2 Salinity

The characteristics of the Gulf St Vincent make it highly susceptible to increases in salinity as a result of high evaporation due to climate change and extreme heat. With salinity levels already exceeding ocean salinities, fish species occupying this region require effective osmoregulatory capability. Juvenile KGW were found to have a broad salinity tolerance and easily tolerated salinity levels from 30 to 50 ppt. While there was a decreasing trend in growth with increasing salinity, growth measurement or condition factor of young of the year (YOY) KGW was not significantly impacted over the 72 d

salinity trial. Overall osmolality levels of YOY KGW exhibited an increasing trend with increasing salinity without showing significant difference between treatments. Many fish species benefit from being raised in salinities below 35‰ (Boeuf and Payan 2001; Imsland et al. 2008). As KGW are a slow growing species, there was no difference observed in this study in terms of growth between salinities. The trends observed suggest that given a longer period, differences may occur as is seen in faster growing species such as *Solea sengalensis* (Arjona et al. 2009).

As observed in the temperature experiments, this trial revealed that tolerance to salinity depended on fish age. Similarly, juvenile California halibut *Paralichthys californicus* also showed difference in salinity tolerance between age classes (Madon 2002). In this study, the 2^+ yr KGW displayed higher osmolality levels than 1 yr old KGW. The 2^+ yr KGW also showed significantly higher osmolality at all salinity levels which was not observed in 1 yr old KGW. Plasma ions were significantly increased in 2^+ yr KGW with increasing salinity as is the trend observed in many other fish species (Gaumet et al. 1995; Woo and Chung 1995; Morgan and Iwama 1998; Gonzalez et al. 2005; Laiz-Carrion et al. 2005). This result together with the reduction of haematocrit in fish agrees with the notion that the increased energy expenditure and increased stress in fish are associated with maintaining osmotic homeostasis in waters exceeding 40 ppt (Boeuf and Payan 2001; Magnussen et al. 2008). The salinity levels used in this study were well within the tolerance range for KGW, but there are some indications that KGW can grow faster at lower salinities. The results tend to suggest that if salinity levels were to rise to the levels tested in this study, then the environment would be approaching a critical point at which the overall fitness of this species would be compromised. Similarly

to the discussion on temperature, migration away from juvenile habitats as fish age suggests that older fish are more likely to be susceptible to extreme environmental conditions. If salinity levels in the northern gulf rise to levels that alter salinity in the southern regions where breeding occurs through the southern flow of water out of the gulf, then it may result in conditions that are detrimental to older fish. However, current salinity conditions are well within both age groups tolerance levels.

6.1.3 Light intensity

The inevitable increases in UV radiation, water temperatures and salinity levels in environments such as the Gulf St Vincent due to climate change or human activity will not only impact fish directly through physiological and biological responses but also indirectly through loss of habitat and associated food sources. An abundance of evidence is found in current literature that highlights how anthropogenic and climate changes will have a significant impact on the state of seagrass beds (Short and Neckles 1999). In many regions around the globe, increases in temperature as well as damage from development and pollution have caused significant die offs of important seagrasses. Many fish species inhabit these shallow water seagrass beds that are exposed to high UV radiation, as they provide shelter for vulnerable juveniles of migrant fish as well as resident fish. In the event of seagrass/shelter loss, cryptic body colouration then becomes even more important as a means of predator avoidance. However, UV radiation directly influences body colour because it increases melanin levels in the skin. Light intensity can also affect fish physiology and behaviour (Boeuf and Le Bail 1999; Puvanendran and Brown 1998). Juvenile KGW typically inhabit shallow water environments subject to high light

intensity so the impact of light on this species was examined in this study to further understand the implications of anthropogenic habitat loss to fish in shallow water.

While light intensity often influences the growth of young fish (Boeuf and Le Bail 1999) no difference in growth or survival was observed in KGW exposed to different light conditions in this study. Behaviourally, light intensity did not affect swimming speed or the amount of aggressive encounters, but did influence schooling behaviour with low light leading to greater schooling, while group flight activity increased at high light intensity. These results are likely related to the reduction of activity under low light intensity in KGW (Connolly 1995) and the compaction of fish shoals which is a means of predator avoidance (Pitcher 1993).

The results of this study reveal that low light intensity reduced the cryptic colourations naturally found on KGW by reducing counter-shading and increasing the overall brightness of the fish. Contrary to this, the higher light intensity resulted in darker spots and greater contrast between dorsal and ventral surfaces of the fish increasing the counter shading and overall camouflage. Crypsis incorporates several forms of camouflage including countershading, background matching, and disruptive colouration (Stevens & Merilaita 2009a). The colour patterns observed under high light intensity may play a dual role in both background matching and disruptive colouration by blending into the environment and creating false edges to obscure the shape of the fish to predators (Rowland 2009; Stevens and Merilaita 2009a; Stevens and Merilaita 2009b. Habitats without the adequate cover or shelter required to obscure the view of predators increase the risk of predation for all seagrass associated fish. In addition, within habitats subjected

to bright illumination, fish such as KGW that have markings that are darkened by increased light intensity will have an advantage in avoiding predation. This advantage can be attributed to the increase in cryptic colouration which is particularly important for slow moving juveniles foraging over bare sand in the absence of seagrass cover.

6.1.4 Resource competition

Destruction of seagrass beds reduces the abundance and diversity of prey species that are typically associated with seagrass habitats. Adult fish stocks are directly influenced by the successful recruitment of juvenile fish from nurseries and one of the many factors influencing survival of new recruits is the competition for food resources. The diet of newly-recruited KGW post larvae was examined and the potential for competition assessed by comparing food preference, niche breadth and diet overlap with numerous other fish species that occupy the same habitat. Within the current environment of the Barker Inlet, there was a high abundance of only two prey types (harpacticoid copepods and amphipods), while other prey items were only available in low abundance. This resulted in low niche breadth and some degree of dietary overlap between species sharing the seagrass habitat. To reduce the degree of food competition, fish species often exhibit a partitioning of resources. In the Barker Inlet, resource partitioning was achieved via spatial, temporal, morphological and ontogenetic differences in feeding in juvenile KGW. As predation was primarily directed towards the most common prey types, it is unlikely that resources would become depleted in the environment as rapid reproductive rates of copepods and amphipods make them a difficult food resource to deplete (Gee 1989; Coull 1999; Moyle and Cech 2004). The current level of competitive interactions

and resource exploitation by fish is unlikely to significantly impact upon the survival of KGW recruiting to nursery areas along the metropolitan coastline. However, the understanding gained through this research on how KGW interact with conspecifics and competitive species in seagrass habitats highlights how habitat destruction will change the trophic relationships between fish species and add pressure on recruiting KGW.

To some extent, the successful partitioning of resources relies on habitat complexity. Habitat structure and seagrass beds are important for both food and shelter. The destruction of seagrass beds via climate change and anthropogenic impacts will result in increased competition for reduced areas of habitat and food resources. In this study, spatial separation was observed between species that had high dietary overlap. Reductions in the quantity of usable habitats will lead to less spatial separation between competing species and remove an important means of partitioning resources. As higher biomass of food sources is usually correlated with high biomass of seagrass, the loss of seagrass beds will inevitably impact on food supply. In this study the diets of KGW were separated from species of the Clinidae and Scorpaenidae families due to differences in mouth morphology and subsequent selection of prey of different sizes. Reduction in food supply will result in fish becoming less selective in their choice of prey type or size. This will lead to a reduction in the amount of trophic partitioning between co-existing fish as the decrease in food supply leads to more opportunistic feeding behaviours. A similar result will occur with the differences in food selection brought about by ontogenetic change as was observed for KGW. A reduction in food supply is likely to cause less trophic segregation between conspecifics with early settlers outcompeting new recruits

for sparse food resources.

6.2 Conclusions

The predicted changes to aquatic ecosystems in relation to climate change will impact on fish in seagrass nurseries in a number of ways. There can be vast differences between species in the response to environmental factors. However, the impacts of temperature and salinity on juvenile KGW recruitment will be low in terms of fish physiological response. Indirect effects such as changes to habitat, food sources and behaviour are likely to be the first areas to be detrimental to the recruitment of KGW and will place increasing pressure on this economically important fish species in the future. Based on the results of previous four data chapters, specific conclusions are summarised as follows to illustrate main findings of this PhD study.

1. Heat shock proteins can successfully be used to detect the response of KGW to changes in temperature at a molecular level, and it may serve as a tool to monitor environmental stress of KGW to climate changes in Gulf St Vincent.
2. The production of HSP69 in juvenile KGW is elevated at 24 °C when compared to the basal level of HSP69 at 18 °C which suggests that current temperatures within the nursery system can exert stress on juvenile KGW and KGW may be living close to their maximum threshold. However, KGW required a short period of acclimatization (96 to 168 h) when prolonged temperature elevation occurred in the ambient environment.
3. Salinity from 30 to 50 ppt had very little impact on the growth performance and survival of KGW juveniles. Therefore, the salinity increase in a dry summer in Gulf

St Vincent is unlikely to have a negative impact on KGW in the short term.

4. KGW showed a general reduction in tolerance to temperature and salinity elevation as fish size increased which coincided with their life cycle. The migratory patterns of KGW involve a southward migration of juveniles from shallow seagrass beds in the northern parts of the Gulf St Vincent to deeper waters at around 3 years, followed by a southward migration concluding at the mouth of the Gulf where breeding occurs (3-4^{+} yr). The temperature and salinity levels at the mouth are lower and more stable than northern regions. These results suggest that KGW seek a more favourable environment in order to breed, and offers a partial explanation for the location of spawning grounds and lack of northward migration observed in this species.
5. Prime habitats in shallow water that are subjected to high light intensities aid in maintaining the cryptic colouration of KGW by increasing countershading and disruptive colouration. This response is likely to enable increased predator avoidance as well as protection from ultraviolet radiation via increased melanin in the skin.
6. Within nursery grounds, KGW juveniles share a similar diet to numerous other species as a result of low prey diversity. Direct competition with other species is likely reduced via temporal and spatial differences in feeding. KGW exhibit a distinct ontogenetic shift in diet which broadens their foraging diversity over age, thus reduces intraspecies competition between different age classes.
7. Overall, KGW exhibit considerable tolerance enabling them to withstand a very broad range of environmental condition and fluctuations and are thus very well adapted to the dynamic conditions experienced within South Australian gulf waters. It is more likely that anthropogenic induced changes to habitat structure and food supply will

play a more critical role in recruitment success than climate change.

6.3 Future research

This study revealed valuable information about KGW and their response to the environment although the depth of the study was limited by the amount of fish that could be captured from wild stock. Numerous areas of study are possible to further expand upon the results obtained from this research.

1. The consequences of the increased energy required to maintain osmotic homeostasis and produce HSPs may divert energy away from growth and ultimately result in slow growth and smaller KGW in fishery catches. Within a nursery ground where fish are subject to predation from larger fishes, slow growth rates will increase the period of time that these fish are susceptible to predation. The relationship between fish size and fecundity is well known, therefore the long term effects of reduced growth rates may lead to smaller animals migrating to spawning grounds and a reduction in the overall egg production and ultimately larval production. As this species is slow growing it is of benefit to conduct long term monitoring on age versus growth to provide better evidence as to how environmental conditions impact on KGW until the point that they begin breeding.
2. The effects of temperature on KGW in the laboratory were found, but the temperature impacts on KGW in the field remain unknown. This research indicates that there was a heat shock response and a period of acclimatizing even at a laboratory condition of 24 °C. Conducting heat shock analysis on fish captured from the field would be necessary to validate if the levels of HSP produced are considered normal as opposed

to what levels are potential harmful. Given that the seasonal variation in temperature is considerably different, establishing the basal level of HSP in relation to seasonal change is essential to using HSP as a reliable bio-indicator of stress in the field. Establishing the impact of temperature on the growth, energy demand, egg quality and larval survival would all be relevant to determining the potential constraints of juvenile recruitment for this species.

3. Increases in salinity may impact on various other factors relevant to juvenile recruitment such as egg and sperm production and viability. There are currently no hatcheries for KGW so it was not possible to obtain larval fish. However, studies on the impact of salinity on the survival and development of larval KGW pre settlement would undoubtedly provide useful predictive information in terms of the impacts of environmental change and how it may constrain settlement and recruitment to seagrass beds. Similar to temperature, longer term studies on the effects of salinity on growth may reveal clearer trends than what was produced in the short term studies of this thesis.
4. All of the KGW were collected from the same area at Barker Inlet. South Australia contains large geographical barriers to fish movement and previous research suggests that Gulf St Vincent and Spencer Gulf are replenished by separate spawning stocks (Fowler and March 2000). Environmental conditions vary slightly depending on location (e.g. Juvenile KGW in upper Spencer Gulf have been reported in water approaching 50 ppt (Ham and Hutchinson 2002)). Therefore, in terms of environmental parameters, comparisons between stocks spawned from different regions will provide a broader understanding of the overall response of KGW and

reveal information about its ability to adapt to changing condition.

5. Light intensity is particularly influential during larval development influencing numerous functions including swimming activity, prey capture rates and swim bladder inflation. Light intensity had no impact on growth and survival of the post larval KGW in this thesis but it may show a more pronounced influence in earlier life stages. Research on the effects of light intensity on larval KGW would be useful in determining potential constraints on KGW recruiting to seagrass beds. This thesis showed the benefits of high light intensity to the cryptic colouration of KGW. Background colour also has an impact on fish colouration but was not investigated. Research into the interaction of light intensity and background colour or habitat type on the cryptic colouration of KGW is another potential area for future investigation that would reveal the effects of environmental conditions on recruitment success.
6. Competition for space may also impact on a species success and is relatively unknown for KGW. Anthropogenic change such as seagrass loss due to pollution is likely to be a key determinant for future KGW stocks. Revealing if there is any direct competition for prime habitats, antagonistic interactions or species displacement between species sharing nursery habitats would provide a clearer picture of the ecosystem in which KGW reside during the critical early stages of their life.

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