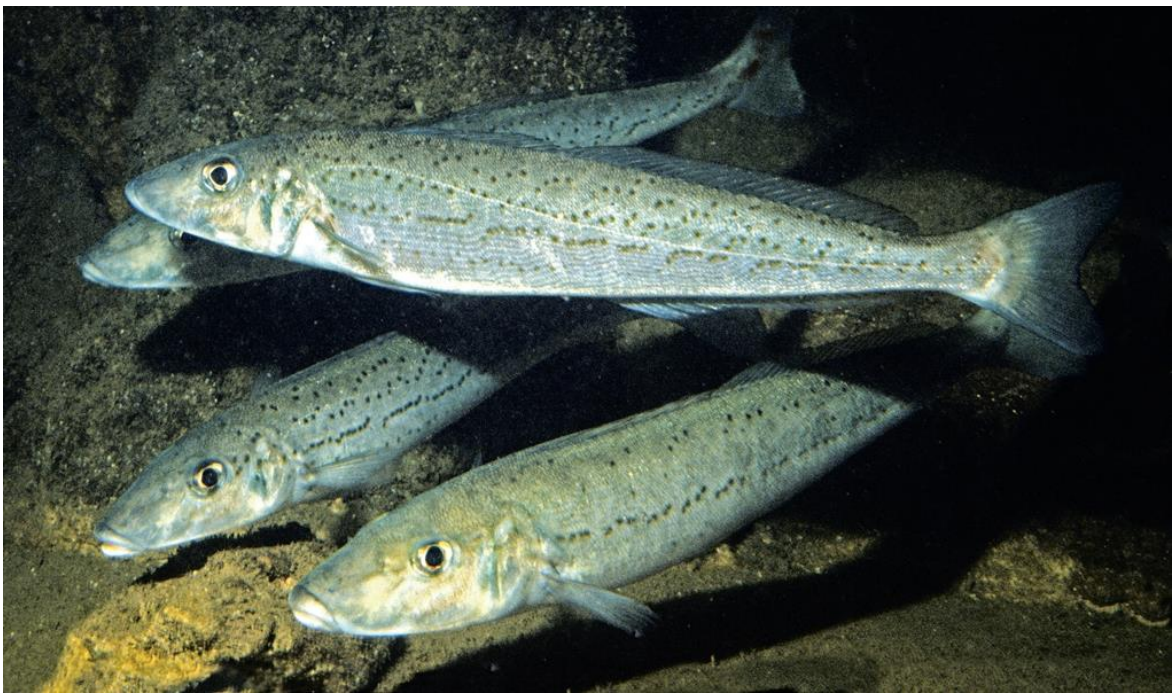
The physiological responses of King George whiting to a changing environment



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Declaration

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Cover image: Adult King George whiting (*Sillaginodes punctatus*)

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Abstract

Environmental variability affects the physiology of marine ectotherms, causing changes to metabolic rate, locomotion and growth. Species that move between habitats with different temperature and salinity for spawning purposes may experience significant changes in their growth rate and physiology compared to those that live in stable environments. Ectotherms have a temperature and salinity range at which growth and survival are optimal. Although, ectotherms are capable of tolerating a range of temperatures and salinities, moving from optimal to extreme ranges can affect oxygen consumption, locomotion and growth. The physiological responses of many marine ectotherms to environmental variability are not well known. King George whiting (*Sillaginodes punctatus*; Sillaginidae) is an important commercial and recreational temperate fish in Southern Australia, with concerns it may be at risk to future climate change. Due to the deficit of information on physiology and growth of this species, they were targeted to evaluate their physiological response to environmental change.

Climate-growth relationships were reconstructed for King George whiting using growth chronologies derived from fish ear bones (otoliths). Otolith samples were collected from Kangaroo Island, Spencer Gulf and Gulf St Vincent in South Australia. A chronological approach was used to examine the inter-annual variation in growth and the influence of region, sea surface temperature (SST), El-Niño Southern Oscillation (ENSO) events (SOI), and recruitment. The growth chronology showed a negative correlation with winter SST. Recruitment and region did not affect growth rate.

The swimming performance and metabolic rate of adult fish was investigated at two temperatures (16°C and 26°C), as well as their potential to recover after a prolonged swimming period, in a resting chamber. Fish were initially swum in a swim chamber, while water velocity

was increased, until exhaustion, then their critical swimming speed (U_{crit}) was calculated. Following exhaustion, fish were transferred into a resting chamber and the maximum metabolic rate (MMR) was calculated. Thereafter, they were allowed to recover in the chamber overnight and their standard metabolic rate (SMR) was measured. The U_{crit} and aerobic metabolic rate were higher at the higher temperature and the fish recovered quicker in warmer water.

A similar study was performed on juvenile fish, but across four temperatures (16, 19, 22 and 25°C) and two salinities (30 and 40ppt), using swim chamber. Metabolic rate of the juveniles was explained by a curvilinear relationship with temperature, but temperature had no influence on U_{crit}. Salinity did not affect the MMR and aerobic scope, but SMR decreased and U_{crit} increased as salinity decreased. The temperature optimum for SMR and aerobic scope was between 16°C and 19°C and their thermal window was between 16°C and 22°C with a critical temperature (T_c) of 25°C.

The effects of temperature and salinity (the same treatments as mentioned above) on otolith elemental composition were investigated as a precursor to tracing environmental history of King George whiting. The concentration of Mg, Mn, Sr and Ba, ratioed to calcium, in juvenile otoliths was influenced by salinity, with a minor effect of temperature and no interaction between temperature and salinity for all element:Ca ratios. This indicated that otolith chemistry maybe useful for reconstructing the salinity history of King George whiting.

I developed methods for evaluating the effects of environmental parameters (e.g. SST, SOI and salinity) on King George whiting growth, physiology and otolith chemistry. Outcomes can be used to assess the growth and metabolic response of King George whiting to temperature and salinity change. The otolith chemistry results can be used for reconstructing the environmental

salinity history, and potentially movement patterns, of King George whiting. The temperature examined did not significantly affect the swimming speed and otolith elemental composition of the fish. A plausible reason for these results might be that the temperature range examined was within the species' optimal thermal tolerance window, but any further temperature increase or decrease at both ends of the thermal window can possibly affect the growth and survival of this species.

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Chapter 1: General Introduction

Photo: Me in Port Vincent, South Australia, the juvenile King George whiting sampling location
Photo credit: Amir Forghani

Chapter 1

Environmental variability and its effects on aquatic ectotherms

Environmental variability can directly affect an organism's physiology, growth and locomotion (Meakin and Qin, 2012), and indirectly disturb the ecosystem structure and function through anthropogenic impacts and global warming (Neuheimer et al., 2011). Environmental factors, such as dissolved oxygen, temperature and salinity, affect an organism's physiology and growth. Aquatic ectotherms have an optimal range of environmental conditions in which their growth and survival are optimum (Pörtner, 2010). Prolonged exposure to environmental stressors (in this study temperature and salinity) out of the species tolerance limit results in reduced growth, and impairment in oxygen consumption and metabolism (Barton, 2002). The negative impacts of environmental variability on species survival and distribution is predicted to become more serious in the near future with changing climate (Lough et al., 2012). The juveniles of many species occupy shallow marine or estuarine waters, where the temperatures and/or salinities vary both spatially and temporally. These environmental parameters can therefore influence a critical phase (early life stages) in the life cycle of many coastal fish species. Understanding the physiological performance of the organism in light of environmental variability would help to develop our knowledge about the aerobic capacity and growth rate of the organism and their ability to tolerate unfavourable environmental conditions.

Temperature change

Temperature can have a profound effect on growth, physiological processes (aerobic metabolism), bio mineralisation (otolith chemistry) and plays an important role in shaping the

distribution and abundance of organisms (Schulte, 2015; Viña, 2002). Generally, physiological responses are related to how an organism maintains function at different temperatures and how they are able to adjust their normal temperature range (thermal window) (Pörtner, 2010). The chemical reactions to temperature can be studied through chemistry and growth of the hard parts of ectotherms (e.g. otoliths).

Salinity

Salinity is a key environmental parameter regulating physiological processes in aquatic ectotherms. Changes in salinity affect osmoregulation and can cause stress (Evans, 2010). Physiological response to salinity is species specific (Uliano et al., 2010), and depends on life history stage (Boeuf and Payan, 2001). Climate change is leading to an increase in water temperature and shifts in rainfall patterns that will ultimately affect salinities in estuaries.

Stenohaline species have a narrow tolerance to salinity and can only live in either fresh or salt water. Euryhaline species have a wide tolerance to salinity and can live in freshwater, brackish water or hypersaline water and tolerate a range of salinities (Farrell, 2011). For some species that move between habitats at different life history stages (e.g. King George whiting), the ambient salinity at settlement plays an important role in determining their growth and recruitment success (Meakin and Qin, 2012). Investigating the impact of salinity change on growth and metabolic rate of aquatic ectotherms will provide an insight into their salinity tolerance and adaptation**.**

Dissolved Oxygen

Dissolved oxygen (DO) in estuaries can vary from hypoxia to supersaturating in a short time period. Decreasing levels of DO (hypoxia) cause a biochemical reaction in fish by producing harmful products in their bodies such as lactic acid (Nakano et al., 1992). On the other hand, increasing levels of DO (super saturation) may cause a decrease in swimming performance and increase in mortality rate (Nebeker and Brett, 1976). Environmental variables such as temperature and salinity affect the oxygen solubility in the water with the solubility of oxygen decreasing with an increase in temperature of the water (Verberk et al., 2011). Therefore, the fish oxygen demand and metabolic rate increases in response to elevated temperature and salinity (Farrell, 2011) provided the temperatures and salinities are within the optimal range for the species. The thermal physiology and oxygen demand of the organism in response to changing DO shapes their aerobic performance in response to climate change (Verberk et al., 2011).

El Niño Southern Oscillation Index

A broader scale atmospheric-oceanographic phenomenon is the El Niño Southern Oscillation (ENSO). This refers to a situation when the seawater temperature in the eastern and central Pacific Ocean becomes warmer than normal (Bjerknes, 1969). This phenomenon happens every three to eight years and is generally associated with the Southern Oscillation Index (SOI) and together termed as ENSO (Bjerknes, 1969). SOI is the pressure difference between Tahiti (17°339 S; 149°379 W) in the South Pacific and Darwin (12°289 S; 130°519 E) in northern Australia (Mantua et al., 1997). Variation in temperature is linked with ENSO (Nicholls, 1991); cool phases of ENSO are related to the La-Niña and warm phases correspond to the El-Niño

event (Nicholls, 1991). ENSO causes a major variation in weather and affects the marine biology, fish stocks and food availability for the marine species (Holton et al., 1989).

Methods to assess effects of environmental variation on aquatic ectotherms

I applied several approaches to evaluate environmental variability on the growth and physiology of a temperate marine fish. Approaches included: growth chronology development, swimming speed and aerobic metabolic rate measurement and otolith chemistry analysis in response to temperature and salinity.

Growth chronologies

Obtaining accurate information about the growth of an organism is important for understanding a species response to environmental variation (Black et al., 2008). Growth studies can help to manage exploited marine fish and assess their susceptibility to over-exploitation especially for the commercial and recreational fish population which are under pressure of over fishing. Growth estimation is commonly associated with the periodic measurement of increments in the hard parts of the animal (e.g. otolith, shell and teeth) (Campana, 2005).

Annual and daily growth increment analysis in otoliths of fish is analogous to tree-ring science (dendrochronology). Otolith growth rate is highly correlated (normally linearly) with the somatic growth rate of the fish and the annual or daily increments represent the individual growth for each assigned year (Thresher et al., 2007). Growth rate is related to species survival, competition, and reproduction (Barber and Jenkins, 2001). For example, mortality rate is growth

related because faster growing individuals are more prone to harvest by fishermen than slower growing individuals, which can cause population size to decline over time (Ricker, 1969).

Long-term growth chronologies are valuable for reconstructing the environmental history of the fish. Further they can be used to forecast the future growth in relation to environmental change and provide a general growth model for the species of interest (Stocks et al., 2011; Black et al., 2008). Some previous studies have used a traditional dendrochronological approach (similar to tree-ring analysis) to understand the correlation between growth increment widths and environmental variability over multiple decades (Black et al., 2011; Matta et al., 2010; Black et al., 2008; Black et al., 2005). This approach uses the cross-dating technique to ensure that the growth increment is assigned to the correct calendar year. Further it assumes that growth is affecting by some limiting environmental factor and that inter-annual growth variations in response to the environmental changes are synchronised (Black et al., 2013; Matta et al., 2010). Other studies have used a mixed modelling approach to explore the relationship between the growth rate and environmental variability (Morrongiello and Thresher, 2015; Morrongiello et al., 2011; Neuheimer et al., 2011; Thresher et al., 2007). Mixed modelling methods (generalised linear mixed model: GLMM) provide an ecologically robust understanding of how fish may respond to climate variability (such as changes in temperature and salinity) compared to more traditional dendrochronological methods which are designed to maximise climate-growth relationships and reduce 'ecological noise' within the data (Morrongiello et al., 2012). In mixed effect modelling methods comparisons and interactions among environmental and non-environmental factors are assessed and the assessments do not rely on averaged data (Morrongiello et al., 2012).

Otolith chemistry and environmental reconstruction

Otolith chemistry has been used as a tool for reconstructing the environmental history of fish (Campana, 1999). The historical information about the environmental conditions (e.g. temperature and salinity) are recorded and saved on the otolith. This information can represent the habitats and environments that the fish inhabited, as well as patterns of movement (Elsdon and Gillanders, 2002). Trace elements such as strontium (Sr), barium (Ba), manganese (Mn), magnesium (Mg) are crystallised and deposited on the calcified structure of the fish (e.g. otolith) (Campana, 1999). The trace elements in otoliths can be used as a natural chemical tag (Elsdon et al., 2008). The chemistry of the otolith can be analysed with a Laser Ablation Inductively Coupled Plasma-Mass Spectrometer (LA ICP-MS) (Campana et al., 1995).

Determining the factors that influence elemental uptake in the otolith can help to reconstruct the environmental history of the species (Elsdon et al., 2008). Environmental and biological parameters have the potential to alter the chemical composition of the otolith. Temperature and salinity are two factors that can affect the otolith chemical composition. These factors are assumed to have a direct influence on bio-mineralisation processes and otolith chemical composition (Elsdon et al., 2008).

Temperature can affect the incorporation of elements into the otolith. Different studies have reported differing results including positive, negative and no relationship between temperature and otolith chemistry (Martin and Thorrold, 2005; Elsdon and Gillanders, 2002). The varying results are assumed to be related to the crystallisation process of elemental incorporation into the otolith. Temperature affects the pH of the blood plasma and endolymph fluid thereby affecting the crystallisation process and consequently the otolith chemical composition (Gauldie et al., 1995). The crystallisation process can also be regulated by physiological processes (Melancon et

al., 2009). In addition, different responses to temperature may be species related (Dorval et al., 2005). Nevertheless, defining the influence of temperature on otolith chemistry is somewhat difficult because temperature is correlated with otolith growth for some species (Fowler et al., 1995).

Another environmental factor that affects the otolith elemental composition is salinity. Previous studies have suggested that Sr and Ba can be used as a marker for tracking the anadromous movement and reconstructing the salinity profile of the fish (Dorval et al., 2005; Kraus and Secor, 2004). Although previous studies have suggested that there is a strong relationship between Sr:Ca and Ba:Ca and water salinity (Panfili et al., 2015; Dorval et al., 2007) individual species behave differently and incorporate elements into their otoliths in different ways. Most previous studies have found no relationship between Mg:Ca and Mn:Ca and salinity (Martin and Wuenschel, 2006; Elsdon and Gillanders, 2002) while some found a negative relationship (Fowler et al., 1995). Overall, the influence of salinity on otolith elemental concentration varies from positive (Stanley et al., 2015; Panfili et al., 2012; Dorval et al., 2007) to negative (Reis-Santos et al., 2013; Elsdon and Gillanders, 2002; Campana, 1999) and no relationship (Gillanders et al., 2012; Miller, 2009; Martin and Wuenschel, 2006) for different fish species. Differing results for different studies implies that the otolith elemental concentration relationship with salinity is likely species specific and might also be physiologically regulated (Walther et al., 2010; Martin and Thorrold, 2005; Boeuf and Payan, 2001).

Swim respirometry

It has been suggested that the physiological responses (e.g. metabolism and swimming) to environmental variability is energetically costly (Durant et al., 2007). Swimming performance can be used to define the species response to environmental variation. Swimming performance and metabolic rate can be assessed using equipment such as a swim chamber in which water velocity can be controlled.

Critical swimming performance (U_{crit}) in response to temperature and salinity

Swimming determines fish survival because it is related to activities such as finding food, escaping from predators and spawning (Peng et al., 2014). Critical swimming performance (U_{crit}) is used to evaluate the swimming ability of fish in defined water velocities until exhaustion. Exhaustion is when the fish can no longer maintain its position in a swim chamber and stops swimming (Farrell, 2008). At each water velocity or step, the fish is normally swum for between 15 and 60 min and U_{crit} is interpolated from the final steps of swimming performance (Clark et al., 2013). Temperature and salinity can affect U_{crit} (Yetsko and Sancho, 2015; Pang et al., 2013; Deslauriers and Kieffer, 2012). Several previous studies have found a bell-shaped relationship between U_{crit} and temperature (Gollock et al., 2006; Claireaux et al., 2005), while MacNutt et al. (2006) found a weak effect of temperature on U_{crit}. Further, changes in plasma ion concentration and osmoregulation in response to salinity assist the fish in maintaining osmotic homeostasis which can affect U_{crit} (Whitehead et al., 2013; Claiborne et al., 1994).

Metabolic response to temperature

Changes in environmental parameters can influence metabolism (Pörtner, 2001). Metabolism has been used to elucidate the link between energy budgets and thermal tolerance (Clark et al., 2013). Maximum metabolic rate (MMR) is the amount of oxygen consumption at U_{crit} and resting or standard metabolic rate (SMR) is the amount of oxygen consumption in a relaxed state ($0BLs^{-1}$ swimming speed) (Roche et al., 2013; Nelson and Chabot, 2011).

The MMR corresponds to maximum aerobic metabolism (oxygen consumption) of the fish during exercise or shortly after fatigue (Clark et al., 2011). Generally following fatigue in a swim chamber, the maximum oxygen consumption is taken and calculated as MMR (Killen et al., 2007). Many species do not naturally swim for a long period of time and then encouraging them to swim in a chamber is almost impossible. Hence, the swim respirometry test is impractical for species that are unwilling to swim and hence an exhaustive chase method can be used as an alternative method for the MMR measurement. This highlights the fact that MMR measurement methods should be tailored to the species of interest (Clark et al., 2013).

Aerobic scope (MMR–SMR) represents the overall capacity of the species to supply oxygen to the tissues (Killen et al., 2011). It has been suggested that aerobic scope of the animal decreases at extreme temperatures (both low and high) but is maximised within the optimal thermal range for each individual (Clark et al., 2013). Further, it has been suggested that in order to examine the physiological responses to environmental stressors, it is useful to go beyond what the species experiences in nature (Clark et al., 2013).

Overview of the study species

In the present thesis King George whiting (*Sillaginodes punctatus*; Sillaginidae) was selected to assess the effects of environmental variation on physiology and growth rate of a temperate fish. King George whiting (*Sillaginodes punctatus*) is the largest species in the family Sillaginidae and occurs along the lower western and southern coasts of Australia (Hyndes et al., 1998). This species is an important commercial and recreational fish in Southern Australia (Fowler et al., 2011). A recent risk assessment categorised King George whiting as medium to high risk to the effects of climate change (Pecl et al., 2014). The juvenile King George whiting live in a shallow and highly variable environment. The highly variable coastal environment can affect the physiology of the juvenile fish. However, there is still a lack of information about the King George whiting physiological response to environmental variability. Further no studies have investigated the swimming performance and metabolic rate of the King George whiting in response to a changing environment. Hence, King George whiting was selected as a good model species for the current project to examine the environmental effects on temperate fish species.

Biology and ecology of King George whiting

King George whiting mature at an age of four (410mm total length in Western Australia and 300-350mm in Southern Australia) and they live for up to 22 years in South Australia and 14 years in Western Australia (Hyndes et al., 1998). They spawn in offshore areas during June to September and the post-larvae are transported to shallow protected embayments that are the nursery grounds (Kailola et al., 1993). Juvenile fish stay in the nursery areas of the Northern gulfs and bays of the West coast of Eyre Peninsula and Kangaroo Island for about one or two years before they move into deeper seagrass areas (Jones et al., 1990). The juveniles grow and

develop and as adults (3-4 years of age) migrate back to deep water (Fowler et al., 2003). Juveniles in shallow nursery areas are vulnerable to temperature/salinity change, thus they can be a good case study to estimate how environmental factors affect their growth, physiology and movement patterns.

King George whiting larvae settle from 80 to 120 days (Fowler and Jones, 2008). Settlement of the larvae is correlated with the zonal westerly winds which influence the rate of the larvae transport and consequently the recruitment success and the productivity of the fishery of this species (Jenkins, 2005). The South Australian population of King George whiting generally spawn in their local habitat and do not migrate to other regions for spawning (Fowler and March, 2000).

The fish from Gulf St Vincent and northern Spencer Gulf move in a southerly direction to the northern sides of Kangaroo Island and Hardwicke Bay in the southeast (Fowler et al., 2014). In contrast, the fish in Kangaroo Island and Southern Spencer Gulf do not move far in a systematic direction (Fowler and March, 2000). Differences between movement patterns of the fish influence their population structure. The population of the fish in Gulf St Vincent and Spencer Gulf is small and includes young fish whereas, in Kangaroo Island and Hardwicke Bay, the population is older (up to 18 years) (Fowler et al., 2014). The species is under pressure from long-term high exploitation as well as changing environmental conditions due to climate change (Fowler et al., 2014).

Fisheries status in South Australia

King George whiting fishery in South Australia is extensive and includes all coastal waters of Gulf St Vincent through Denial Bay. The South Australian catch makes the highest contribution to the national catch of King George whiting (Fowler et al., 2014). South Australia's catch is twice the harvested biomass of this species in Victoria and Western Australia (Fowler et al., 2014). The highest exploitation rate of King George whiting occurs during late summer and autumn with the monthly peak catch in July (Fowler et al., 2014). The commercial catch in Gulf St Vincent and Spencer Gulf are based on the minimum legal size of the fish (270mm) (Fowler and March, 2000). The total annual commercial catch for King George whiting has varied over the period between 1984 and 2013 (Fowler et al., 2014). However, there has been a significant declining trend from 1992 to 2013. The highest annual commercial estimate of catch in 1992 was 776t and then it declined to 428t in 2000. Since 2000 a further gradual decline to about 253t in 2013 was observed (Fowler et al., 2014).

Thesis outline

Principle objective

The purpose of this thesis was to develop a current understanding about the physiology and biology of a temperate fish in response to a changing environment. I used several different methods to examine the biological (otolith growth and chemistry) and physiological (swimming and metabolic rate) responses of King George whiting to environmental variability. Additionally, I defined the thermal tolerance window for aerobic scope for the fish. I also measured the critical swimming performance (U_{crit}) of the fish in response to temperature and salinity. Attempts were

made to develop an understanding about the effects of environmental changes on a temperate fish species and to help predict their responses to a changing environment.

Specific objectives

Specific objectives were to:

i. Reconstruct the relationship between fish growth and a number of environmental and biological variables including, sea surface temperature (SST), El-Niño Southern Oscillation events (Southern oscillation index; SOI), and recruitment.

ii. Assess the swimming performance and scope for activity of adult King George whiting, as well as their potential to recover after a prolonged swimming period under two extreme temperature treatments (16°C and 26°C).

iii. Assess the metabolic rate and U_{crit} of juvenile King George whiting across a range of temperatures (16, 19, 22 and 25ºC) and two levels of salinity (30 and 40ppt).

iv. Determine how temperature and salinity influence the otolith chemistry of King George whiting as a precursor for determining the environmental history of fish.

Notes on structure and style

Chapter 2-5 of this thesis present original data written in a style suitable for publication in scientific journals. Whilst it has been attempted to maintain a logical flow of ideas throughout the thesis, each chapter can be read independently. Tables and figures are embedded within relevant chapters and cited references are listed at the end of each chapter of the thesis. The

thesis contains a list of co-authors, their associated affiliation along with their contribution to the thesis and their permission to include the chapters in this thesis. Each chapter also has acknowledgements at the end. Chapters 2-5 are as follows:

Chapter 2: Determining climate-growth relationships in a temperate fish: a sclerochronological approach.

Growth is a key factor driven by environmental variation. Differences in the width of otolith growth increments of the fish species can reflect the effect of environmental variation on growth. Hierarchical models were developed to assess the inter-annual growth variation of King George whiting (*Sillaginodes punctatus*; Sillaginidae) in response to environmental variables such as, sea surface temperature (SST), El-Niño Southern Oscillation events (SOI) and recruitment. Models were applied to a data set of otolith increment measurements from King George whiting.

Chapter 3: The influence of temperature on the metabolic rate and swimming speed of a temperate fish.

Understanding changes in performance and metabolism in response to extreme temperature (both low and high) is important for understanding how a changing environment may influence fish physiology. The physiological response to temperature is highly species specific. I hypothesised that metabolic rate and swimming performance of adult King George whiting (160-323 ± 0.1g, 290-340 ± 0.1mm TL, 2-3 years old) would be higher in warm temperatures. To address this hypothesis, the maximum and minimum metabolic rate as well as critical swimming

performance of adult King George whiting were examined under two temperatures (16°C and 26°C). The higher temperature is close to the adult tolerance limit and the lower temperature reflects winter ambient temperatures.

Chapter 4: Metabolic rate and swimming behaviour of a juvenile temperate fish in relation to temperature and salinity.

Ectotherms can tolerate a range of temperatures and salinities in their environment. Aquatic species have an optimal range of temperature and salinity in which they can be aerobically active and survive. Moving from optimal to extreme ranges can result in anaerobic metabolism and loss of performance. To assess the tolerance limit of juvenile King George whiting (40-60 mm TL, 0.4-0.5g) to temperature and salinity, I measured metabolic rate and critical swimming performance (U_{crit}) of juvenile fish at a range of temperatures (16, 19, 22 and 25ºC) and two levels of salinity (30 and 40ppt). The main purpose was to find their optimal temperature for aerobic performance.

Chapter 5: The effects of temperature and salinity on otolith chemistry of King George whiting.

Otoliths are used as a tool to study movement and life history of fish. The otolith chemical composition can be related to different environmental conditions that fish have lived in. In this chapter, I designed a controlled laboratory experiment to examine the individual and interactive effects of temperature and salinity on the otolith chemistry of juvenile King George whiting. I chose to examine the effect of four different temperatures (16, 19, 22 and 25ºC) and two levels of

salinity (30 and 40ppt) on otolith concentration ratios to calcium for magnesium (^{24}Mg), manganese (^{55}Mn), strontium (^{88}Sr) and barium (^{138}Ba).

Chapter 6: General discussion

Chapter 6 contains a general discussion of the former chapters. In this chapter, a summary of the findings and the potential for future research is discussed.

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Chapter 2: Determining climate-growth relationships in a temperate fish: a sclerochronological approach

Me, working with Image-pro plus software. Southern Seas Ecology Laboratories, The University of Adelaide. (Photo credit: Amir Forghani)

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Determining climate-growth relationships in a temperate fish: a sclerochronological approach

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Abstract

Otoliths of fish can provide long-term chronologies of growth. Differences in the width of the annual growth increments can reflect the effects of environmental variability on somatic growth rate. We used generalized linear mixed models (GLMM) to evaluate the influence of region, sea surface temperature (SST), El Niño–Southern Oscillation events, and recruitment on the otolith growth of King George whiting (*Sillaginodes punctatus*), a commercially and recreationally important fish species in Southern Australia. Growth increment data spanned 25 years (1985 to 2010). The optimal model demonstrated that mean winter SST was negatively correlated to growth, and as the winter SST increased the average width of the growth increments declined. There were no regional growth differences and recruitment was not correlated with growth. Understanding long-term temperature-growth relationships is crucial for disentangling the effects of climate change and other parameters on fish growth, and thus predicting how populations will change in the future.

Key words: Climate change, fish growth increment, otolith chronology, King George whiting (*Sillaginodes punctatus*).

Introduction

Climate change is a major threat to global biodiversity and ecosystem functioning (Solomon et al., 2007), and its effects are already evident across a range of marine environments and biota (Hobday and Lough, 2011; Parmesan and Yohe, 2003). Understanding how fish populations respond to a changing environment is essential for predicting the future effects of climate on fish growth and survival (Morrongiello et al., 2012). Despite this, there is still a paucity of long-term ecological data available for marine fish species (Thresher et al., 2007), especially in the Southern hemisphere. Regions such as Australia are experiencing relatively rapid rates of global warming (Hobday and Lough, 2011). All fish species have a temperature range at which growth and survival are optimal (Pörtner and Farrell, 2008), hence a detailed understanding of the effects of temperature on individual fish species is important for predicting how they may change in the future (Gillanders et al., 2012; Neuheimer et al., 2011; Thresher et al., 2007).

A growing number of studies have used otoliths to elucidate long-term patterns in fish growth in response to biological (e.g. age, gender) and external (e.g. environmental variables) factors (Morrongiello and Thresher, 2014; Black et al., 2008; Hagen and Quinn, 1991). Otoliths have annual growth increments that can be used to reconstruct growth histories of individuals and populations (Black et al., 2008). Time-dependent changes in otolith growth increments have been investigated for a number of species using generalized linear mixed models (GLMM) including, golden perch (*Macquaria ambigua*) (Morrongiello et al., 2011), tiger flathead (*Platycephalus richardsoni*) (Morrongiello and Thresher, 2014) and smallmouth bass (*Micropterus dolomieu*) (Weisberg et al., 2010). Mixed modelling methods provide an ecologically robust understanding of how fish may respond to climate variability compared to more traditional dendrochronological methods (Matta et al., 2010; Thompson and Hannah, 2010;

Black et al., 2008), which are designed to maximise climate-growth relationships and reduce 'ecological noise' within the data (Morrongiello et al., 2012).

King George whiting (*Sillaginodes punctatus*; Sillaginidae), is an important commercial and recreational fish species found in temperate Southern Australia (Hyndes et al., 1998; Kailola et al., 1993). King George whiting spawn in offshore areas and the post-larvae are transported to shallow protected embayments that are the nursery areas. The juveniles grow and develop and as adults migrate back to deeper water (Fowler et al., 2002). There is little known about the effects of climate change and recruitment on King George whiting growth. We reconstructed historical climate growth relationships for this species using otolith growth chronologies (Morrongiello and Thresher, 2014; Weisberg et al., 2010). The objectives were addressed using a mixed modelling approach to examine: 1) inter-annual variation in growth and; 2) the influence of a number of environmental variables including, sea surface temperature (SST), El Niño–Southern Oscillation events, and recruitment on growth variation.

Materials and Methods

Sample collection

Transverse otolith sections from King George whiting were sourced from archived collections held at the South Australian Research and Development Institute (SARDI), Aquatic Sciences. The samples had been collected from Spencer Gulf (SG) (-34.30 ºN, 136.98 ºE), Gulf St Vincent (GSV) (-34.92ºN, 138.59 ºE) and the northern coastline of Kangaroo Island (KI) (-35.65 ºN, 137.63 ºE) in South Australia between 1995 and 2010 (Table 1). Fish were obtained from

recreational fishers, commercial catch, and scientific surveys. They ranged in length from 324 to 563 mm (Table 1).

Table 1. Details of otoliths from King George whiting collected from three regions in South Australia.

Location	*Number of otoliths*	*Age range (years)*	*Size range - Total length(mm)*	*Biochronology length (year)*	*Sampling year range*
Kangaroo Island	107	6-16	367-563	18	1995-2010
Spencer Gulf	145	6-17	324-553	10	2003-2010
Gulf St Vincent	54	6-17	400-544	9	2004-2010

Otolith preparation and growth estimation

The transverse sections had been previously prepared for age analysis. Each otolith had been embedded in clear, polyester resin and allowed to cure overnight before being sectioned through the core. The transverse section was mounted on a microscope slide using resin, and viewed using a dissecting microscope (Leica® DMLB) with transmitted light and 25x magnification. The section was lightly coated with immersion oil to accentuate the otolith macrostructure and aged by counting annual increments (opaque zones) from the core to the edge. The growth increments have been previously validated as forming on an annual basis. To ensure a sufficiently long growth chronology, the study was restricted to fish aged six years and older. Growth increment widths were measured using 'Image-Pro® Plus software' (version 7.0), along a transect towards the proximal edge from the core to the last complete increment (Fig. 1)

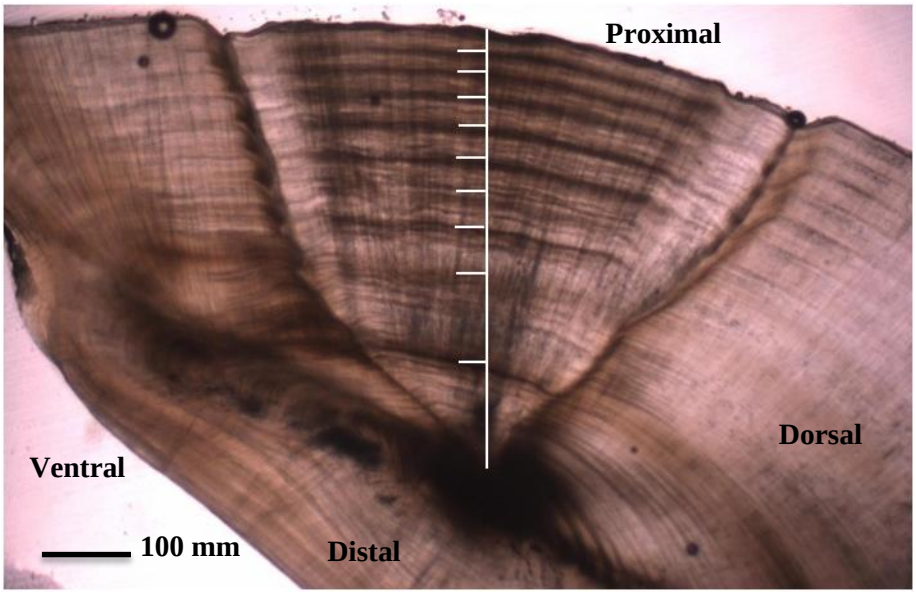

Fig. 1. Transverse section of a sagittal otolith of a 9+ year fish. The opaque zones of annual increments are indicated by the white dashes along the transect towards the proximal edge.

In South Australia, King George whiting spawn in autumn, with a birth date in May and a larval phase of approximately 5 months (June-November) (Fowler and Short, 1998; Fowler and Short, 1996). They lay down their first opaque zone in October of the following year (Fowler and Short, 1996), representing the first 16 months of life rather than 12 months. Therefore, the first growth increment from the core was not included in the analysis. Based on the timing of growth increment formation, the biological growth year of King George whiting was defined from the 1st October of each year to 30th September of the following year (i.e. 2007/08 was therefore referred to as 2007). To ensure the correct calendar year was assigned to each growth increment, marginal increments were classified visually, as narrow, wide, or intermediate and subsequent rules applied depending on the month of capture.

Assuming that the biological growth year for King George whiting runs from 1st October to 30th September, the 1st October was assumed to be the formation date of each increment. For most fish, one year was subtracted from year-of-capture, as the marginal increment was not included in the analysis. Additionally, based on the width of the marginal increment and month-of-capture, further rules were applied to ensure the correct year was assigned to the last full year of growth (Table 2).

Table 2. Rules applied for interpreting marginal growth increments in otoliths of King George whiting to determine the year of capture. MI = marginal increment

Marginal increment category and month of capture	**Rule**
Fish caught 3 months before 1st October with a narrow MI	Year-of-capture
Fish caught 3 months after the 1st October with a wide MI	Year-of-capture minus 2 years
All fish with a medium MI	Year-of-capture minus 1 year
All fish caught between January and June regardless of increment width	Year-of-capture minus 1 year

Growth predictors

Recruitment data were derived from an age-structured stock assessment model of King George whiting from 1985-2007 (McGarvey et al., 2007) (Fig. 2), but were only available for two of the three regions, SG and GSV. Estimates of sea surface temperature (SST) for each region were obtained from the monthly interpolated Hadley sea surface temperature (HadISST), which was derived from the National Aeronautics and Space Administration "Climexp" website (http://climexp.knmi.nl) over the period 1985-2010 (Fig. 3). The SST data represents the changes experienced by the fish in its natural habitat. SST data were averaged across the annual growth year and also across four biologically-relevant 'seasons': spring (October-December), summer (January-March), autumn (April-June) and winter (July-September). SST data were also averaged across all regions when relevant to the analysis Monthly Southern oscillation index (SOI) data were used as a measure of El-Niño South Oscillation events, and were obtained from the Bureau of Meteorology website (BOM) (http://www.bom.gov.au) for the period from 1985-2010 (Fig. 4).

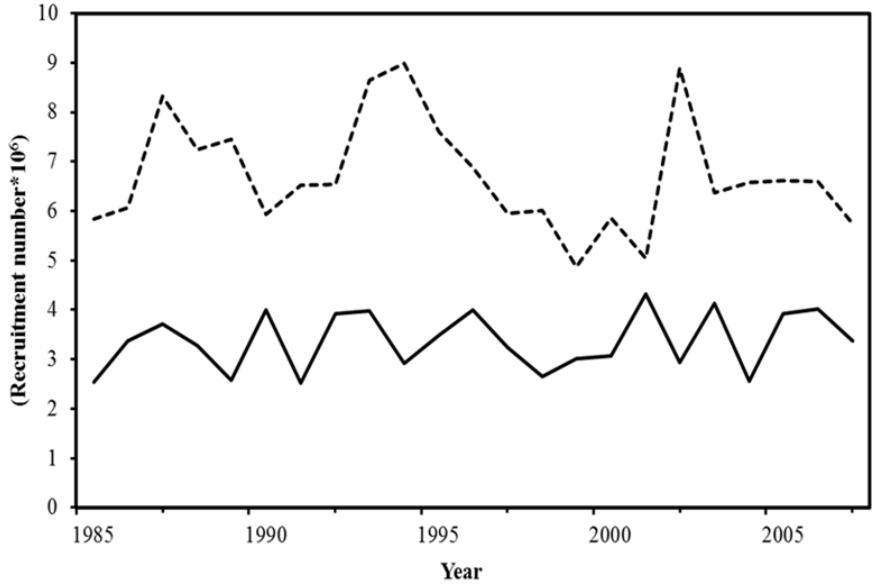

Fig. 2. Recruitment data for fish from the Spencer Gulf (SG; dashed line) and Gulf St Vincent (GSV; solid line) between the years of 1985 and 2007 determined from an age-structured stock assessment model.

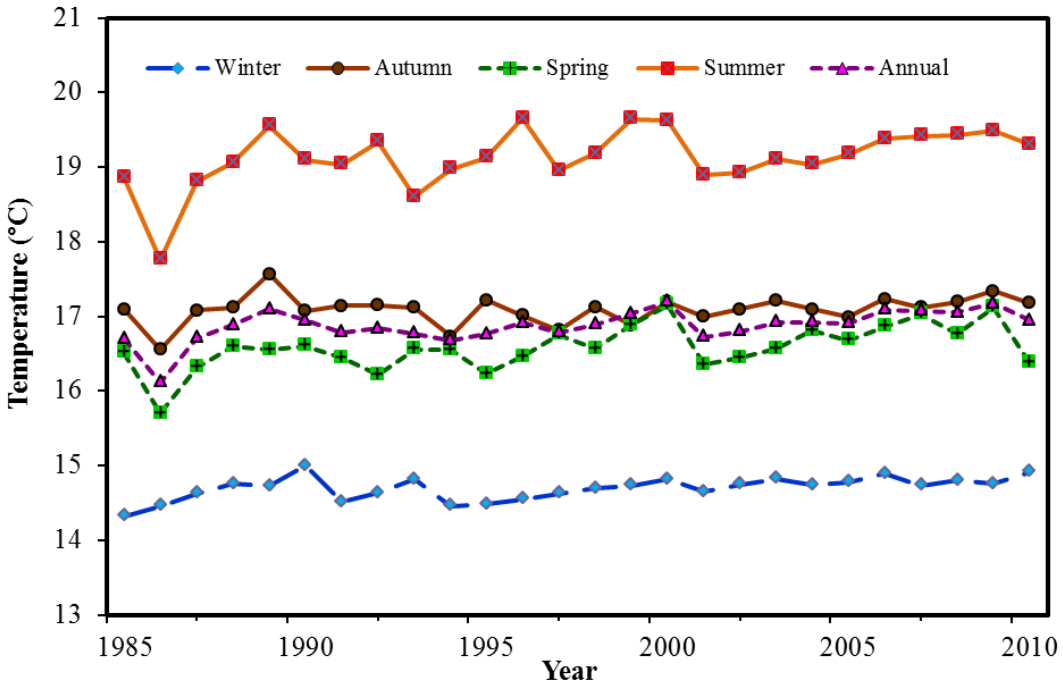

Fig. 3. Mean SST of the regions (GSV, SG and KI) for annual, winter, summer, spring and autumn growth seasons, for the period from 1985 to 2010.

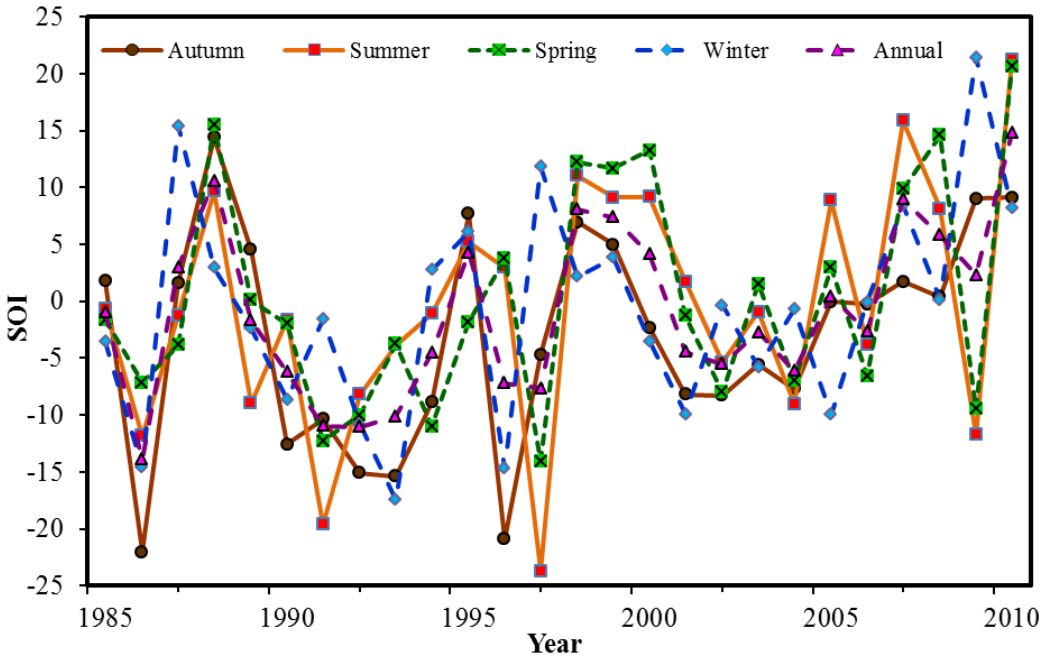

Fig. 4. Mean SOI of the regions (GSV, SG and KI) for annual, winter, summer, spring and autumn growth seasons, for the period from 1985 to 2010.

Modelling approach

Inter-annual growth variation was analysed by GLMM for two separate datasets (reduced and full; see below). The study design required repeated measurements of each fish. To account for the pseudoreplication arising from the repeated measurements for the same individuals, each unique fish identifier was treated as a random effect in the model (Pinheiro and Bates, 2000). The model was used to investigate the relationship between annual otolith growth increments and the environmental covariates of SST and SOI, recruitment and region (location of capture). Annual growth increment data were natural log transformed to satisfy the model assumption for normal distribution of the variances.

Models were fitted using a stepwise forward procedure with the optimal model at each step selected based on lowest Akaike information criterion (AIC) (Burnham and Anderson, 2002). In addition to the unique fish identifier, growth year was also investigated as a random term in the model to provide an estimate of above or below average growth for a given calendar year (Table 3). To test for regional differences in growth, the first two measurements of annual increments were initially excluded from the analysis as juvenile fish (< 3 years of age) migrate between regions (Fowler et al., 2002); this was the reduced model (Table 4). Recruitment data were also fitted to a reduced model for the two regions where recruitment data were available (SG and GSV). If the regional and recruitment variables did not improve the model, a full model that included all growth increment measurements was used to predict the growth variation (Table 4). The R package (R core Team 2014) lme4 was used to fit the mixed-effects models (Pinheiro and Bates, 2000).

Table 3. Description of variables used for modelling otolith growth of King George whiting (KGW).

Variable name	*Description*
Random effects	
Fish ID	Fish identification code
Year	Biological growth year (October 1st to the following September 30th) in which each growth increment was formed
Fixed effects	
Age	Age of each growth increment
SOI	Southern Oscillation Index (SOI), annual and seasonal averages derived from monthly records. A measure of El Niño–Southern Oscillation events
SST	Sea Surface Temperature (ºC; SST), annual and seasonal averages derived from monthly records
Region	Spencer Gulf, Gulf St Vincent and Kangaroo Island
Recruitment	Recruitment data from age-structured stock assessment model of KGW from 1985-2007 (McGarvey et al., 2007) from Spencer Gulf and Gulf St Vincent

Table 4. Description of the models used in the mixed modelling analysis. | = denotes random *Age* slopes for each random *FishID* and Year intercept. The first two increment measurements were initially excluded in reduced models, while the full models included the first two increment measurements.

Model	Random effects	Fixed effects
Reduced model		
R1	1|FishID	Age
R2	1|FishID, 1|Year	Age
R3	1|FishID, 1|Year	Age, Region
R4	1|FishID, 1|Year	Age, Recruitment
Full model		
F1	1|FishID	Age
F2	1|FishID, 1|Year	Age
F2.1	1|FishID, 1|Year	Age, Spring SST
F2.2	1|FishID, 1|Year	Age, Summer SST
F2.3	1|FishID, 1|Year	Age, Autumn SST
F2.4	1|FishID, 1|Year	Age, Winter SST
F2.5	1|FishID, 1|Year	Age, Annual SST
F3	1|FishID, 1|Year	Age, Spring SOI
F3.1	1|FishID, 1|Year	Age, Summer SOI
F3.2	1|FishID, 1|Year	Age, Autumn SOI
F3.3	1|FishID, 1|Year	Age, Winter SOI
F3.4	1|FishID, 1|Year	Age, Annual SOI
	1|FishID, 1|Year	
F4	1|FishID, 1|Year	Age, Winter SST, Spring SOI
F4.1	1|FishID, 1|Year	Age, Winter SST, Summer SOI
F4.2	1|FishID, 1|Year	Age, Winter SST, Autumn SOI
F4.3	1|FishID, 1|Year	Age, Winter SST, Winter SOI
F4.4	1|FishID, 1|Year	Age, Winter SST, Annual SOI

Results

King George whiting varied in age between 6 and 17 years. The oldest samples were from GSV and KI, whilst fish from SG were marginally younger (Fig. 5). There was a strong relationship between fish length and otolith radius (r^2: 0.84, Fig. 6) suggesting that increment width measurements provided a robust index of somatic growth.

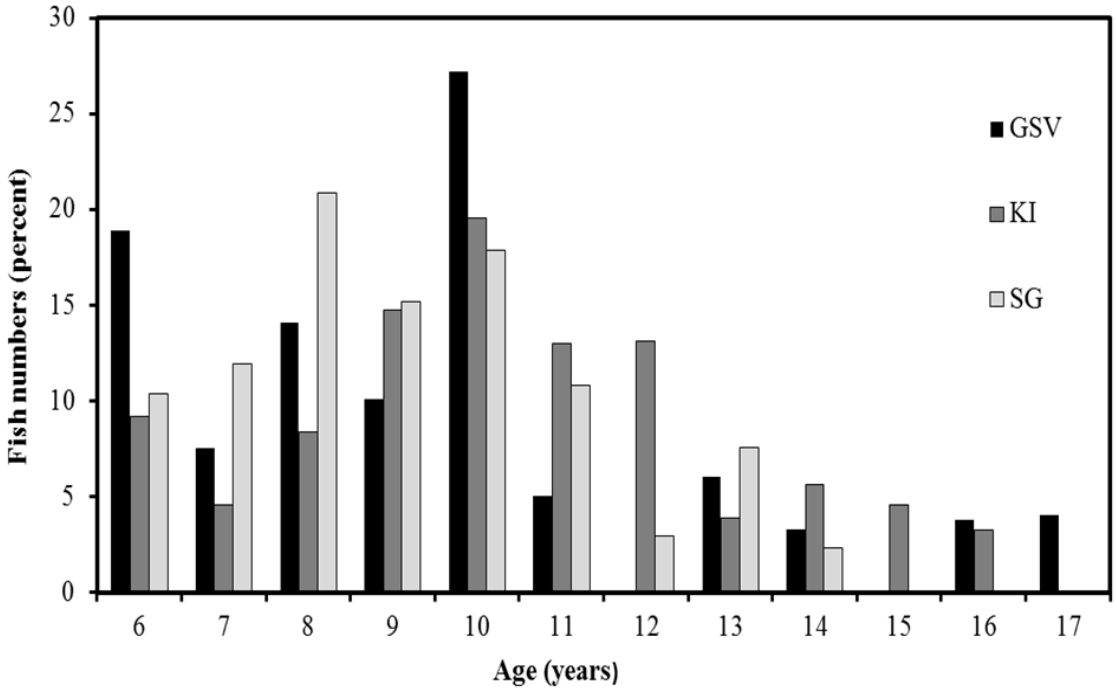

Fig. 5. The number of fish (%) sampled by age class from Gulf St Vincent (GSV), Kangaroo Island (KI) and Spencer Gulf (SG).

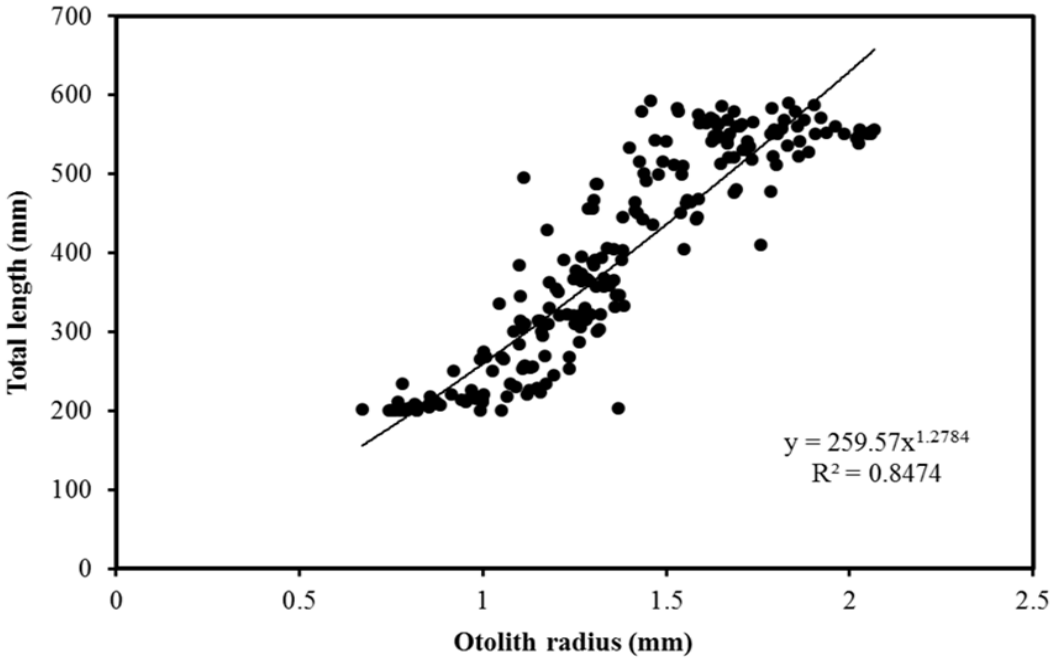

Fig. 6. Relationship between fish size (total length) and otolith radius (mm) for King George whiting.

Age as a fixed effect and year and FishID as random intercepts were the most effective combination of factors that explained growth variation in King George whiting, based on the most strongly supported model (R2, AICc value, -350.99) (Table 5). The width of growth increments declined as fish got older. The addition of region and recruitment did not improve the fit of the model. Therefore, the analysis was re-run using all the growth increment measurements including the first two years of growth (full model).

Table 5. Results of the reduced data model fitted to King George whiting growth increment data. The optimal model is highlighted in **bold**. Res.LL = log restricted likelihood estimation.

Model name	K	AICc	ΔAICc	AICcWt	Res.LL
R2	**17**	**-350.99**	**0**	**0.94**	**192.8**
R3	18	-345.11	5.88	0.05	190.89
R4	18	-340.79	10.2	0.01	188.73
R1	16	157.36	508.35	0	-62.41

For the full model, the optimal model included FishID and Year as random effects and age and winter SST as fixed effects (F1.4, AICc value, -432.11, Table 6). The addition of SOI, or both SST and SOI, did not improve model fit for any of the time periods investigated (Table 6). The chronology showed year-to-year variation, although there was substantial individual variability in growth (Table 7). The optimal model was similar to the dataset that excluded the first two years of growth and the only environmental variable that improved model fit was winter SST, albeit it was weak and negative. The negative relationship between winter temperature and growth suggests that as winter SST increased growth rate declined (Fig. 7; Table 7). Annual growth estimates, based on random year effects of the optimal model (F1.4), indicated that average fish growth for all three regions increased steadily from 1991 to 2000 (good growth years) and then declined from 2000 until 2010 (poor growth years) (Fig. 8).

Table 6. Results of the full data model fitted to King George whiting growth increment data. All models included the maximum fixed intrinsic effect *Age*. The optimal model is highlighted in **bold**. Res.LL = log restricted likelihood estimation.

Model name	*AICc*	*ΔAICc*	*AICcWt*	*Res.LL*
F1.4	**-432.11**	**0**	**0.89**	**236.23**
F1.2	-426.99	5.12	0.07	233.67
F2.5	-422.46	9.65	0.01	232.42
F2.4	-422.38	9.74	0.01	232.38
F2.3	-422.30	9.81	0.01	232.34
F2.1	-421.44	10.68	0	231.91
F2.2	-421.26	10.86	0	231.82
F1.1	-420.95	11.17	0	230.65
F1.5	-420.43	11.68	0	230.39
F1.10	-419.15	12.97	0	229.75
F1.8	-418.91	13.20	0	229.63
F1.9	-418.70	13.41	0	229.53
F1.6	-418.03	14.09	0	229.19
F1.3	-417.78	14.33	0	229.07
F1.7	-417.77	14.34	0	229.06

Table 7. Parameter estimates (± standard error (SE)) and variance components (± standard deviation (SD)) associated with fixed effects and random effects for the optimal full data model (F1.4).

Random effects	*Variance*	*SD*	
FishID	0.22813	0.4776	
Year	0.05499	0.2345	
Residual	0.02631	0.1622	
Fixed effects	*Estimate*	*SE*	*t value*
(Intercept)	3.60923	1.03517	3.487
Winter_SST	-0.2117	0.07021	-3.015

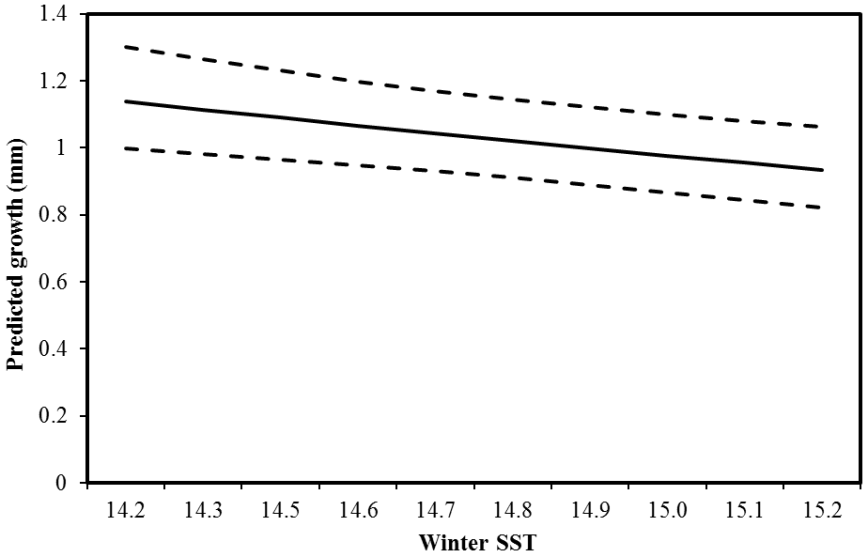

Fig. 7. Predicted effect (± 95% CI) of winter SST on inter-annual growth variation of King George whiting (*Sillaginodes punctatus*) based on model F1.4 (best fitted full data model). Dashed lines represent 95% CI.

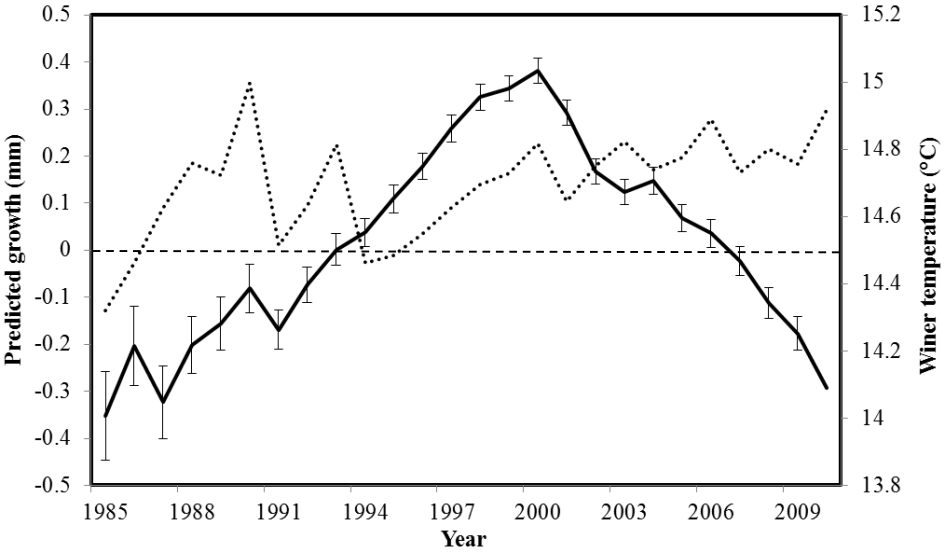

Fig. 8. Coefficient (year) estimates with (±SE) for King George whiting growth increments sampled from all three regions (Spencer Gulf, Gulf St. Vincent and Kangaroo Island). Estimate of random year effect is from model F1.4. Winter sea surface temperature (SST) is also shown to illustrate correlation with inter-annual growth variation. Winter SST was selected according to the best fitted model (F1.4) (solid line: predicted growth over time (mm), dotted line: winter SST, horizontal dashed line: average growth across the time period examined).

Discussion

We investigated inter-annual growth variation for King George whiting and whether growth was related to environmental variation. Results show that increasing winter SST was associated with a decline in growth. Fish growth did not vary among regions and was not influenced by recruitment or El Niño–Southern Oscillation events. Further, no relationship was found between growth and seasonal temperature variations, with the exception of winter.

Many otolith chronology studies show positive growth-temperature relationships, which differs from our results. Postitive growth-temperature relationships have been observed for marine [e.g. tiger flathead, *Platycephalus richardsoni* (Morrongiello and Thresher, 2014), parore, *Girella tricuspidata* (Gillanders et al., 2012)], estuarine [e.g. rock flathead, *Platycephalus laevigatus* (Coulson et al., 2014)], and freshwater [e.g. golden perch, *Macquaria ambigua* (Morrongiello et al., 2011)] fish species in Australian and New Zealand. A positive linear growth-SST relationship was found for the rock flathead between September and March (austral spring and summer) and similarly summer temperature was associated with increased growth of parore (Gillanders et al., 2012). A negative growth-SST relation in winter (April to August) (Coulson et al., 2014) was found for the rock flathead. Further a negative growth-SST relation was recently found for black bream (*Acanthopagrus butcheri*) in Tasmania (Southern end of species distribution) (Doubleday et al., 2015), which is similar to what we found for the King George whiting. Although, the effect of temperature on growth rate of tiger flathead (*Platycephalus richardsoni*) was relatively small, substantial spatial growth variation in response to temperature was found (Morrongiello and Thresher, 2014). Results from previous studies indicate that responses to climate change are species specific. However, temperature increase can benefit growth up to a critical threshold and temperatures above a threshold then have an adverse impact

on growth. Therefore, if samples are collected from regions near the thermal limits of the species range, then a negative response to temperature may be expected (Gillanders et al., 2012).

A plausible reason for the inverse impact of winter SST on growth rate of King George whiting may be because winter temperature was near the lower thermal limit of the fish (Meakin et al., 2014). However, King George whiting is tolerant of temperature change as indicated by responses of heat shock proteins in juvenile fish (Meakin et al., 2014). Another reason was likely due to the direct influence of temperature on expression and fitness of phenotypic traits of this species (Baumann and Conover, 2011). Besides direct influences of temperature on growth [e.g. change in metabolism and expression and fitness of many phenotypic traits (Baumann and Conover, 2011), there are several indirect factors that can affect growth. Indirect factors include changes to food availability or critical habitats, and predator-prey relationships (Pörtner and Peck, 2010). Climate change caused perturbations to food webs which ultimately changed the growth rate of yelloweye rockfish (*Sebastes ruberrimus*) in the northern hemisphere (Black et al., 2008). Warmer water can reduce food availability and or increase food consumption and consequently cause competition for food and a decline in growth (Morrongiello and Thresher, 2014).

Variation in estimated recruitment of King George whiting was not correlated with growth suggesting that density dependent effects (e.g. competition for food) are not associated with growth variation for this species (Grant et al., 2009). The King George whiting fishery is dependent on recruitment variability based on larval supply (Jenkins, 2005). Larval supply and post-larvae abundance is highly related to the larval growth rate and settlement as well as water temperature (Jenkins and King, 2006). Variability in larval growth and mortality exert variation in recruitment (Houde, 1989). Based on this concern, it is assumed that larval survival depends

on temperature. Larvae are more likely to starve in warmer waters than in colder waters. Since, juvenile King George whiting is more tolerant to temperature change in comparison to adults (Meakin et al., 2014), then changes in temperature would be less likely to affect larval survival. Therefore, a greater number of larvae recruit to juveniles and then adults. The King George whiting fishery status in South Australia is not favourable due to heavy exploitation as well as a changing environment due to climate change (Fowler et al., 2014). In addition, nursery grounds for settlement of their post-larvae are limited and vulnerable to climate change. Warming has threatened the survival of the larval fish in seagrass areas (Smith et al., 2012). These factors may affect the recruitment and growth rate of this species in the near future (Smith et al., 2012).

El Niño–Southern Oscillation (ENSO) events were not associated with the growth rate of King George whiting. Few studies have investigated the effects of ENSO events on marine fish. A study on yellowfin tuna (*Thunnus albacares*) larvae in the Panama Bight indicated no relationship between ENSO and larval growth rate (Wexler et al., 2007). Similarly ENSO events were not highly correlated to each other in some fresh water species [e.g. golden perch (*Macquaria ambigua*) (Morrongiello et al., 2011)] as well as estuarine-dependent species [e.g. black bream (*Acanthopagrus butcheri*) (Doubleday et al., 2015)]. Although El-Niño causes warmer SST in late summer and winter and La-Niña does not interfere significantly with SST anomalies, it has been predicted that temperature will increase during ENSO events by up to ~1°C and >2°C by 2030 and 2100 respectively. Our results indicated that warmer winter SST temperature (which is associated with ENSO), could reduce the growth rate of King George whiting. Further, an increase in ENSO events will result in intense tropical cyclones, more extreme rainfall, longer drought periods and higher sea level which may affect the population

dynamics, fisheries catches, and total fecundity and reduce the habitats of marine species (Holbrook et al., 2009).

Salinity can also indirectly affect the growth rate of a fish. King George whiting were collected from inverse estuaries (Kämpf et al., 2009) where salinity increases away from the estuary mouth during summer months. Gulf St Vincent and Spencer Gulf in South Australia are large inverse estuaries, which are largely isolated from external waters during summer through the boundary layer that forms at their mouths, but this breaks down during winter when there is significant outflow of dense, highly saline water into the outside oceanic waters (Lennon et al., 1987). Reduction in water exchange between the gulf and open ocean during winter results in salt accumulations at the bottom of the gulf and thereby increase in salt budget over winter. Salinity increase in the gulfs may affect the growth increments of the fish. Unfortunately, salinity data were not available to test how salinity affects growth of King George whiting.

Conclusion

Our study provides information about the possible effects of climate change on fish growth over a multi-decadal time period. The application of mixed-effect models provides a useful means to evaluate climate-growth relationships retrospectively in fish using otolith archives. This is beneficial for tracking environmental effects and changes in population dynamics (e.g. recruitment) on growth rate of targeted species. Current climate has not yet significantly affected the growth of the King George whiting, but any further temperature increase or decrease at both ends of the species thermal window may affect the growth and survival of the fish.

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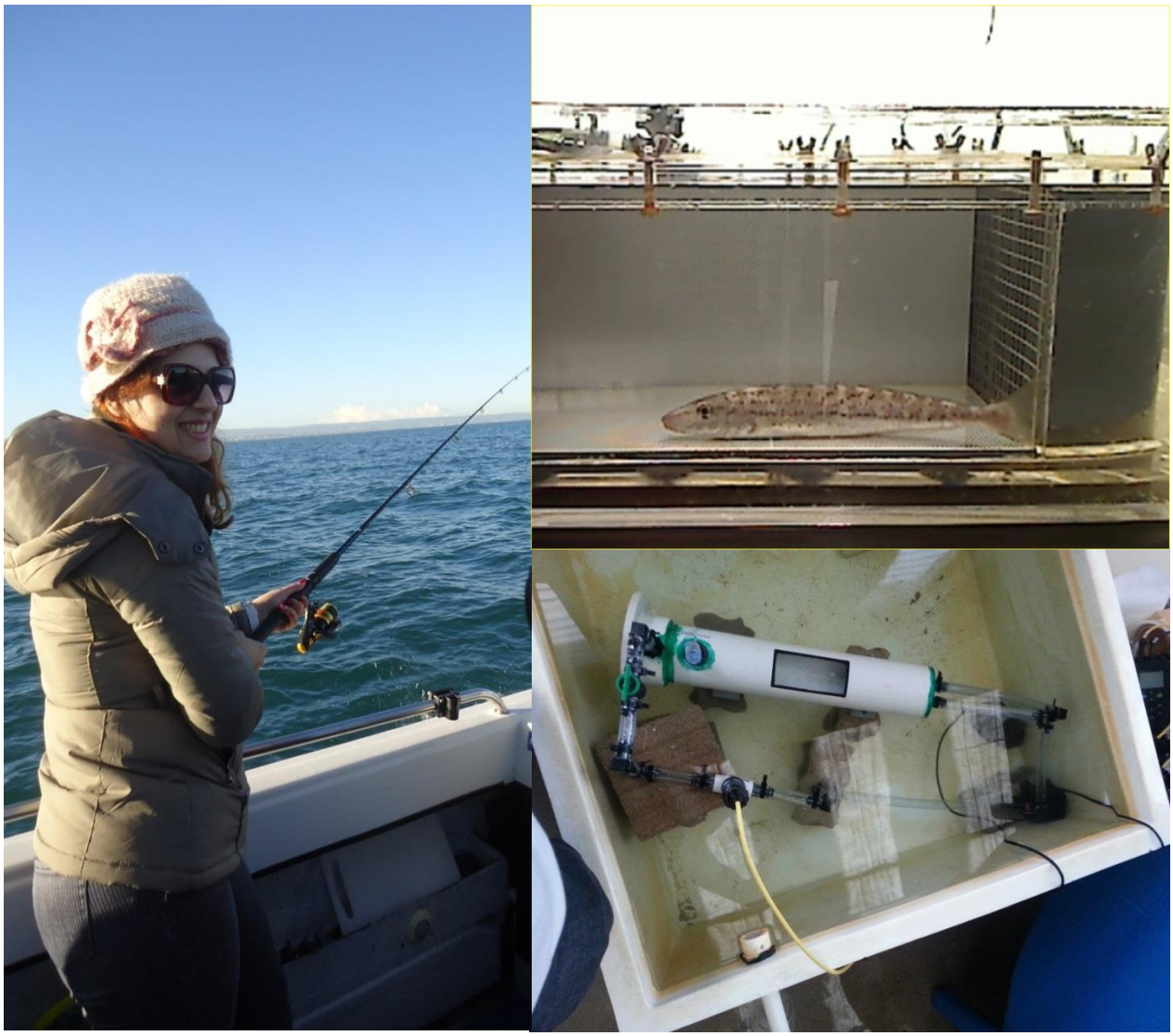
Chapter 3: The influence of temperature on the metabolic rate and swimming speed of a temperate fish



Left: Me, North Haven, Adelaide catching some adult KGW for the experiment (Photo credit: Kayla Gilmore)

Right top: King George whiting adult in a swim chamber (Photo credit: Nastaran Mazloumi)

Right bottom: A resting chamber (Photo credit: Amir Forghani).

Statement of Authorship

Title of Paper	**The influence of temperature on the metabolic rate and swimming speed of a temperate fish**
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Principal Author

Name of Principal Author (Candidate)	Nastaran Mazlouni
Contribution to the Paper	Performed analysis on all samples, interpreted data, wrote manuscript and will be acted as corresponding author
Signature	Date: Sept 2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that the candidate's stated contribution to the publication is accurate and that permission is granted for the publication to be included to the candidature thesis.

Name of Co-Author	Jacob Johansen
Contribution to the Paper	Assisted with intellectual development, provided suggestions, comments and feedback on manuscript drafts as well as experimental design
Signature	Date:

Name of Co-Author	Zoe Doubleday
Contribution to the Paper	Assisted with intellectual development and field work, as well as provided suggestions, comments and feedback on manuscript drafts
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Contribution to the Paper	Acted as principal supervisor and assisted with intellectual development, provided suggestions, comments and feedback on manuscript drafts
Signature	Date: Sept 2015

The influence of temperature on the metabolic rate and swimming speed of a temperate fish

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Abstract

There has been considerable interest in how temperature influences the metabolic rate and swimming speed of fish, as most biological activities of fish is dependent on their ability to move. These physiological parameters were measured in adult King George whiting (*Sillaginodes punctatus*), a temperate fish, under two temperature regimes (16 and 26 ºC). Fish were swum in a Brett-type swim chamber until they reached maximum swimming capacity, whereby the critical swimming speed (U_{crit}) was calculated. Subsequently, fish were immediately transferred into a resting chamber where maximum and standard metabolic rate (MMR and SMR) were measured, allowing the aerobic scope of activity to be calculated. At higher temperatures, fish consumed more oxygen, recovered quicker, and had a higher aerobic scope of activity compared to fish at the lower temperature. Increased swimming speed and improved metabolic performance of fish at the higher temperature may mean that under increasing temperatures due to climate change, fish may be able to survive more successfully.

Key words: King George whiting (*Sillaginodes punctatus*), critical swimming speed (U_{crit}), climate change, standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope.

Introduction

Global ocean warming is a current threat to marine systems (Perry et al., 2005) and is predicted to intensify in the future (Boyd et al., 2010). Aquatic ecosystems are vulnerable to climate change due to direct influences of temperature on species physiology (Lee et al., 2003), distribution patterns, abundance (Last et al., 2011) and population dynamics (Doney et al., 2012). As different species can respond differently to environmental change, a detailed understanding of the effects of thermal change on individual fish species is important for developing conservation and fisheries management strategies (Neuheimer et al., 2011).

Changes in temperature can affect the behavioural and physiological characteristics of fish, such as swimming performance and aerobic metabolic rate (Gillanders et al., 2011; Pörtner et al., 2001). Most of the critical biological activities that fish undertake during their life time, like predator avoidance, finding food and migration are dependent on their ability to move (Farrell, 2007; Schneider and Connors, 1982). Swimming performance and aerobic metabolism have been used to elucidate the link between energy budgets and thermal tolerance (Clark et al., 2013; Green and Fisher, 2004; Luna-Acosta et al., 2011). Most prior studies have been on northern hemisphere species such as salmonids (Farrell, 2007); few studies have investigated the effect of temperature on the swimming ability of species in the Southern hemisphere. Climate change hotspots occur throughout the world (Diffenbaugh and Giorgi, 2012). For example the marine region of south-east Australia is one of the hotspots that experienced warming at a much greater rate than the global average (Hobday and Pecl, 2014). Therefore a greater understanding of the potential impacts of increasing temperature on species in the Southern hemisphere is required.

Critical swimming speed (U_{crit}) is widely used to assess the prolonged swimming performance of fish and its aerobic capability to overcome temperature change (Beamish, 1978; Brett, 1964; He

et al., 2013). In this assessment, each fish is allowed to swim while water velocity is increased incrementally followed by a recovery time (Jain et al., 1998). At each water velocity or step, the fish is normally swum for between 15 and 60 min and U_{crit} is interpolated from the final steps of swimming performance (Farrell, 2007) or shortly after fatigue (Clark et al., 2013). Measurements of oxygen consumption can be used to estimate metabolic rate.

Studies of metabolic rate have aimed to find a link between fish biology and climate change by calculating the aerobic scope of activity (difference between maximum and standard metabolic rate) for the species of interest (Price et al., 2012). Standard metabolic rate (SMR) is the amount of oxygen consumed at a steady resting state (zero swimming speed) (Nelson and Chabot, 2011). Several methods exist for SMR calculation, which require the fish to be in a relaxed state. Recent approaches use a normal frequency histogram of oxygen consumption and extract the lowest values to report as SMR (Clark et al., 2013; Nelson and Chabot, 2011).

We targeted King George whiting (*Sillaginodes punctatus*) from the family Sillaginidae, an important commercial and recreational fish species in Southern Australia, to evaluate the species' physiological response to temperature change. King George whiting spawn in deep waters and the post-larvae are transported to shallow coastal areas. After reaching maturity they migrate back to deeper waters (Meakin et al., 2014). A recent risk assessment categorised King George whiting as medium to high risk to climate change (Pecl et al., 2014). The maximum tolerable temperature for King George whiting adults is between 24°C and 26°C (Meakin et al., 2014). Based on the species biology and distribution, we hypothesize that the swimming speed and metabolic rate of King George whiting would be higher in warmer water. We tested this hypothesis by assessing the swimming performance and scope for activity of King George whiting, as well as their potential to recover after a prolonged swimming period under two

temperatures (16°C and 26°C); the higher temperature is close to the adult tolerance limit and the lower temperature reflects average winter temperatures (Meakin et al., 2014).

Material and Methods

Fish collection and maintenance

Adult King George whiting (160-323± 0.1g, 29-34± 0.1cm TL, 2-3 years old) were collected from the wild (North Haven, South Australia) in July 2013 and transported to the South Australian Research and Development Institute (SARDI), Aquatic Sciences Division at West Beach, South Australia. Upon arrival, fish were held at the same temperature as the collection site (16°C) in a 1000L tank for 2 weeks for adaptation to laboratory conditions. Fish were then randomly distributed into two different flow-through 600L tanks and temperature increased in one tank at a rate of $1°Cd^{-1}$ until it met the desired experimental temperature (26ºC). Fish were then held at experimental temperatures for 3 weeks of acclimation before being used in experiments. Photoperiod was kept at a 12-hour light and 12-hour dark cycle to simulate the natural light cycle. Fish were fed on cockles (*Katelysia scalarina*) three times a week.

Experimental setup

The experiment was designed to investigate the U_{crit}, MMR, SMR and aerobic scope of activity for adult King George whiting at two different temperatures (16ºC and 26ºC, n=10). Fish were initially swum in a 90L Loligo® swim chamber, with a 70×20×20cm working section. Water velocity was adjusted by a digital controlled motor. The velocity of the water was calibrated using a voltage signal recording program (flow speed calibration), before starting the swim tests.

The water temperature inside the swim chamber was kept at the experimental temperature using an external heater/chiller unit and a submersible heater. Water temperature did not fluctuate more than 0.5ºC during each swim trial. Fish were fasted for 48 hours prior to starting the swim trial to ensure a post absorptive state and avoid energy loss due to food digestion (Jourdan-Pineau et al., 2010). Following fasting, fish weight, length and girth were measured and fish were transferred to the swim chamber.

Measurement of critical swimming speed

The fish was introduced into the swim chamber 24h before the start of the swim test and allowed to acclimate. Following acclimation, water velocity was increased by 0.25 body lengths/s every 30 minutes until the fish stopped swimming due to exhaustion. During each thirty minute period the system remained sealed for 7 minutes and was flushed for 3 minutes to ensure adequate oxygen within the water. Exhaustion was defined as the time when the fish could no longer swim and maintain position within the chamber for >30s. U_{crit} was then calculated using Brett's equation (Brett, 1964):

$$U_{crit} = U + (t/t_i \times U_i) \quad \textbf{(1)}$$

Where U is the last speed expressed in cms^{-1}, U_i is the velocity increment expressed in cms^{-1}, t is the time swum in the final velocity increment and t_i is the set time interval for each velocity increment (30 minutes).

Measurement of metabolic rate

Following exhaustion in the swim chamber fish were immediately transferred into a custom made resting chamber (5.7L) and allowed to recover, which took around 17 hours. When first placed in the resting chamber the MMR was measured from the highest oxygen data, and then oxygen was recorded as the fish recovered, such that SMR could be obtained. A similar protocol to the swim trial was used where the resting chamber was sealed for 7 minutes and then flushed for 3 minutes. The water flow rate (300 L/h) was constant over the recovery period such that the fish slowly relaxed without any disturbance. The length of time to recovery was recorded, which was based on when the oxygen consumption rate became stable and did not change significantly over time. Oxygen was estimated using an oxygen probe (LDO®, Luminescent Dissolved Oxygen model with a range of 0 to 200 % air saturation and accuracy of ± 0.2ppm above 5ppm) which was placed in the pipes used for circulating water (Fig. 1) and was associated with HQ40d portable meter package software. The oxygen concentration inside the chamber did not decrease below 85%.

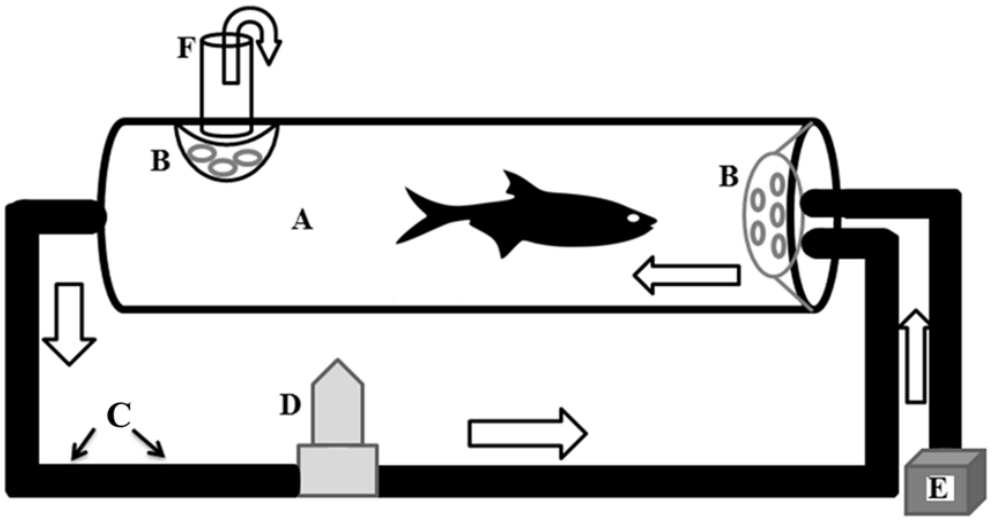

Fig. 1. A schematic of the resting chamber showing (A) the test section where the fish is placed, (B) baffles which facilitate laminar water flow, (C) recirculating flush pump, (D) oxygen probe, (E) pipes for water flow, and (F) overflow chimney.

To determine MMR, the slope of decrease in oxygen through time was calculated by linear regression. All slopes had an $r^2 > 0.95$ and were included in subsequent analyses. MO_2 values were calculated according to the following equation:

$$MO_2 = 60 \text{slope Vol/m} \quad (2)$$

Where slope represents the amount of oxygen consumption for each cycle of measurement, Vol is the volume of the resting chamber (5.7L) minus the volume of the fish and m is the body mass of the fish (kg). The slope was calculated using the Chart and Scope software from AD instruments. A normal distribution curve was fitted to the oxygen data (bin size = 1mg O_2 kg^{-1} h^1). We then averaged the lowest mode of MO_2 values to get the most precise estimate of SMR. The highest oxygen consumption values recorded at the start of the recovery period were used as MMR (Clark et al., 2012). MMR generally occurs shortly after fatigue (Shultz et al., 2011);

therefore our approach of measuring MMR in the resting chamber should provide an accurate representation of maximum MO_2 consumption. Aerobic scope was expressed as the difference between SMR and the MMR (Clark et al., 2013).

Temperature quotients (Q_{10}) were calculated using the formula provided by Kieffer et al. (2014): $Q_{10} = (k_2/k_1)^{10/(t2 - t1),}$ where k_1 and k_2 are the rates of oxygen consumption at the lowest temperature (t_1) and the highest temperature (t_2), respectively.

Statistical analysis

The effect of temperature on U_{crit}, SMR, MMR, aerobic scope and time to recovery were analysed using a t-test to determine whether there were significant differences between temperature treatments. SPSS statistics 22.0 was used for all data analyses.

Results

The U_{crit} was higher at 26ºC than at 16ºC (t-test, t=-2.5, p=0.02) (Fig. 2), suggesting that U_{crit} is temperature dependent. The oxygen consumption rate was significantly different between the two temperature treatment groups (t=-27.80, $p<0.001$) with fish in warmer temperatures consuming twice as much oxygen (Fig. 3).

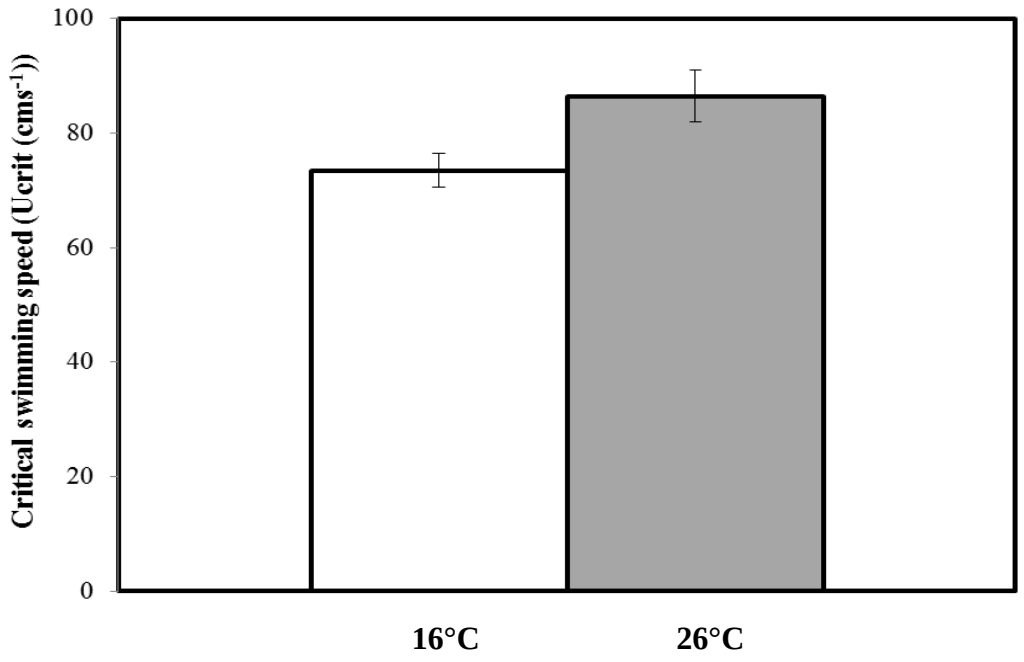

Fig. 2. The effect of temperature on U_{crit} of adult King George whiting (mean ± SE.).

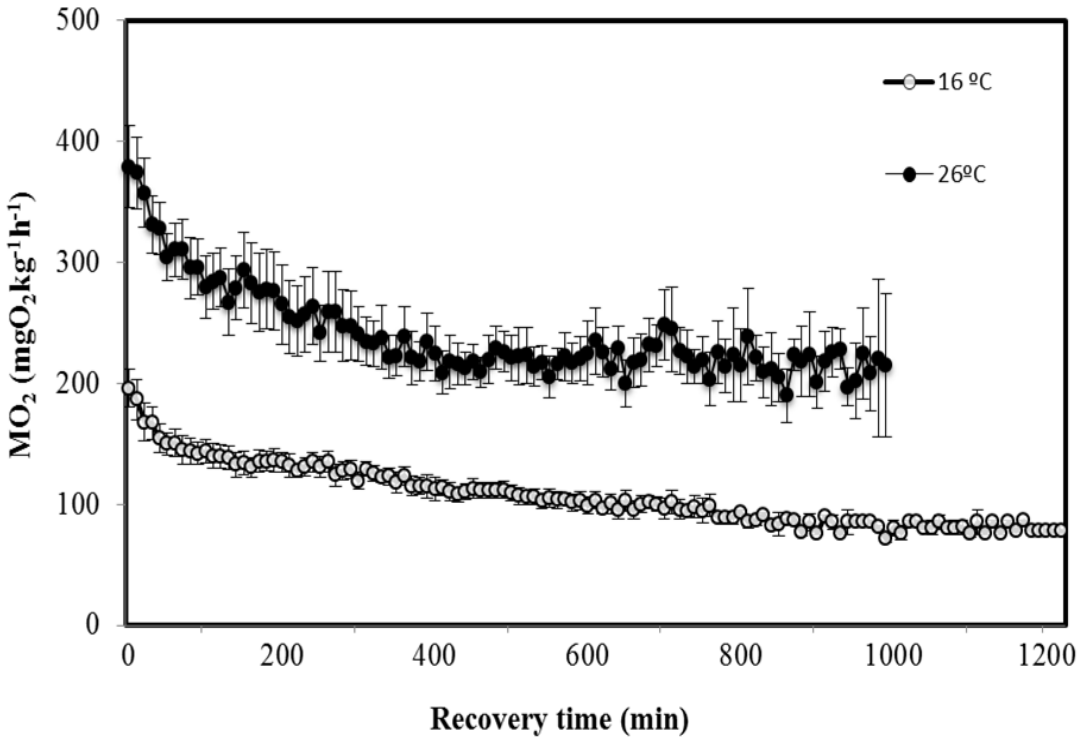

Fig. 3. The oxygen consumption of fish over time after being placed in the resting chamber at two different temperatures (mean ± S.E., n=10 fish for 16ºC and 7 fish for 26ºC).

Fish at the higher temperature (26ºC) recovered quicker than fish at the lower temperature (16ºC), although there was no significant difference in recovery time between temperatures (8±0.04h versus 10±0.01h respectively, t = -2.30, p=0.4). Aerobic scope was greater at 26ºC than at 16ºC (approximately double) (Fig. 4). All three variables differed significantly between temperatures (SMR, t=-7.43, p<0.001; MMR, t= -7.08, p<0.001; aerobic scope, t= -3.8, p<0.001) with the higher temperature being approximately double that of the lower temperature (Fig 4).

The Q_{10} for SMR and MMR was 2.06 and 2.07 respectively. This indicates that the metabolic rate of King George Whiting was temperature dependent, as an increase in temperature of about 10°C increased the metabolic rate about two-fold.

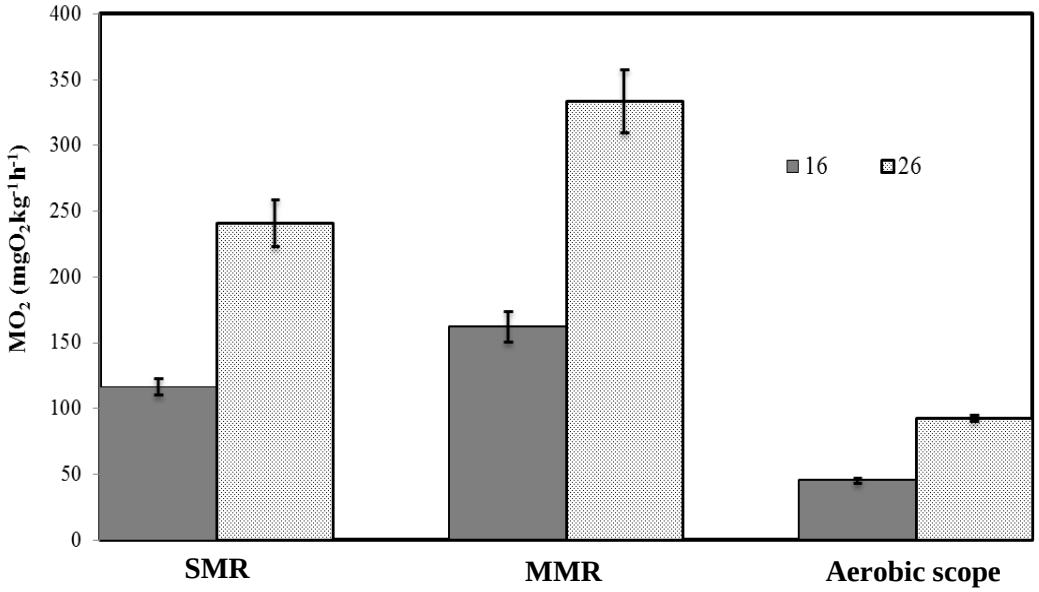

Fig. 4. The effect of temperature on metabolic rate of adult King George whiting at two different temperatures (16ºC and 26ºC) showing SMR, MMR and aerobic scope (mean ±S.E, n=10 fish for 16ºC and 7 fish for 26ºC)**.**

Discussion

We evaluated the effect of temperature on swimming speed and metabolic rate of King George whiting. The U_{crit}, SMR, MMR and aerobic scope of fish was higher for fish from higher temperatures, which supports our hypothesis. Fish swum faster and consumed significantly more oxygen in warmer water suggesting that an increase in temperature to around 26^{o}C may be beneficial to King George whiting movement and metabolism, however the fish would need more energy to sustain itself.

The effect of temperature change on critical swimming speed (U_{crit})

The U_{crit} in fish is a proxy for assessing the maximum aerobic capacity of fish (Beamish, 1978; Plaut, 2001; Soofiani and Priede, 1985). U_{crit} in King George whiting increased when fish were exposed to a 10^{o}C temperature increase, which is comparable to previous studies on a range of species including juvenile short nose sturgeon (*Acipenser brevirostrum*), juvenile qingbo (*Spinibarbus sinensis*) and various salmonid species (Deslauriers and Kieffer, 2012; Lee et al., 2003; Pang et al., 2013). The relationship between swimming performance and temperature, ranges from curvilinear (e.g. juvenile short nose sturgeon) (Deslauriers and Kieffer, 2012) to bell-shaped (e.g. salmonids) (MacNutt et al., 2004) depending on whether the highest temperature is above the point where there is a loss of physiological performance (Clark et al., 2013; Pöertner and Farrell, 2008). We only used two temperatures for our study therefore it was not possible to determine the shape of the curve between swimming performance and temperature (Pöertner and Farrell, 2008).

An increase in U_{crit} at higher temperatures might be due to greater metabolic power produced by red muscle fibres (Day and Butler, 2005). At higher temperatures the capillary density of red muscle fibres is enhanced leading to increased adenosine triphosphate (ATP) generation (He et al., 2013; Martin and Johnston, 2006), but if temperatures exceed the thermal limit of the fish, the reverse pattern may be found. In addition, mitochondrial densities also increase (Davie et al., 1986), hence aerobic enzyme activity is boosted to fuel fish locomotion (Guderley and St-Pierre, 2002; Watabe, 2002). Elevated water temperatures could also affect the fish gill morphology, so that the gill surface area is greater allowing for higher oxygen uptake (Fu et al., 2011). Hence, the fish will be able to absorb more oxygen during and after activity (Eme et al., 2009; Gallaugher et al., 2001; Liu et al., 2009).

Maximum metabolic rate (MMR) and standard metabolic rate (SMR) in response to elevated temperature

The SMR of the fish at higher temperatures was double the amount of oxygen consumption compared to the fish at lower temperatures, which is similar to previous studies on salmonids, juvenile common carp (*Cyprinus carpio*) and dark barbel catfish (*Peltebagrus vachelli*) (He et al., 2013; Lee et al., 2003; Li et al., 2010; Liu et al., 2009). Temperature quotient (Q_{10}) values also indicate that the increase in temperature form 16°C to 26°C doubled the metabolic rate for the adult King George whiting. This increase may be due to increased gill surface area allowing more sustained swimming activity, extreme cardiac performance, and accelerated blood circulation. SMR is used to define the functional capacity of fish in respect to temperature change. King George whiting had significantly higher MMR and U_{crit} in warmer water compared

to cooler water. Similar factors to those listed under MMR may also explain variation in SMR between temperatures.

Although many studies of aquatic organisms have investigated different methods to measure SMR (Table 1), there is still an absence of reliable methods for measuring the SMR of fish (Farrell, 2011). In our study we applied a new method for measuring SMR, which involved transferring fatigued King George whiting from the swim chamber into a custom-designed resting chamber. This approach allowed oxygen to be reliably measured as the fish recovered and became inactive (zero activity). We generated a frequency histogram and extracted the average of the lowest mode (oxygen values) (Farrell, 2011). Our SMR values were consistent with previous studies on juvenile Atlantic cod (Luna-Acosta et al., 2011; Schurmann and Steffensen, 1997). Our approach was necessary because while the swim chamber we used was suitable for King George whiting in terms of fish length, the overall volume of the internal chamber was too great to detect changes in oxygen consumption.

Table 1: Approaches used to calculate SMR from previous literature and the current study. BLs^{-1}(body lengths per second)

SMR measurement approach	*Species*	*Source*
Generating frequency distribution of MO_2 values at $U=0.75BLs^{-1}$ and averaging the lowest mode (bin-size=$5mgO_2kg^{-1}h^{-1}$)	Coral reef fish (*Scolopsis bilineata)*	Roche et al., 2013a
Averaging the lowest 10% of the data and excluding the outliers	Review paper, Brown trout, Golden grey mullet (*Liza aurata*)	Clark et al., 2013a, Norin and Malte, 2011, Killen et al., 2011b
Averaging the lowest 6 values of the data and excluding the outliers	Bone fish (*Albula vulpes*)	Shultz et al., 2011
Generating frequency distribution of MO_2 values at $U=0BLs^{-1}$and averaging the lowest mode (bin size=$1mgO_2kh^{-1}h^{-1}$)	King George whiting (*Sillaginodes punctatus*)	Current study

The effect of elevated water temperature on aerobic scope of activity and recovery time

Aerobic scope of activity for King George whiting increased two fold (from 45.35±1.99 mgO_2 kg^{-1} h^{-1} to 92.74±2.2 mgO_2 kg^{-1} h^{-1}) as the temperature increased. This significant improvement was likely due to increased capacity for oxygen exchange and enhanced cardio-respirometry function in warmer temperatures (Owerkowicz and Baudinette, 2008).

The fish took 10±0.01h at 16ºC and 8±0.04h at 26ºC to recover, which was the time when the oxygen consumption rate reached a stable minima. During the recovery time, the fish will replenish the consumed oxygen and will calm down to reach its minimum metabolic rate. So

although fish consumed more oxygen at higher temperatures they were able to recover just as quickly as fish from lower temperatures. King George whiting survived and had increased oxygen consumption at higher temperatures with the highest temperature used in our study (26^{o}C) likely within their thermal tolerance, at least for adult fish. Given the limited number of samples available we were not able to test a range of temperatures and determine the critical upper temperature where growth and metabolism may be impaired. Thermal tolerance curves based on oxygen consumption, may explain how ocean warming can shape the thermal window of different fish species (Pörtner et al., 2001). It can also elucidate the optimum level of performance between the low and high pejus temperatures (pejus=the point where performance declines with increasing temperature) as well as lethal temperature (Pörtner et al., 2001). Future research should endeavour to use a broader range of temperatures to investigate these factors.

Conclusion

King George whiting are likely to experience different temperatures while migrating between warm estuarine waters and cooler offshore waters. Elevated oxygen consumption rate, faster swimming performance, and higher aerobic scope occurred in warmer water suggesting that increased water temperature may increase the survivorship of individuals (e.g. increase movement capacity and thus ability to avoid predators and find food). Hence, this research assists with better understanding the impacts of short term climate change on a migrant marine fish species. It has also made an important step into understanding the fish metabolic rate to elevated temperature beyond traditional approaches that have been used in some previous literature (Pörtner et al., 2001). Further research should focus on finding the optimum and lethal temperatures for this species to evaluate its tolerance to future ocean warming.

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Chapter 4: Metabolic rate and swimming behaviour of a juvenile temperate fish in relation to temperature and salinity

Top right: Me in Aquarium room, The University of Adelaide (Photo credit: Amir Forghani)

Top left: Me in Barker Inlet, Adelaide (Photo credit: Zoe Doubleday)

Bottom right: Port Vincent, Adelaide. Juvenile King George whiting sampling (Photo credit: Amir Forghani

Bottom left: juvenile King George whiting in a swim chamber (Photo credit: Nastaran Mazloumi).

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By signing the Statement of Authorship, each author certifies that the candidate's stated contribution to the publication is accurate and that permission is granted for the publication to be included to the candidature thesis.

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Metabolic rate and swimming behaviour of a juvenile temperate fish in relation to temperature and salinity

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Abstract

Environmental variability (e.g. temperature and salinity fluctuation) can affect the metabolism and swimming behavior of aquatic organisms. We investigated the influence of four different temperatures (16, 19, 22 and 25°C) and two different salinities (30 and 40ppt) on aerobic performance and critical swimming speed (U_{crit}) of juvenile King George whiting (*Sillaginodes punctatus*), a commercially and recreationally important temperate fish in Southern Australia. Following three weeks acclimation, fish were swum in a swim chamber to estimate the standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope and critical swimming performance (U_{crit}). We then used generalized linear mixed models (GLM) to investigate the metabolic rate and swimming ability of juvenile fish in response to temperature and salinity. Differences in metabolic rate (SMR and MMR) were largely explained by a curvilinear relationship with temperature, but temperature did not affect U_{crit}. Both SMR and MMR increased with increasing temperature from 16 to 22°C and then decreased from 22 to 25°C. This suggests that temperatures between 16 and 19°C are optimal for aerobic performance of juvenile King George whiting and a shift to higher temperatures (>22-25°C) may result in loss of performance. The thermal optimum for aerobic activities of juvenile fish was consistent with

their natural temperature range. Salinity did not affect MMR and aerobic scope, but SMR decreased with a decrease in salinity and U_{crit} increased with decreasing salinity. Evaluating the physiological responses of juvenile fish to environmental parameters is beneficial for understanding their optimal tolerance for aerobic metabolism and locomotion.

Key words: Standard metabolic rate (SMR), maximum metabolic rate (MMR), critical swimming speed (U_{crit}), climate change, King George whiting, thermal tolerance window

Introduction

Environmental variability can directly affect habitat selection and species' physiological characteristics (Brett, 1971). Variations in environmental conditions can indirectly affect key prey species and food web dynamics (Pörtner and Peck, 2010), population structure (Pöertner and Knust, 2007), and species distribution and migrations (Grebmeier et al., 2006). These changes can be understood at organismal (physiology), individual (behaviour), population (mortality-growth balance) and ecosystem levels (productivity and food web interactions) (Pörtner and Peck, 2010). At the organismal level, changes in environmental conditions affect species tolerance, metabolic rate and locomotion in an unpredictable way (Meakin and Qin, 2011; Moser, 1989).

The salinity tolerance of fish can be regulated by osmoregulation and metabolic rate which are important for maintaining ionic balance with their surrounding environment (Evans, 2010). These responses are species specific and enable an animal to adapt to new environmental conditions (Uliano et al., 2010; Fiol and Kültz, 2007). Metabolic response to salinity is highly dependent on life history, habitat and age of the organism (Boeuf and Payan, 2001; Morgan and Iwama, 1991). Species that live in environments with extreme salinity changes may experience significant decreases or increases in swimming performance compared to those that live in stable salinities (Yetsko and Sancho, 2015; Kammerer et al., 2010). In addition, studies have shown that a species metabolic rate increases with increasing salinity, but is influenced by life history stage (Uliano et al., 2010). With changes in freshwater input associated with drought conditions, waters may become more saline. Despite this, physiological responses of fish to salinity changes that are associated with drought and warming have not generally been studied.

Ectotherms are capable of tolerating a range of temperatures, but moving from optimal temperatures to extremes can cause anaerobic metabolism and loss of performance (Lee et al., 2003b). The capacity of organisms to cope with different temperatures is generally referred to as thermal tolerance (Angilletta, 2009; Huey and Stevenson, 1979). Optimal oxygen consumption within the thermal tolerance of species (between high and low pejus temperature, pejus=getting worse) characterizes their aerobic performance (Pörtner, 2002; Frederich and Pörtner, 2000), which is the difference between the maximum and standard metabolic rate. Calculation of aerobic scope has enabled researchers to investigate the optimum temperature tolerance (T_{opt}) in which organisms can perform aerobically (Clark et al., 2013).

Maximum metabolic rate (MMR) corresponds to maximum oxygen consumption of the fish during exercise or shortly after fatigue (Clark et al., 2011; Korsmeyer and Dewar, 2001), and standard metabolic rate (SMR) is the amount of oxygen used by the fish in a relaxed state ($0BLs^{1}$, resting). The amount of oxygen used in a relaxed state, is the minimum that the fish requires to survive and live sustainably (Fry and Hart, 1948). These metabolic rate metrics may vary across different species and temperatures (Behrens et al., 2012). Previous research analysed the aerobic metabolic rate of the same individuals at each temperature [e.g. salmonids including: sockeye salmon (*Oncorhynchus nerka*) (Eliason et al., 2011) and coho salmon (*Oncorhynchus kisutch*) (Lee et al., 2003a)], while other studies have tested different individuals at a range of temperatures [e.g. juvenile barramundi (*Lates calcarifer*) (Norin et al., 2014), Atlantic cod (*Gadus morhua*) (Tirsgaard et al., 2015) and tropical damselfish (*Acanthochromis polyacanthus*) (Donelson and Munday, 2012)]. The sensitivity of species to different temperatures requires further investigation as there is still a paucity of information regarding thermal tolerance for most

species (Pörtner, 2002). An understanding of the low and high tolerance limits of a species is necessary to find how they may respond to changing environmental conditions.

Environmental variability may also affect swimming performance (Hein and Keirsted, 2012). Swimming performance determines an organism's survival (Pang et al., 2013), as it is related to its ability to find food, escape from predators and undertake spawning migrations (Peng et al., 2014; Grigaltchik et al., 2012). Measurement of sustained critical swimming performance (U_{crit}) can be used to assess prolonged swimming speed (Farrell, 2008; Beamish, 1978), the speed at which maximum sustainable oxygen uptake occurs (Gregory and Wood, 1999). This metric is species specific (Nelson and Chabot, 2011; Hammer, 1995) and may be influenced by salinity and temperature (Yetsko and Sancho, 2015; Deslauriers and Kieffer, 2012).

To assess the effects of temperature and salinity on King George whiting we measured metabolic rate and U_{crit} of juvenile fish at a range of temperatures (16, 19, 22 and 25ºC) and two levels of salinity (30 and 40ppt) under controlled laboratory conditions. Our main objective was to determine the optimum temperature and salinity for juvenile King George whiting.

Methodology

Fish collection

Juvenile King George whiting (40-60mm TL, 0.4-0.5 g, n=64) were collected in December 2014 from Port Vincent on the east coast of Yorke Peninsula (Gulf St Vincent), South Australia (34°46′0″S 137°51′0″E). Samples were collected by beach seine (6m spread, 2mm mesh). Following capture, fish were placed into containers equipped with aeration and transferred to the aquarium room at The University of Adelaide. Upon arrival, fish were held in a 100L tank for 10 days to acclimate to laboratory conditions. The holding tank was continuously supplied with air-

equilibrated seawater. Temperature and salinity were held at the same temperature and salinity as the collection site (20°C and 40ppt).

We aimed to simulate temperature fluctuations similar to those juvenile fish might experience in the wild (Fig. 1). Estimates of monthly sea surface temperature from the South Australian Gulfs (Spencer Gulf and Gulf St Vincent) over 5 years (2010-2014) were downloaded and processed from the Integrated Marine Observing System (IMOS) data portal (http://www.imos.org.au). Based on these data, we chose four temperatures (16, 19, 22 and 25°C, n= replicate tanks per temperature) spanning the range of temperatures along the gulfs and two salinities (30 and 40ppt, n=2 replicate tanks per salinity).

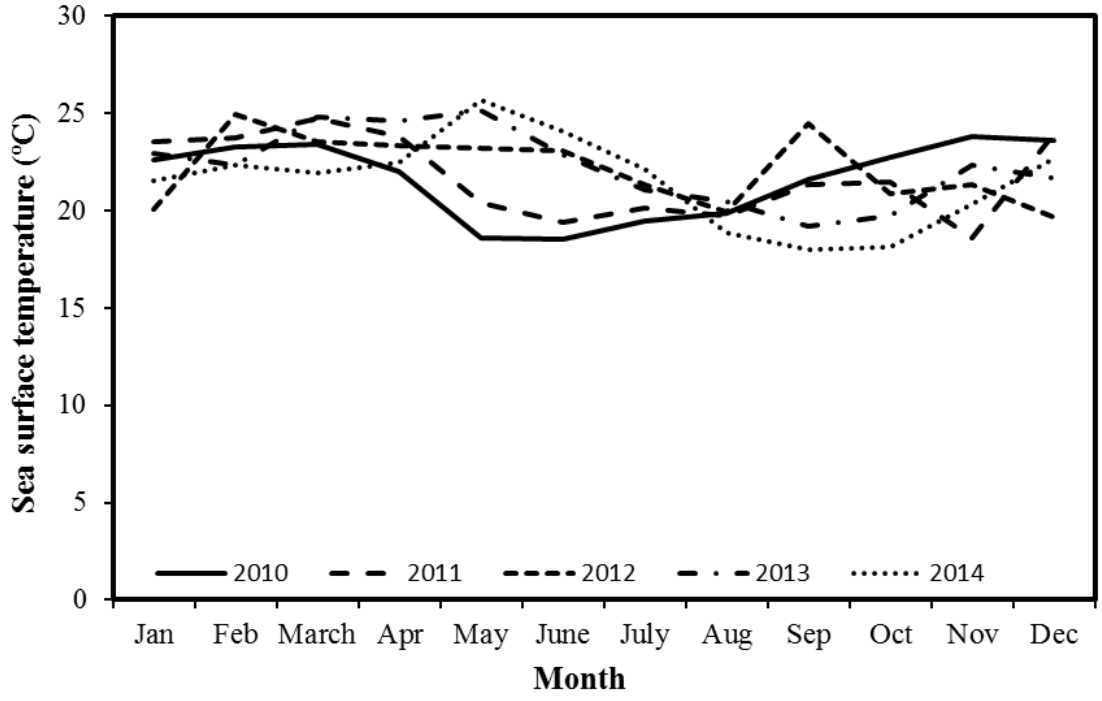

Fig. 1. Monthly sea surface temperature data (SST) from the South Australian Gulfs (Spencer Gulf and Gulf St Vincent) over a 5 year period (2010-2014).

Experimental procedures

Fish were randomly assigned to replicate 40L tanks at a nominal density of 4 fish per tank. Each tank was covered with a clear Plexiglas lid to minimize evaporation thereby keeping experimental salinities constant. All experimental tanks were placed in water baths that were connected to chillers and portable heaters. Water was oxygenated with air pumps via air stones. Temperature was increased or decreased in tanks at a rate of 1°C d^{-1} and salinity decreased by 2ppt d^{-1} until they met the desired experimental temperatures and salinity. Fish were then left for a minimum of 3 weeks in holding tanks to acclimate to the new conditions. Photoperiod was kept at 12h light and 12h dark cycle to simulate the natural light cycle. Temperature and salinity, as well as ammonia and nitrite levels in seawater were monitored on a daily basis using an electronic water quality unit (YSI Sonde, 556 MPS) and ammonia and nitrite test kits. Half of the seawater in tanks was exchanged every other day, ensuring that the ammonia level in the water never exceeded 0.25ppm.

To investigate the effect of different temperatures and salinity on MMR, SMR, aerobic scope and U_{crit}, acclimated fish were randomly selected after 48h of fasting and introduced to a 170ml Loligo® swim chamber (swim chamber dimensions: 26.4mm inner diameter × 100mm length, Loligo Systems, Copenhagen, Denmark). The swim chamber was filled with well-aerated, filtered and UV-sterilized seawater and maintained at a constant temperature equivalent to the experimental temperature of each fish via a heater/chiller unit. Oxygen levels in the swim chamber were recorded using an optical fiber dipping probe oxygen mini sensor (PreSens, Regensburg, Germany) fed into the AutoResp software via a Witrox instrument (Loligo® Systems). The velocity of the water was calibrated using a digital flow tracking system before

starting the swim test. The chamber was regularly rinsed and cleaned with bleach between measurements of each fish.

Metabolic rate measurement

At the start of each experiment, the fish was left undisturbed in the chamber at a swimming speed of $0BLs^{-1}$ for at least 10h while monitoring oxygen. This was used to determine the minimum oxygen consumption while the fish was resting, and corresponded to when the oxygen consumption rate stabilised and did not change greatly over time. MO_2 was measured for 900s followed by a 10s wait and 300s flush; this was repeated four times at each swimming speed. Water temperature did not fluctuate from the experimental temperature by more than 0.5ºC during each swim trial, and the oxygen concentration inside the chamber did not decrease below 85%. MO_2 was calculated by the AutoResp software as the slope of oxygen consumption over time for each measurement cycle using the following equation (all slopes had $r^2 > 0.95$ and were included in subsequent analyses):

$MO_2 = 60 slope\ Vol/m$

Where slope represents the amount of oxygen consumed for each cycle of measurement ($mgO_2kg^{-1}h^{-1}$), Vol is the volume of the resting chamber (1.7L) minus the volume of the fish (L) and m is the body mass of the fish (kg).

SMR values were initially calculated using one of three approaches: 1) averaging the lowest 10% of MO_2 values ($SMR_{\_low}$) (Schurmann and Steffensen, 1997), 2) averaging the last 10% of the MO_2 values (just before starting the swim test) ($SMR_{\_last}$) (Binning et al., 2013), and 3) the $SMR_{\_hist}$ approach, which involved fitting a double normal distribution curve (bin size=

$5mgO_2kg^{-1}h^{-1}$) to the frequency distribution of oxygen values at a swimming speed of $0BLs^{-1}$ (resting). The mode with lowest MO_2 was used as SMR (Roche et al., 2013; Nelson and Chabot, 2011; Svendsen et al., 2011). Given there were no significant differences between the three approaches ($F_{2, 189}$=1.20, P=0.30), $SMR_{\_hist}$ was selected as the most suitable approach for this study. This method has also been recommended in recent literature (Clark et al., 2013; Nelson and Chabot, 2011) and is based on large numbers of observations relative to the other approaches therefore is most suitable for measurements at low swimming speeds (e.g. $0BLs^{-1}$ for standard metabolic rate) (Johansen and Jones, 2011).

Swimming performance

After the fish reached resting, the swim test was started. The back grid of the swim chamber was covered by a black plastic sheet, which encouraged the fish to swim. The swim test involved increasing water velocity by $0.3BLs^{-1}$ every 60 minutes until fatigue to provide a good estimate of U_{crit}. Fatigue was defined as a time when the fish could no longer swim and maintain position within the chamber for >30s to ensure that the fish was totally exhausted (Johansen and Jones, 2011). U_{crit} was calculated from the following equation (Brett, 1964):

1) $U_{crit} = U + (t/t_i \times U_i)$

Where U is the last swim speed expressed in BLs^{-1}, U_i is the velocity increment expressed in cms^{-1} and t is the time that fish were swum in the final velocity increment, and t_i is the set time interval for each velocity increment (60 min).

To determine MMR, MO_2 concentration was measured at U_{crit} (maximum sustained activity) and the highest MO_2 value at U_{crit} was calculated as MMR (Clark et al., 2013; Plaut, 2001;

Brett, 1964). Aerobic scope (MMR – SMR), or the absolute oxygen consumption of the fish, was calculated and compared within the different treatment groups.

Temperature quotient calculations (Q_{10})

The temperature quotient (Q_{10}), which is a measure of the sensitivity of the species to a 10ºC temperature increase (Farrell, 2011), was calculated for each temperature using the formula provided by Kieffer et al. (2014):

1) $Q_{10} = (k_2/k_1)10/^{(t_2 - t_1)}$

Where k_1 and k_2 are the rates of oxygen consumption at temperatures t_1 and t_2 respectively (n =8 fish per group).

Statistical analysis

We analysed the relationship between metabolic rate (SMR, MMR and aerobic scope) or swimming speed (U_{crit}) and environmental parameters [temperature (16, 19, 22 and 25°C) and salinity (30, 40ppt)] using general linear mixed effect models (GLMM, *lme4* package in R). Separate GLMMs were examined for temperature, salinity and the interaction between temperature and salinity. Models were fitted using a stepwise forward procedure with the optimal model at each step selected based on lowest Akaike information criterion corrected for small sample size (AICc) (Burnham and Anderson, 2002). Replicate tanks were treated as a random term and temperature and salinity as fixed terms in all models. MO_2 values were natural log transformed to satisfy the model assumption for normal distribution of variances. The same models were also used for U_{crit}. Predicted effects of the most influential fixed term for U_{crit} and

metabolic rates were also estimated using the *effects* package in R (Fox, 2003; Pinheiro and Bates, 2000). A regression analyses were used to determine the relationship between the oxygen consumption (during swimming) and temperature, salinity and swimming speed (BLs^{-1}) for the juvenile fish.

Results

Juvenile King George whiting showed a significant correlation between oxygen consumption and temperature, salinity and swimming speed (BLs^{-1}) (Table 1). Oxygen consumption increased with increasing swimming speed (BLs^{-1}), but was lower at the low salinity (30ppt) relative to high salinity (Fig. 2a, b).

Table 1. Estimates of coefficients of oxygen consumption of King George whiting juveniles at different temperatures and salinities.

Coefficients	**Estimate**	**SE**	**t.value**	**Pr(>|t|)**
Intercept	7.85	37.40	0.21	0.83
Temperature	19.75	1.19	16.56	< 0.001
Salinity	5.66	0.79	7.17	< 0.001
Swimming speed (BLs^{-1})	65.74	5.81	11.29	< 0.001

The most strongly supported model for SMR had temperature and salinity as independent fixed terms. For aerobic scope and MMR, the optimal model only had temperature as an independent fixed term and for U_{crit} the optimal model only had salinity as an independent fixed term. The addition of a temperature × salinity interaction did not improve any of the models for SMR, MMR, aerobic scope and U_{crit} (Table 2).

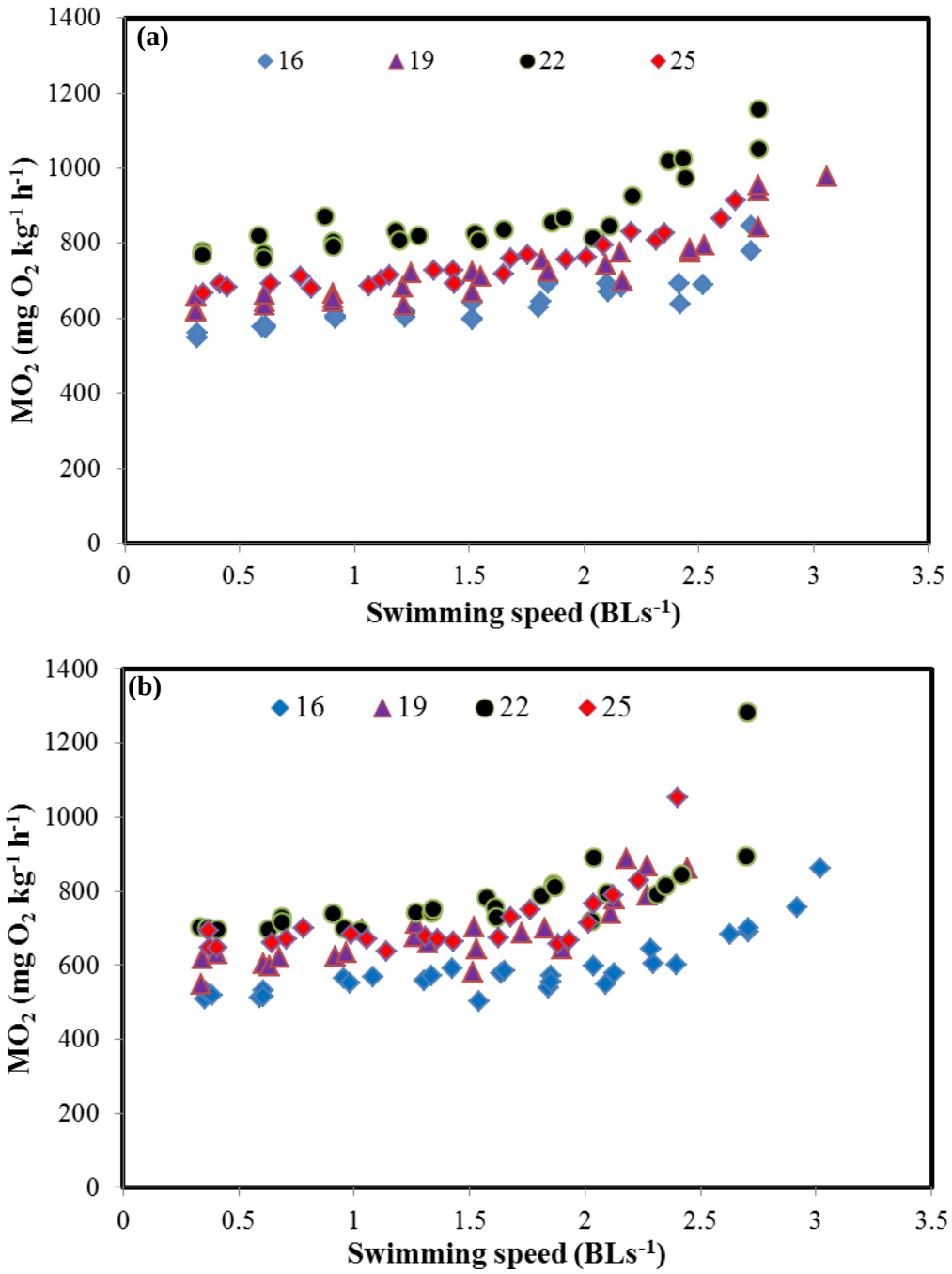

Fig. 2. The relationship between oxygen consumption (mg O_2 kg^{-1} h^{-1}) and swimming speeds (BLs^{-1}) of juvenile King George whiting (*Sillaginodes punctatus*) at four different temperatures and two different salinities. (a) 30ppt and (b) 40ppt for each temperature.

Table 2. Results of models fitted to juvenile King George whiting standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope, and critical swimming speed (U_{crit}). The optimal model (bold) was based on the lowest AICc value. Res.LL = log restricted likelihood estimation. Models included tank as a random term and temperature and salinity as fixed terms. (For the models (×) indicate an interaction between salinity and temperature, whereas (+) indicates independent temperature and salinity terms).

	Models	***K***	**AICc**	**∆AICc**	**Res.LL**
SMR	**Temperature + salinity**	**7**	**10.01**	**0.00**	**2.99**
	Temperature	6	16.14	6.13	-1.33
	Temperature × Salinity	10	22.84	12.83	0.65
	Salinity	4	36.22	26.21	-13.77
MMR	**Temperature**	**6**	**5.99**	**0.00**	**3.74**
	Temperature + salinity	7	10.46	4.47	2.77
	Salinity	4	10.55	4.57	-0.94
	Temperature × salinity	10	24.83	18.84	-0.34
Aerobic scope	**Temperature**	**6**	**122.95**	**0.00**	**-57.14**
	Salinity	4	125.13	2.18	-55.83
	Temperature + salinity	7	129.73	6.77	-56.86
	Temperature × salinity	10	136.69	13.74	-56.27
U_{crit}	**Salinity**	**4**	**-21.58**	**0**	**15.13**
	Temperature	6	-9.91	11.67	11.69
	Temperature × salinity	7	-3.71	17.87	9.85
	Temperature + salinity	10	11.78	33.36	6.19

SMR and MMR increased with increasing temperature from 16ºC to 22ºC followed by a decline from 22ºC to 25ºC for both salinities (Fig. 3a, b, Table 3). However, SMR values were lower at the lower salinity and increased by 12.5 % with increasing salinity from 30ppt to 40ppt (Fig. 3a). Predicted effects plot of aerobic scope against temperature, demonstrated that upper and lower pejus temperatures (T_p) for aerobic performance of juvenile King George whiting were 22°C and 16°C respectively (Fig. 3c). There was a positive correlation between the aerobic scope and temperature at 19°C and 22°C and a negative correlation between 22°C and 25°C (Fig. 3c, Table 3). This implies that optimal temperature for aerobic performance of the juveniles was close to the upper pejus temperature (Fig. 3c) and the onset of anaerobic metabolism and loss of performance may occur at temperatures greater than 25°C for juvenile fish. Critical swimming performance (U_{crit}) was not influenced by temperature, but could best be explained by salinity. The U_{crit} increased with decreasing salinity and had a negative correlation with salinity (Fig. 4, Table 3).

Temperature quotient (Q_{10}) values were estimated and extrapolated between 16 and 19°C, 19 and 22°C, and 22 and 25°C for SMR, MMR and aerobic scope (Table 4). The highest Q_{10} values were between 16°C and 19°C for SMR and aerobic scope and between 19°C and 22°C for MMR. The Q_{10} values for SMR and aerobic scope showed that the temperature optimum for SMR and aerobic activity is between 16°C and 19°C. Hence, the thermal window for juvenile King George whiting is between 16°C and 22°C (Fig. 3c). The optimum temperature for MMR was between 19°C and 22°C, implying that the fish consumes more oxygen at elevated temperatures (19-22ºC).

Table 3. Parameter estimates (± SE) and variance components (± SD) associated with fixed effects and random effects for the optimal models shown in Table 2.

	Random effects:	***Variance***	***SD***	***t.value***
SMR	Tank	Intercept	1.465e-05	0.00
	Residual		4.181e-02	0.20
	Fixed effects:	***Estimate***	***SE***	
	Intercept	5.86	0.05	102.44
	Temperature 19ºC	0.32	0.07	4.51
	Temperature 22 ºC	0.52	0.07	7.32
	Temperature 25 ºC	0.44	0.07	6.18
	Salinity 40	0.19	0.05	3.74
MMR	***Random effects:***	***Variance***	***SD***	
	Tank	0.00	0.00	
	Residual	0.04	0.20	
	Fixed effects:	***Estimate***	***SE***	
	Intercept	6.61	0.05	127.73
	Temperature 19 ºC	0.23	0.07	3.24
	Temperature 22 ºC	0.31	0.07	4.26
	Temperature 25 ºC	0.15	0.07	2.10
Aerobic scope	***Random effects:***	***Variance***	***SD***	
	Tank	Intercept	0.00	0.00
	Residual		0.31	0.55
	Fixed effects:	***Estimate***	***SE***	
	Intercept	5.76	0.13	41.24
	Temperature 19 ºC	0.16	0.19	0.84
	Temperature 22 ºC	0.01	0.19	0.07
	Temperature 25 ºC	-0.28	0.19	-1.46
U_{crit}	***Random effects:***	***Estimate***	***SD***	
	Intercept	0.00	0.00	
		0.03	0.17	
	Fixed effects:	***Variance***	***SE***	
	Intercept	0.88	0.03	27.79
	Salinity 40ppt	-0.03	0.04	-0.82

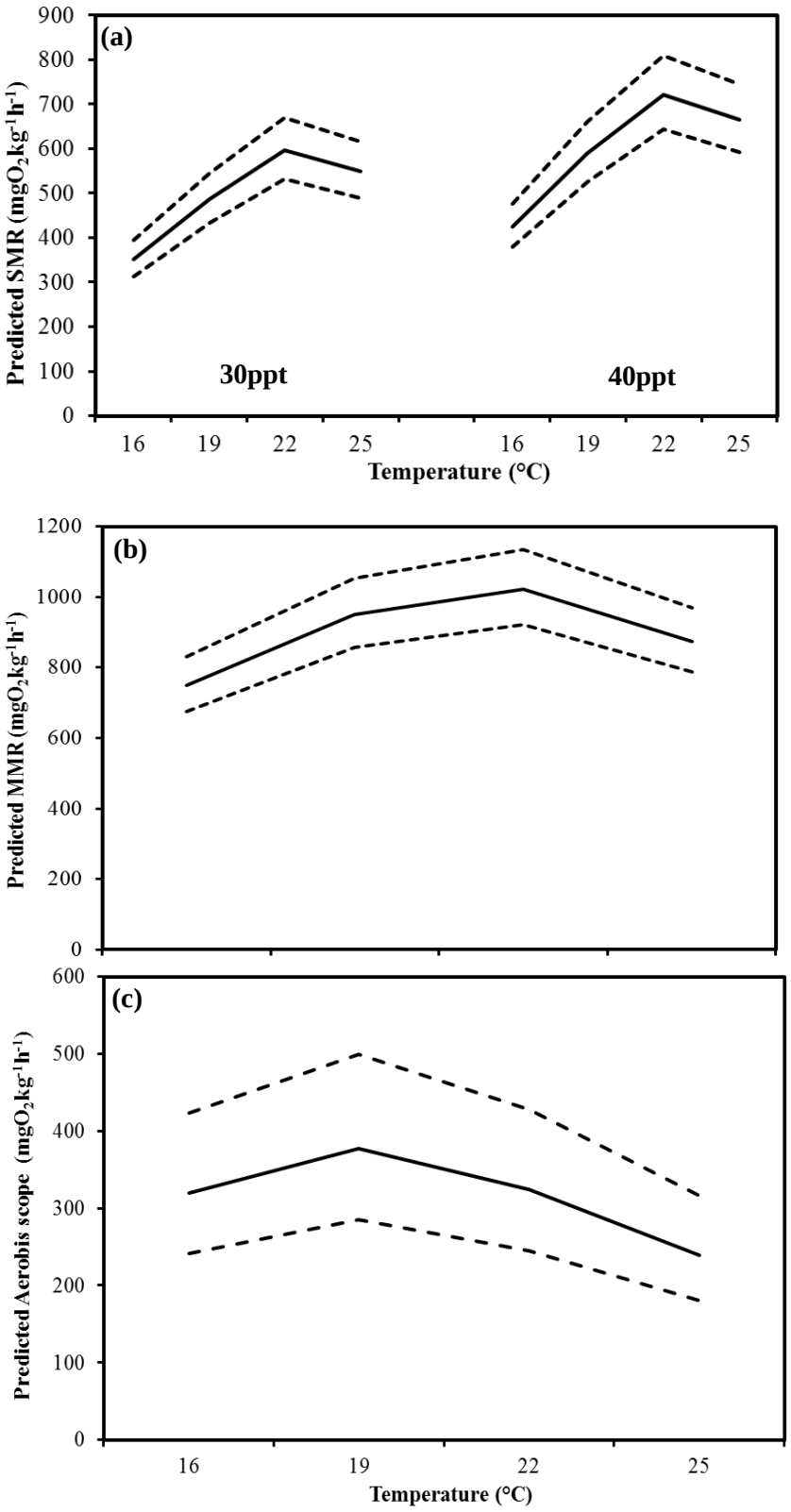

Fig. 3. Predicted effect plots (± 95% CI, dashed line) of temperature and salinity for (a) standard metabolic rate (SMR), and temperature for (b) maximum metabolic rate (MMR), and (c) aerobic scope. All plots are based on the optimal model (see Table 2).

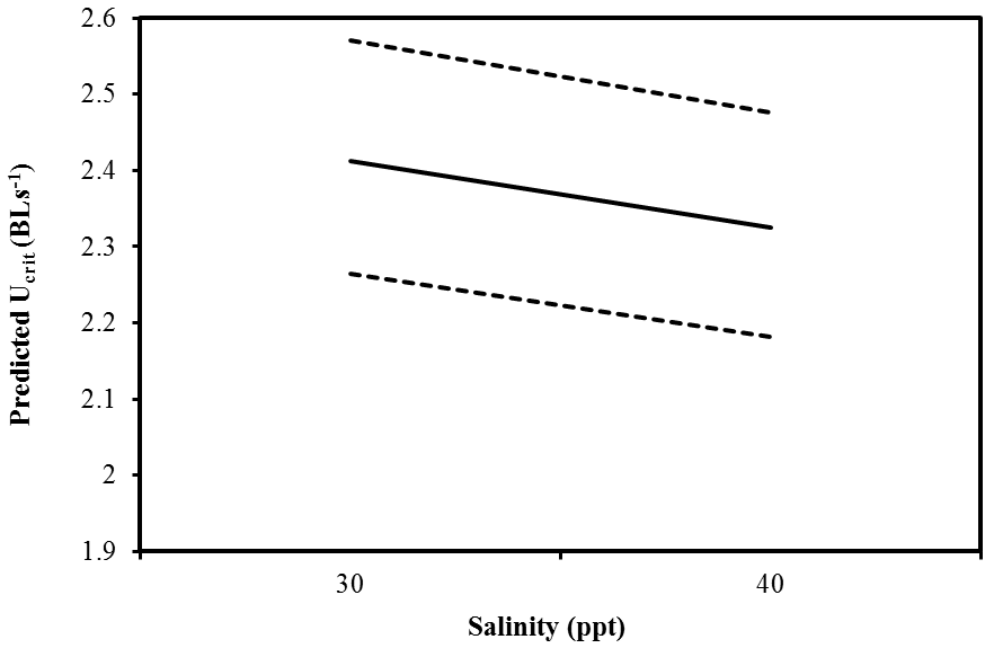

Fig. 4. Predicted effect (± 95% CI, dashed line) plot of salinity on critical swimming speed (U_{crit}) of juvenile King George whiting based on the optimal model (see Table 2).

Table 4. Temperature quotient (Q_{10}) values for juvenile King George whiting acclimated to various temperatures. Highest Q_{10} values are highlighted in bold for maximum metabolic rate (MMR), standard metabolic rate (SMR) and aerobic scope.

Q_{10}	**Temperature (°C)**		
	16-19	**19-22**	**22-25**
MMR	0.2	**7.0**	1.4
SMR	**2.7**	1.8	0.7
Aerobic scope	**1.4**	0.7	0.3

Discussion

We assessed the physiological sensitivity of juvenile King George whiting to temperature and salinity. Temperature affected SMR, MMR and the aerobic performance of the fish, but had little influence on U_{crit}. Additionally, salinity affected U_{crit} and SMR. The juvenile fish had lower SMR and higher U_{crit} at lower salinity. The thermal window and temperature optima for the juvenile fish approximated an aerobic scope curve. The optimal temperature for aerobic performance was similar to the seasonal environmental temperatures where fish were collected. Aerobic scope was higher at 19ºC and decreased as temperature increased, whereas both SMR and MMR increased until 22ºC and then decreased thereafter.

Temperature and salinity effects on aerobic metabolic rate

The temperature where the fish were collected never exceeded 25ºC or dropped below 17ºC over the 5 year period in which we had temperature data, thus our temperatures spanned the range expected for King George whiting in this region (see Fig. 1). The body temperature and metabolic rate of poikilothermic animals (e.g. fish) follows their ambient environmental temperature. Between the upper and lower critical temperature of each species there is a range in which their survival and reproduction are optimum and the species are found in their greatest abundance. This range is the optimum temperature for the aerobic performance of the species and optimises the oxygen supply to tissues and thereby survival (Farrell, 2011; Pörtner, 2010). Optimal temperature can be estimated from the relationship between Q_{10} and the acclimation temperature (Kita et al., 1996). Based on the Q_{10} values, the optimal temperature for SMR and aerobic scope was between 16 and 19ºC and for MMR between 19 and 22ºC, which was just below the upper pejus temperature (25ºC). Based on the outcomes from the present study, depression in aerobic scope at 22-25ºC did not result in loss of performance (see Fig. 3c). This suggests that oxygen limitation (anaerobic metabolism) only

occurs at critical temperatures (Clark et al., 2013), and for King George whiting this temperature was greater than 25ºC.

Different optimal temperatures for SMR, MMR and aerobic scope of the juvenile fish can be partially explained by the idea of multiple performance-multiple optima (MPMO) (Clark et al., 2013). Based on this idea, it is assumed that different physiological processes happen at different temperatures. For juvenile King George whiting the optimum aerobic physiological performance is maximised at temperatures between 16 and 22ºC. However, the idea of MPMO suggests that several other functions such as growth, reproduction and behaviour, which might not be optimised at the same temperature as the aerobic scope, may have different optimal temperatures (Clark et al., 2013).

The results from this study demonstrated that juvenile fish could maintain functional metabolic rate to sustain their activity level within their thermal tolerance window. The schematic diagram of the thermal window of performance indicates that metabolic depression at both ends of the thermal envelope results in passive tolerance (Pörtner, 2010) (Fig. 5a).

Metabolic depression and consequently the passive tolerance range occurs when organisms are exposed to extreme temperatures beyond the critical temperature (T_c, onset of anaerobic metabolism) and possibly start reaching the denaturation temperature (T_d), which causes a loss of structural integrity at the molecular level (Pörtner and Peck, 2010). Passive tolerance at critical temperatures is also associated with stress responses such as stress hormone secretion (e.g. cortisol) (Angilletta, 2009), enzyme activity breakdown (Schulte et al., 2011) and production of heat shock protein (HSP) to enhance thermotolerance (Meakin and Qin, 2011).

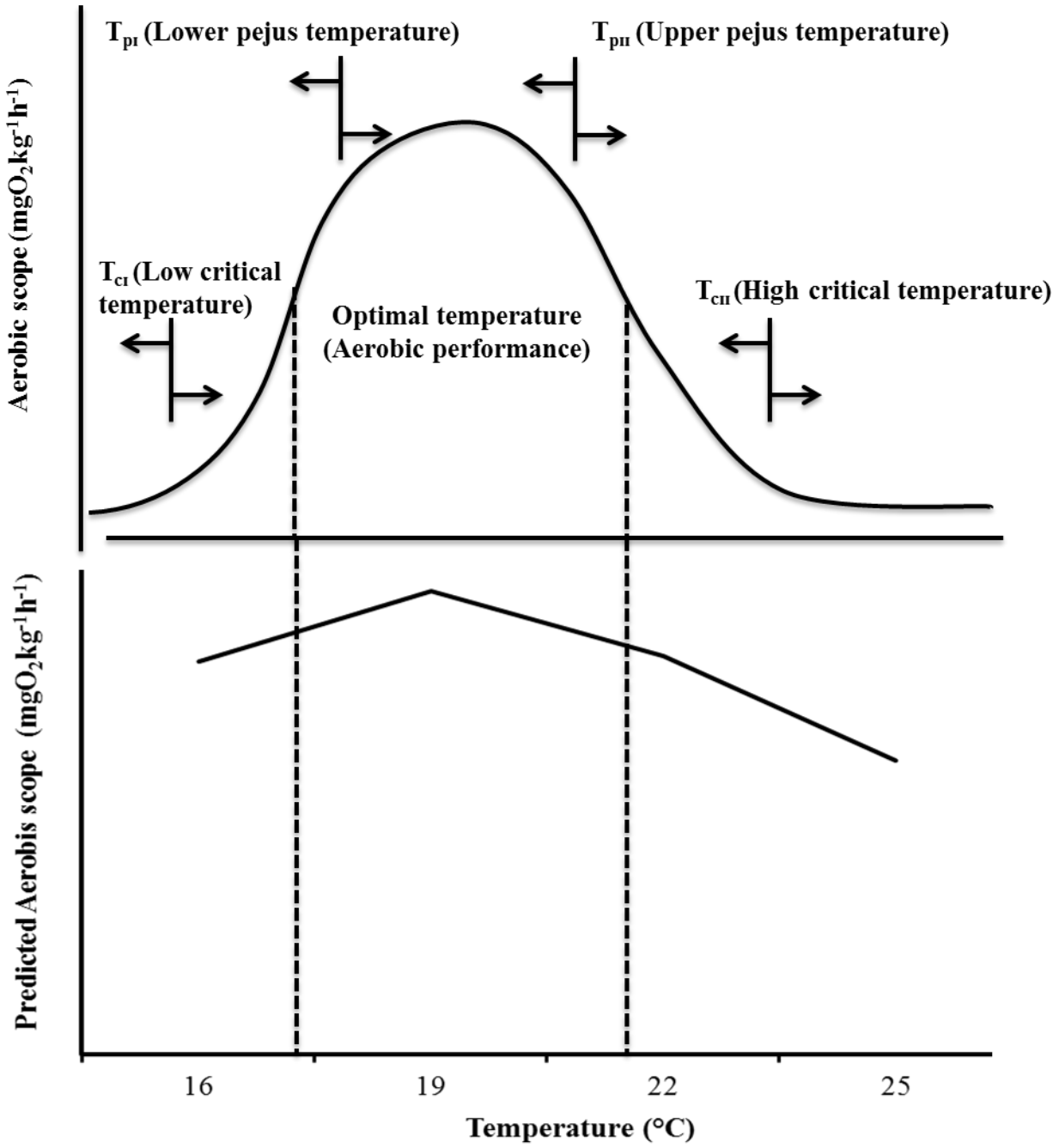

Fig. 5. (a) Schematic diagram of aerobic performance for different temperatures showing pejus and critical temperatures (redrawn from Pörtner and Farrell, 2008) and (b) aerobic scope of activity for juvenile King George whiting (*Sillaginodes punctatus*).

Extreme temperatures interfere with biochemical reactions in ectotherms (Hochochka and Somero, 2002), which results in thermal sensitivity of function at cellular and organismal levels (Rome et al., 1992). Further, the biochemical mechanism, such as the capacity of the

cells and tissues for carrying oxygen and maximum cardiac performance, are reduced at extreme temperatures (Pörtner and Peck, 2010; Farrell, 2002). The capacity of the cardiovascular system becomes limited at extreme temperatures beyond the thermal tolerance of ectotherms (beyond the pejus temperature). Hence, oxygen would not be sufficient to supply the tissues at critical temperatures. Consequently, an imbalance between the oxygen supply and demand will occur. Loss of performance is a preliminary sign of the imbalance between the oxygen supply and demand which might stem from thermal stress (at pejus temperature, T_p) or denaturation by hypoxia (at critical temperature, T_c) (Pörtner and Peck, 2010).

Enzymes are protein based and the protein connection with other reactants can be easily broken at extreme temperatures. Therefore, extreme temperatures destroy the enzyme function, which will affect the locomotion of the fish. Understanding the thermal tolerance in fish species and measuring their metabolic rate in response to environmental stressors helps understand their physiology and behaviour (Rijnsdorp et al., 2009). Nonetheless, the effect of chronic thermal exposure (weeks to years) on aerobic scope is yet to be understood.

The U_{crit} of juvenile King George whiting was not influenced by temperature. This result was similar to Chinook salmon (*Oncorhynchus tshawytscha*) where temperature had little effect on U_{crit} (MacNutt et al., 2006). Several previous studies have found a bell-shaped relationship between U_{crit} and temperature (Gollock et al., 2006; Claireaux et al., 2005). The poor temperature relationship with U_{crit} of the juvenile fish in our study may be because, 1) the selected temperatures were not broad enough to show any significant change in U_{crit}, or 2) the species thermal tolerance limit was not exceeded; if we had increased or decreased the temperature further (<16ºC and >25ºC) then we may have seen a change in U_{crit}.

Salinity effect on U_{crit} and SMR

Response to salinity change depends largely on the developmental stage and habitat the fish inhabits. Morgan and Iwama (1991) described four metabolic responses to salinity, 1) no change in metabolic rate, 2) minimum change in metabolic rate, 3) linear salinity/metabolic rate relationship and, 4) maximum change in metabolic rate. Our results were similar to (1) for MMR and (2) for SMR.

The first response of ectotherms to environmental salinity is a change in plasma cortisol and catecholamine in blood plasma and the second response is an increase in plasma metabolites (e.g. glucose and lactate) (Farrell, 2011). Cortisol plays an important role in regulating the metabolic processes (Herrera et al., 2012). Hence, changes in plasma cortisol levels provide potential information about the physiological status of the fish under changing environmental salinity (Laiz-Carrión et al., 2002). In addition, osmoregulatory processes in response to salinity changes usually causes metabolic expenditure and an increase in oxygen consumption rate (Morgan and Iwama, 1991). Metabolic response to salinity is related to the life history of the species (Herrera et al., 2012). Similar responses to salinity have been reported for Senegalese sole (*Solea senegalensis*) (Herrera et al., 2012) and tilapia (*Oreochromis mossambicus*) (Kammerer et al., 2010). However, no change in MMR with a decrease in salinity was found in juvenile King George whiting, indicating that the maintenance of osmotic homeostasis may not induce significant stress or metabolic costs at U_{crit} in lower salinity.

The higher U_{crit} at low salinity suggests that juveniles can modify their gill morphology to offset the osmotic imbalance in hypo-osmotic situations (Whitehead et al., 2013). The physiological reason behind the increased U_{crit} in response to decreased salinity is that the sodium, chloride, potassium and osmolality all decrease in less saline water and thereby the fish allocate more energy for swimming (Gonzalez et al., 2005; Canario et al., 2005).

Consequently, the decrease in solute contents of blood will reduce the osmotic activity and the fish consume less energy to maintain their ionic balance and thereby swim faster (Magnussen et al., 2008).

Meakin and Qin (2011) found that King George whiting can survive and grow in salinities up to 50ppt and are unlikely to be influenced by fluctuations in salinity. However, they only tested the salinity tolerance of fish over 72 days which might not be long enough to evaluate the influence of salinity on fish survival and physiology. The swimming ability of fish is species specific and can be explained by a number of factors including development stage, phylogeny and methodology (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997). Some species of fish larvae are not good swimmers because they have a low amount of mitochondria in their muscles (Bellwood and Fisher, 2001; Crockett and Sidell, 1990). In addition, the ability of juvenile King George whiting to maintain exercise following a decrease in salinity can be associated with their outstanding capacity for homeostatic regulation of plasma osmolality and tissue water balance.

Conclusion

Activities such as swimming and metabolism are dependent on the physiological capability of the organism for aerobic activities. Although the thermal window of species matches their aerobic scope of activity, environmental stressors such as temperature and salinity can disturb the thermal window and aerobic scope relationship. In the present study, juvenile King George whiting aerobic metabolism was optimal between 16 and 19ºC and fish swum faster at salinity of 30ppt. The juvenile fish were tolerant to both salinities, as well as the range of temperatures investigated.

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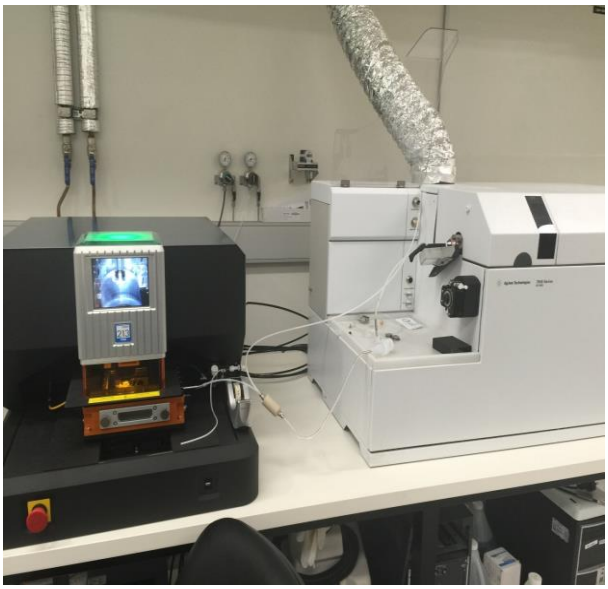
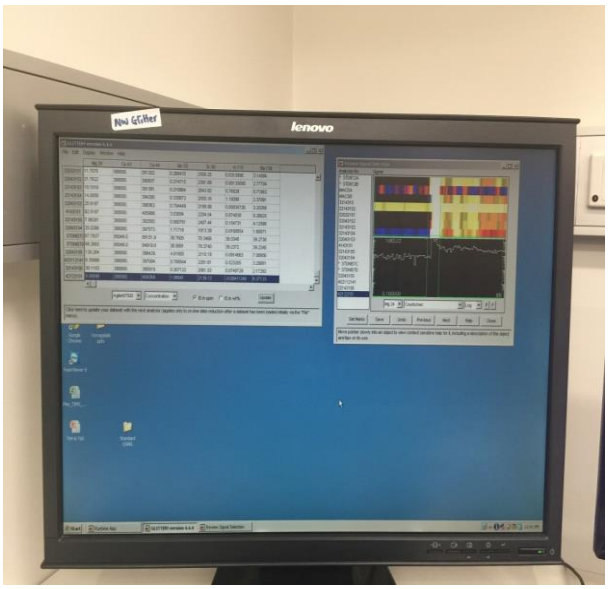
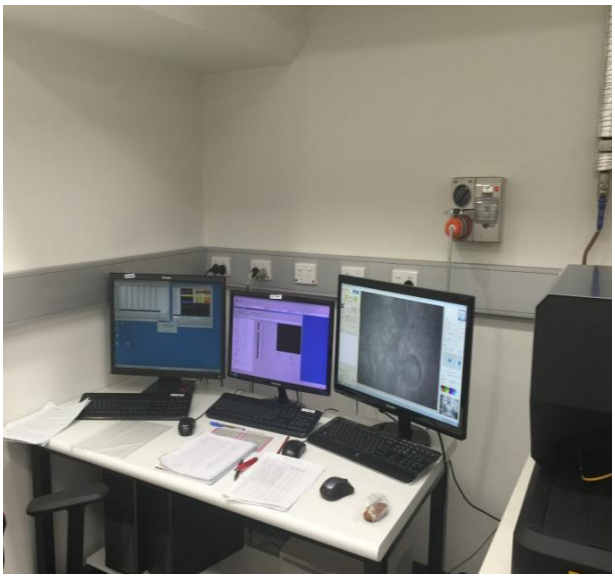
Chapter 5: The effects of temperature and salinity on otolith chemistry of King George whiting







Laser ablation Inductively Coupled Plasma-Mass Spectrometer (LA ICP-MS) and the Glitter software, Adelaide microscopy (photo credit: Nastaran Mazloumi).

Statement of Authorship

Title of Paper	**The effects of temperature and salinity on otolith chemistry of King George whiting**
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Principal Author

Name of Principal Author (Candidate)	Nastaran Mazloumi
Contribution to the Paper	Collected the fish samples, performed analysis on all samples, interpreted and analysed data, wrote manuscript and will be acted as corresponding author
Signature	Date

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that the candidate's stated contribution to the publication is accurate and that permission is granted for the publication to be included to the candidature thesis.

Name of Co-Author	Zoe Doubleday
Contribution to the Paper	Assisted with intellectual development, experimental design and laboratory analyses, as well as provided suggestions, comments and feedback on manuscript drafts
Signature	Date 29/9/15

Name of Co-Author	Bronwyn Gillanders
Contribution to the Paper	Acted as principal supervisor and assisted with intellectual development, provided suggestions, comments and feedback on manuscript drafts
Signature	Date

The effects of temperature and salinity on otolith chemistry of King George whiting

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Abstract

Otolith chemistry is used widely to reconstruct the environmental histories of fish. Examining the relationships between environmental conditions and otolith chemistry is an essential first step towards accurately reconstructing environmental histories, with lack of information potentially resulting in the erroneous interpretation of fish movement and the environments they have inhabited. We evaluated the influence of seawater temperature and salinity on the otolith chemistry of juvenile King George whiting (*Sillaginodes punctatus*), a commercially and recreationally important fish species in Southern Australia. Juveniles were reared under controlled laboratory conditions at four temperatures (16, 19, 22 and 25°C) and two levels of salinity (30 and 40ppt) for 90 days. Otoliths were analysed for barium (^{138}Ba), strontium (^{88}Sr), magnesium (^{24}Mg) and manganese (^{55}Mn) using laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS), and ratioed to calcium (^{43}Ca). Otolith chemistry data were analysed using generalized linear mixed models (GLMM). Analyses showed that Mg:Ca and Mn:Ca increased with increasing salinity, whereas Sr:Ca and Ba:Ca decreased with increasing salinity. Temperature only had a minor influence on elemental concentration and therefore elemental chemistry was not useful for reconstructing temperature histories in this species. The influence of salinity on otolith chemistry suggests that otolith chemistry could be used as a potential tool for reconstructing the salinity and movement history of King George whiting from estuaries to open coast regions.

Key words: Otolith chemistry, King George whiting, temperature, salinity, LA-ICPMS

Introduction

The life history and movement patterns of fish have been reconstructed using otolith elemental chemistry (Reis-Santos et al., 2013; Campana, 1999). Elemental concentrations in otoliths vary with environmental factors (e.g. temperature, salinity, water chemistry) (Elsdon and Gillanders, 2003), but may also be influenced by physiology (e.g. metabolic rate) (Gaetani and Cohen, 2006). Understanding how otolith chemistry is related to environmental variability is a necessary prerequisite for reconstructing the environmental conditions that a fish has experienced (Elsdon et al. 2008).

Otoliths are composed of calcium carbonate usually in the form of aragonite (Campana, 1999; Thorrold et al., 1997). Trace elements (e.g. Sr, Ba, Mg and Mn) are incorporated into the calcium carbonate matrix of the otolith often in relation to the ambient water chemistry or the environmental conditions surrounding the organism (Bath et al., 2000; Thorrold et al., 1997). Thus, if water conditions vary among areas then the trace elements within the otolith can be used as a natural tag. The advantage of using trace elements as a natural tag is that their composition represents a permanent record of the entire life of the fish and that variation across the otolith can be related to the age of the fish (Campana and Thorrold, 2001; Beck et al., 1992).

Otoliths are not in direct contact with the physical environment (i.e. water) and barriers, such as the gills and the ear membrane (endolymphatic fluid); regulate the uptake of trace elements (Campana and Thorrold, 2001). Hence, environmental and biological parameters have the potential to alter the chemical composition of the otolith, such that there may not be a direct relationship between water chemistry and otolith chemistry (Elsdon et al., 2008). Temperature and salinity are two major environmental factors that affect the rate at which

elements replace calcium in the aragonite matrix (Elsdon and Gillanders, 2003; Wei et al., 2000; Fowler et al., 1995). Temperature also affects the pH of the blood plasma and endolymph fluid thereby affecting the crystallisation process and consequently the otolith chemical composition (Romanek and Gauldie, 1996; Gauldie et al., 1995). Salinity can also affect the otolith elemental composition by mediating the elemental uptake into the blood, endolymph and otolith (McCormick, 2001). For fish species that move across salinity gradients osmoregulation demands can alter the ion transport rate across their gill membrane (Miller, 2011; Martin and Wuenschel, 2006).

Previous studies have investigated the relationship between temperature and/or salinity and otolith elemental concentration and have indicated varying results including positive, negative and in some cases no significant relationship for different species (Elsdon and Gillanders, 2005; Dorval et al., 2005; Kraus and Secor, 2004; Payan et al., 1997). Several elements have been used as a marker of movement between habitats with salinity gradients (e.g. Sr and Ba) (Milton and Chenery, 2005). These elements are used for reconstructing anadromous migrations (Trudel et al., 2010; Secor et al., 2001; Kalish, 1990). For example, a positive Sr:Ca correlation with salinity, where otolith Sr:Ca is low in freshwater and increases in marine waters, is commonly reported (Kraus and Secor, 2004). In contrast, a negative correlation between Ba:Ca and salinity has been reported (Stanley et al., 2015; Elsdon and Gillanders, 2005; Dorval et al., 2005).

Some studies have indicated that Sr:Ca in otoliths is temperature dependent (Radtke and Shafer, 1992; Townsend et al., 1992). Temperature significantly affected the elemental concentration of Mg, Mn, Sr and Ba in otoliths of juvenile Atlantic cod (*Gadus morhua*), but salinity had no significant effect on Mg:Ca. The influence of temperature and salinity on otolith Mn:Ca and Mg:Ca also varies among species from positive (Dorval et al., 2007) to negative (Miller, 2009) and no influence (DiMaria et al., 2010; Martin and Thorrold, 2005).

The varying results from different species indicate that the relationship between the element:Ca ratio and salinity and temperature is species specific.

For species that live in dynamic environments, such as estuaries and shallow nursery areas, otoliths may be used to reconstruct environmental histories provided the relationship between the environmental parameters and otolith chemistry is known. Given that the effects of environmental variables on otolith chemistry are species specific (Gillanders and Kingsford, 2003), evaluating how local environmental conditions affect otolith chemistry for different fish species is essential for accurate environmental reconstruction. Herein, we designed a controlled laboratory experiment to examine the individual and interactive effects of temperature and salinity on the otolith chemistry of juvenile King George whiting (*Sillaginodes punctatus*).

Methodology

Study species

King George whiting (*Sillaginodes punctatus*) is endemic to temperate Southern Australia (Hyndes et al., 1998; Kailola et al., 1993). Adults spawn in coastal areas in early spring and the post larvae are transported by ocean currents to shallow seagrass beds (juvenile habitats) (Jenkins and Wheatley, 1998; Jenkins and May, 1994). Juveniles in shallow nursery areas are exposed to fluctuations in temperature and salinity and are thus good candidates to study the influence of environmental factors on otolith elemental composition.

Experimental procedure

Juvenile King George whiting, 40-60mm in total length, were collected in December 2014 from Port Vincent, Gulf St Vincent, South Australia (34.77° S, 137.85° E). Samples were collected by beach seine (6m spread, 2mm mesh) and placed into containers equipped with aeration for transfer to The University of Adelaide. Upon arrival, fish were held in a 100L tank for 10 days to acclimate to laboratory conditions. The holding tank contained natural seawater and temperature and salinity conditions were matched to the collection site (20°C and 40ppt). On completion of acclimation, fish were randomly assigned to 40L tanks at a density of 4 fish per tank. Each tank was covered with a clear Plexiglas lid to minimize evaporation. Treatments consisted of four different temperatures (16, 19, 22 and 25ºC) and two salinities (30 and 40ppt) with two replicate tanks per treatment (Table 1). Estimates of monthly sea surface temperature (SST) from the South Australian Gulfs (Spencer Gulf and Gulf St Vincent) over 5 years (2010-2014) were downloaded and processed from the Integrated Marine Observing System (IMOS) data portal (http://www.imos.org.au). Temperatures selected were based on temperatures that the species experience in nature (Fig. 1). The selected salinities were based on marine and estuarine conditions. The highest salinity (40ppt) was reflective of the inverse estuary that the fish were collected from and the lower salinity (30ppt) reflected a typical estuary with freshwater input; both salinities are where King George whiting might be found within their range.

Table 1. Summary of rearing conditions for each treatment tank for juvenile King George whiting (data are displayed as mean ± standard error, n=3; TL= total length and BW= body weight, n=64).

Treatment	Tank	Temperature (°C)	Salinity (ppt)	TL (mm)	BW (g)
16ºC, 30‰	1	16.01±0.07	30.22±0.07	46±0.10	0.47±0.04
	2	16.13±0.06	29.83±0.09	45±0.20	0.45±0.06
19ºC, 30‰	1	19.26±0.04	30.35±0.04	48±0.23	0.42±0.02
	2	19.34±0.04	30.21±0.05	47±0.47	0.42±0.04
22ºC, 30‰	1	21.77±0.09	29.89±0.03	46±0.23	0.42±0.03
	2	21.64±0.04	30.45±0.08	42±0.14	0.37±0.02
25ºC, 30‰	1	24.66±0.10	30.11±0.05	45±0.30	0.37±0.04
	2	24.49±0.13	29.84±0.09	49±0.19	0.38±0.22
16ºC, 40‰	1	16.34±0.04	40.22±0.04	53±0.44	0.62±0.11
	2	16.22±0.03	40.20±0.03	43±0.23	0.35±0.05
19ºC, 40‰	1	19.27±0.03	39.93±0.06	45±0.25	0.35±0.02
	2	19.31±0.00	39.89±0.08	43±0.23	0.40±0.04
22ºC, 40‰	1	21.90±0.03	40.34±0.09	43±0.32	0.38±0.03
	2	21.95±0.05	39.99±0.05	47±0.32	0.40±0.04
25ºC, 40‰	1	25.05±0.04	40.27±0.02	42±.014	0.37±0.03
	2	24.82±0.10	40.30±0.09	45±0.20	0.40±0.04

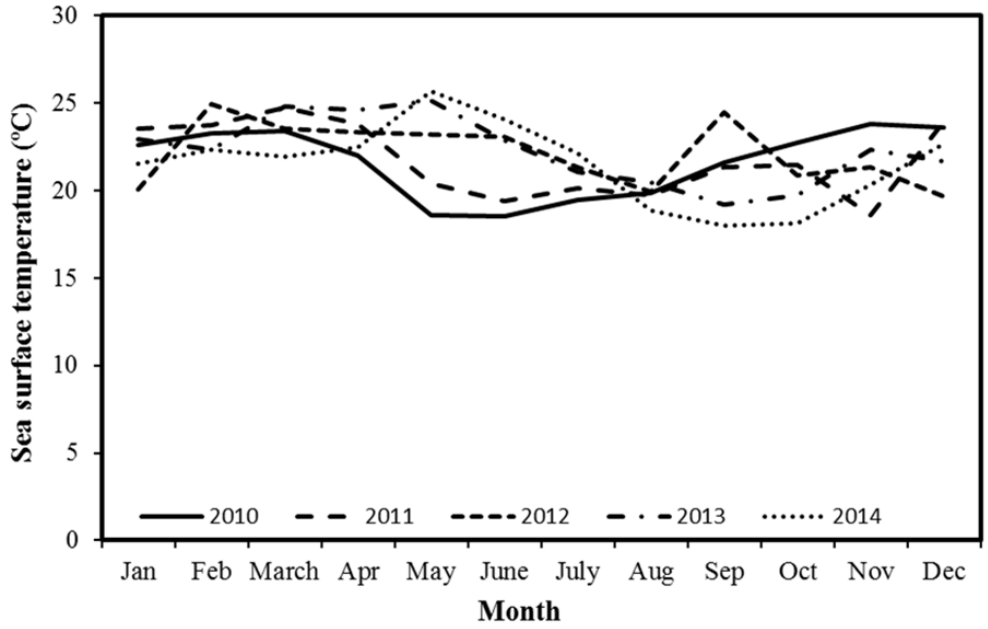

Fig. 1. Monthly sea surface temperature data (SST) from the South Australian Gulfs (Spencer Gulf and Gulf St Vincent) over a 5 year period (2010-2014).

All experimental tanks were placed in water baths that were connected to heater-chiller units. Temperature was increased/decreased in tanks at a rate of 1°C d^{-1} and salinity decreased by 2ppt d^{-1} until they met the desired experimental temperatures (16, 19, 22 and 25ºC) and salinity (30ppt) and fish were kept in their respective experimental conditions for a minimum of 90 days. Water was oxygenated with air pumps via air stones. Photoperiod was kept at a 12h light and 12h dark cycle to replicate the natural light cycle. Temperature and salinity were monitored on a daily basis using an electronic water quality unit (YSI Sonde, 556 MPS). In addition, ammonia and nitrite levels were checked on a daily basis with ammonia and nitrite test kits. Half of the seawater in tanks was exchanged every other day, ensuring that the ammonia level in the water never exceeded 0.25ppm. Throughout the duration of the experiment, juveniles were fed twice daily with frozen blood worms and any accumulated detritus was siphoned out daily. Following exposure to experimental conditions for 90 days, fish were euthanized in an ice and seawater slurry and stored frozen until the otoliths were

extracted. Some of the juveniles (n=5) were marked at the beginning of the experiment with alizarin complexone ($C_{19}H_{15}NO_8$) at a concentration of 35mg·L^{-1} for 24h to determine experimental otolith growth (see below).

Water elemental concentration

Water samples were taken in triplicate from each replicate tank over the course of the experiment (at the beginning, middle and end of the 90 day rearing period). Water samples were collected using a 25mL syringe, filtered through a 40µm filter, preserved with 2% nitric acid, and then refrigerated. Water samples were analysed for ^{24}Mg, ^{55}Mn, ^{138}Ba and ^{88}Sr and ^{43}Ca using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) at the National Measurement Institute (NMI), Australia. Mean elemental concentrations were calculated for each tank (µmol for ^{138}Ba and ^{55}Mn and mmol for ^{24}Mg and ^{88}Sr) and ratioed to ^{43}Ca. The analytical accuracy of elements for water samples averaged across all samples was 97 (Ca), 100 (Mg), 100 (Mn), 110 (Sr), and 110% (Ba).

Otolith preparation and analysis

Total length of each fish was measured before dissection. Sagittal otoliths were extracted and washed with Milli-Q water and allowed to dry for 24h before being stored in micro centrifuge tubes. The otoliths were embedded in clear, polyester resin and allowed to cure overnight before being sectioned through the core. The sections were then mounted on microscope slides using thermoplastic glue (crystal bond). Otolith sections of the alizarin marked fish (n=5) were examined under a fluorescent microscope with transmitted light (Leica model DMLB). The distance between the alizarin mark and the edge of the otolith was measured and used to ensure that there was an adequate amount of experimental otolith growth for

analysis using laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS) (Fig. 2).

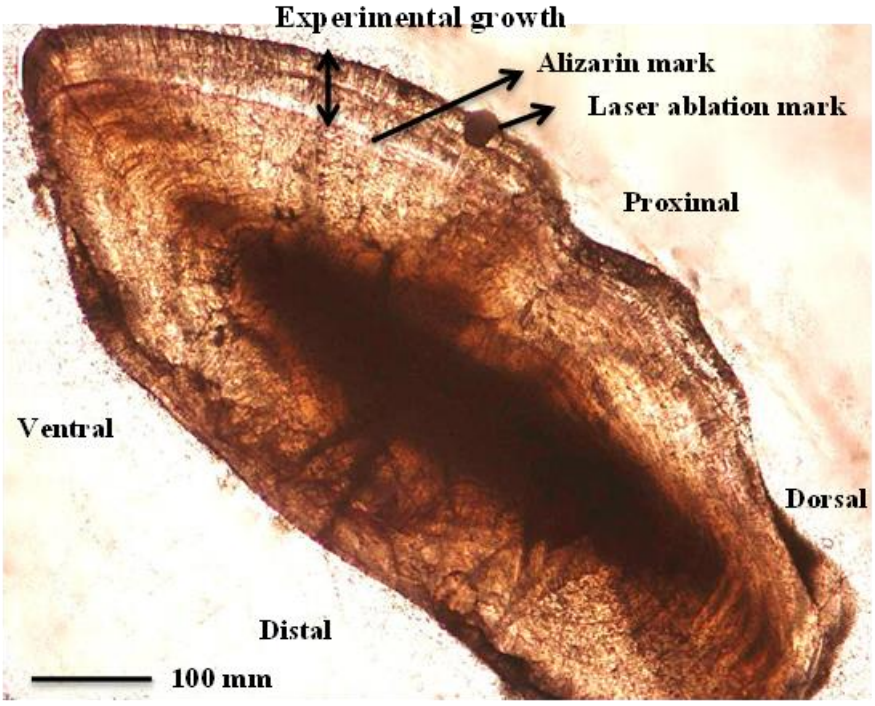

Fig. 2. Transverse section of a juvenile King George whiting sagittal otolith showing growth during the experimental period, the laser ablation crater (30µm) and alizarin mark.

Elemental concentration of ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba, ^{24}Mg and Indium (^{115}In) in otoliths were analysed using an Agilent 7500cs ICP-MS coupled to a Merchantek UP213 (New Wave Research) Nd:YAG deep ultraviolet laser microprobe, with a pulse rate of 5Hz. The edge of the otolith (i.e. experimental growth) was ablated using a 30µm spot. Indium was measured to ensure that only otolith material was ablated since indium was added to the resin. A reference standard, NIST612, was analysed every 10 samples throughout the session and the carbonate standard, MACS-3 was also analysed at the beginning and end of the session to account for instrument drift and determine precision of measurements. The dwell times (ms) for each element were: ^{138}Ba (300), ^{88}Sr (200), ^{55}Mn (300), ^{24}Mg (100) ^{43}Ca (100), and ^{115}In (50). Elements were ratioed to ^{43}Ca and expressed in µmol/mol for ^{138}Ba and ^{55}Mn and in

mmol/mol for ^{24}Mg and ^{88}Sr. The analytical accuracy of elements based on replicate analyses of the NIST612 were was 100% for all isotopes.

Statistical analysis

A two-way ANOVA was used to examine the effects of temperature and salinity on juvenile King George whiting total length and weight among different treatments. We analysed the effects of temperature (16, 19, 22 and 25°C), salinity (30, 40ppt) and their interaction on otolith elemental composition using general linear mixed effects models (GLMM, lme4 function in R) (Pinheiro and Bates, 2000). Element:Ca ratios were log transformed to satisfy the model assumption for normal distribution of variances. Models were fitted using a stepwise forward procedure with the optimal model at each step selected based on lowest Akaike information criterion (AIC) (Burnham and Anderson, 2002) corrected for small sample size (AICc). Replicate tanks were treated as a random term and temperature and salinity as fixed terms. Linear regression analyses were used to determine the relationship between the elemental ratio in the rearing water and the elemental ratio in the juvenile otoliths.

Results

Rearing conditions

The rearing temperature and salinity were constant (temperature ± 0.05ºC and salinity ±0.06ppt) throughout the experiment, and generally conformed to the actual temperature and salinity of the rearing conditions (Table 2). Elemental ratios in rearing water were not manipulated, but showed some variation among treatments (Table 2). For example, Sr:Ca increased with salinity, whereas Ba:Ca decreased with salinity and Mg:Ca and Mn:Ca were

relatively constant. The length and weight of the fish at time of sacrifice did not vary among treatments ($p > 0.05$) (Table 3).

Table 2. Summary of the triplicate water samples within the treatment tanks (data are displayed as mean ± standard error, n=3).

		Trace elements							
Treatment	**Tank**	**Mn**	**Mn:Ca**	**Mg**	**Mg:Ca**	**Sr**	**Sr:Ca**	**Ba**	**Ba:Ca**
16ºC, 30‰	1	3.46±0.35	7.87 ± 0.57	1.44 ± 0.16	4.88 ± 0.03	5.23 ± 0.03	7.50 ± 0.25	13.00 ± 0.00	11.88 ± 0.36
	2	4.41±2.01	8.65 ± 4.30	1.29 ± 0.18	4.76 ± 0.07	5.50 ± 0.60	6.17 ± 0.15	11.66 ± 1.86	8.90 ± 2.23
19ºC,30‰	1	1.60±0.10	4.09 ± 0.60	1.31 ± 0.08	4.86 ± 0.07	5.20 ± 0.20	7.39 ± 0.23	15.00 ± 0.58	5.63 ± 0.21
	2	2.20±1.64	3.53 ± 0.13	1.24 ± 0.16	4.83 ± 0.08	6.00 ± 0.59	6.30 ± 0.18	12.93 ± 2.71	6.37 ± 1.31
22ºC, 30‰	1	3.03±0.47	3.69 ± 0.39	1.54 ± 0.07	4.85 ± 0.05	5.33 ± 0.19	7.45 ± 0.17	13.33 ± 0.67	13.73 ± 0.47
	2	3.63±0.38	3.81 ± 3.05	1.18 ± 0.11	4.76 ± 0.06	5.80 ± 0.21	6.20 ± 0.54	15.33 ± 0.33	9.03 ± 0.54
25ºC, 30‰	1	2.30±1.21	6.29 ± 3.90	1.16 ± 0.13	4.87 ± 0.05	5.23 ± 0.03	7.69 ± 0.08	13.66 ± 0.33	5.95 ± 0.18
	2	1.65±0.15	3.56 ± 2.55	1.28 ± 0.23	4.88 ± 0.04	5.40 ± 0.20	6.39 ± 0.59	14.00 ± 0.00	4.84 ± 0.59
16ºC, 40‰	1	2.43±0.45	6.37 ± 0.86	1.19 ± 0.08	4.84 ± 0.03	6.93 ± 0.12	7.08 ± 0.28	8.26 ± 0.07	11.43 ± 1.34
	2	2.20±0.23	7.37 ± 1.04	1.24 ± 0.13	4.95 ± 0.03	6.26 ± 0.58	7.31 ± 0.24	9.60 ± 1.20	12.36 ± 0.57
19ºC, 40‰	1	3.73±2.28	4.48 ± 1.36	1.03 ± 0.13	4.91 ± 0.04	7.33 ± 0.13	7.27 ± 0.13	8.90 ± 0.32	5.60 ± 0.02
	2	2.70±1.91	2.57 ± 0.38	1.21 ± 0.13	4.79 ± 0.08	7.26 ± 0.18	6.68 ± 0.64	8.60 ± 0.47	5.31 ± 0.62
22ºC, 40‰	1	2.73±0.82	5.12 ± 2.73	1.03 ± 0.11	4.88 ± 0.09	7.10 ± 0.15	7.50 ± 0.27	8.56 ± 0.15	12.48 ± 0.23
	2	1.80±0.25	3.08 ± 0.47	1.15 ± 0.12	4.74 ± 0.01	7.40 ± 0.26	6.26 ± 0.16	9.20 ± 0.55	10.39 ± 0.66
25ºC, 40‰	1	1.10±0.57	1.84 ± 0.99	1.14 ± 0.14	4.98 ± 0.05	7.33 ± 0.13	7.64 ± 0.23	8.83 ± 0.30	5.87 ± 0.16
	2	0.90±0.90	1.24 ± 1.24	1.10 ± 0.16	4.82 ± 0.06	7.20 ± 0.00	6.54 ± 0.32	9.00 ± 0.00	5.21 ± 0.26

Units: Ba, Mn (µg/l), Ba:Ca (µmol/mol), Mn:Ca (µmol/mol), Mg, Sr (mg/l), Mg:Ca (mmol/mol), Sr:Ca (mmol/mol).

Table 3. Results from the two-way ANOVA analysis on the effects of temperature and salinity on juvenile King George whiting total length and weight among different treatments. Data were pooled among replicate tanks. df, degrees of freedom; MS, mean square.

	df	MS	F ratio	p value
Total length (mm)				
Temperature	3	0.16	1.52	0.67
Salinity	1	0.04	0.15	0.69
Temperature×Salinity	3	0.42	1.36	0.26
Residual	57	0.31		
Weight (g)				
Temperature	3	0.03	2.94	0.04
Salinity	1	0.00	0.01	0.91
Temperature×Salinity	3	0.00	0.43	0.72
Residual	57	0.01		

Effect of temperature and salinity on otolith chemistry

Otolith element concentrations varied with salinity, but there was little effect of temperature on otolith elemental concentration (Fig. 3, Table 4). The most strongly supported model included salinity as a fixed term and replicate tanks as a random intercept (Table 4). However, for Ba:Ca, there was a similar level of support for the model that included temperature as a fixed term (ΔAICc 0.25). The addition of a temperature×salinity interaction did not improve any of the models (Table 4). A negative correlation with salinity was detected for Sr:Ca and Ba:Ca and a positive correlation was detected for Mn:Ca and Mg:Ca (Fig. 4, Table 5). Further, there was little variation in element:Ca ratios among the replicate tanks in each treatment group (Table 5).

Table 4. Results of models fitted to juvenile King George whiting otolith Mn:Ca, Mg:Ca, Sr:Ca and Ba:Ca. The optimal models (bold) were based on the lowest AICc value. Res.LL = log restricted likelihood estimation, K=number of parameters. Models included the tank term as a random intercept and temperature and salinity as fixed terms (for the models (×) indicates an interaction between salinity and temperature, whereas (+) indicates independent temperature and salinity terms).

Elements	***Models***	***K***	***AICc***	***ΔAICc***	***AICcWt***	***Res.LL***
Mn:Ca	**Salinity**	**4**	**138.83**	**0**	**0.78**	**-65.07**
	Temperature+Salinity	7	142.15	3.31	0.15	-63.06
	Temperature	6	143.68	4.85	0.07	-65.09
	Temperature×Salinity	10	147.84	9	0.01	-61.8
Mg:Ca	**Salinity**	**4**	**119.99**	**0**	**0.76**	**-55.65**
	Temperature+Salinity	7	122.39	2.40	0.23	-53.17
	Temperature	6	128.51	8.52	0.01	-57.5
	Temperature×Salinity	10	130.72	10.73	0	-53.25
Sr:Ca	**Salinity**	**4**	**-88.28**	**0**	**1**	**48.47**
	Temperature	6	-73.94	14.33	0	43.69
	Temperature+Salinity	7	-69.64	18.63	0	42.8
	Temperature×Salinity	10	-51.60	36.68	0	37.84
Ba:Ca	**Salinity**	**4**	**126.42**	**0**	**0.47**	**-58.88**
	Temperature	6	126.67	0.25	0.42	-56.61
	Temperature+Salinity	7	129.34	2.91	0.11	-56.69
	Temperature×Salinity	10	136.10	9.68	0	-56.02

Table 5. Parameter estimates and variance components (± standard deviation, (SD) and standard error (SE)) associated with fixed effects and random effects, respectively, for each of the optimal models. For all element:Ca data, the optimal models only included salinity.

Trace elements	***Random effects:***	***Variance***	***SD***	***t.value***
Mn:Ca	Tank	0.04	0.20	
	Residual	0.43	0.66	
	Fixed effects:	***Estimate***	***SE***	
	(Intercept)	0.04	0.19	0.22
	Salinity40	0.41	0.17	2.47
	Random effects:	***Variance***	***SD***	
Mg:Ca	Tank	0.06	0.25	
	Residual	0.31	0.56	
	Fixed effects:	***Estimate***	***SE***	
	(Intercept)	-2.86	0.20	-14.24
	Salinity40	0.46	0.14	3.24
	Random effects:	***Variance***	***SD***	
Sr:Ca	Tank	0.00	0.01	
	Residual	0.01	0.11	
	Fixed effects:	***Estimate***	***SE***	
	(Intercept)	0.99	0.02	49.03
	Salinity40	-0.05	0.03	-1.97
	Random effects:	***Variance***	***SD***	
Ba:Ca	Tank	0.00	0.00	
	Residual	2.56	1.60	
	Fixed effects:	***Estimate***	***SE***	
	(Intercept)	3.08	0.28	10.90
	Salinity40	-0.58	0.40	-1.47

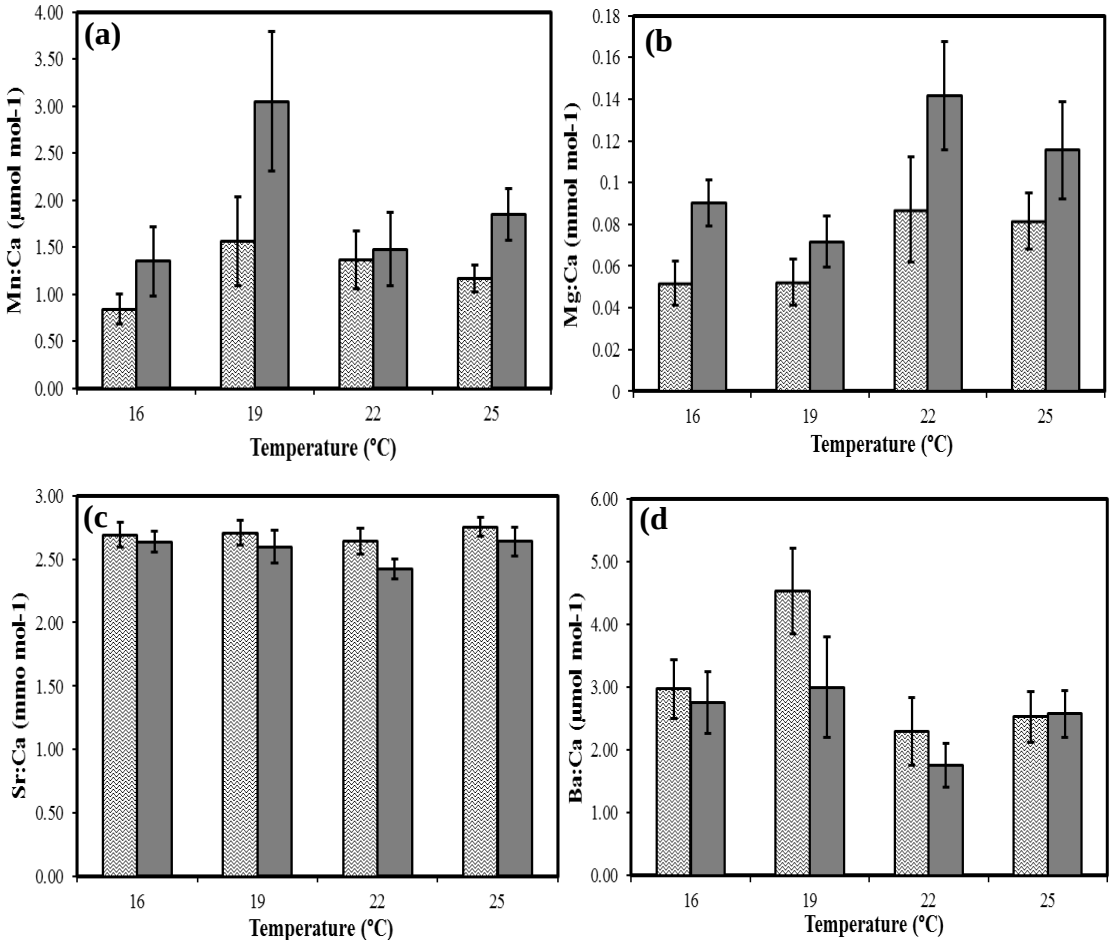

Fig. 3. Mean concentrations (±SE) of (a) Mn:Ca, (b) Mg:Ca, (c) Sr:Ca, and (d) Ba:Ca in otoliths of juvenile King George whiting (*Sillaginodes punctatus*) reared under experimental treatments of temperature and salinity, with replicate tanks pooled (n=8 individuals per treatment). Light grey bars 30ppt, dark grey bars 40ppt.

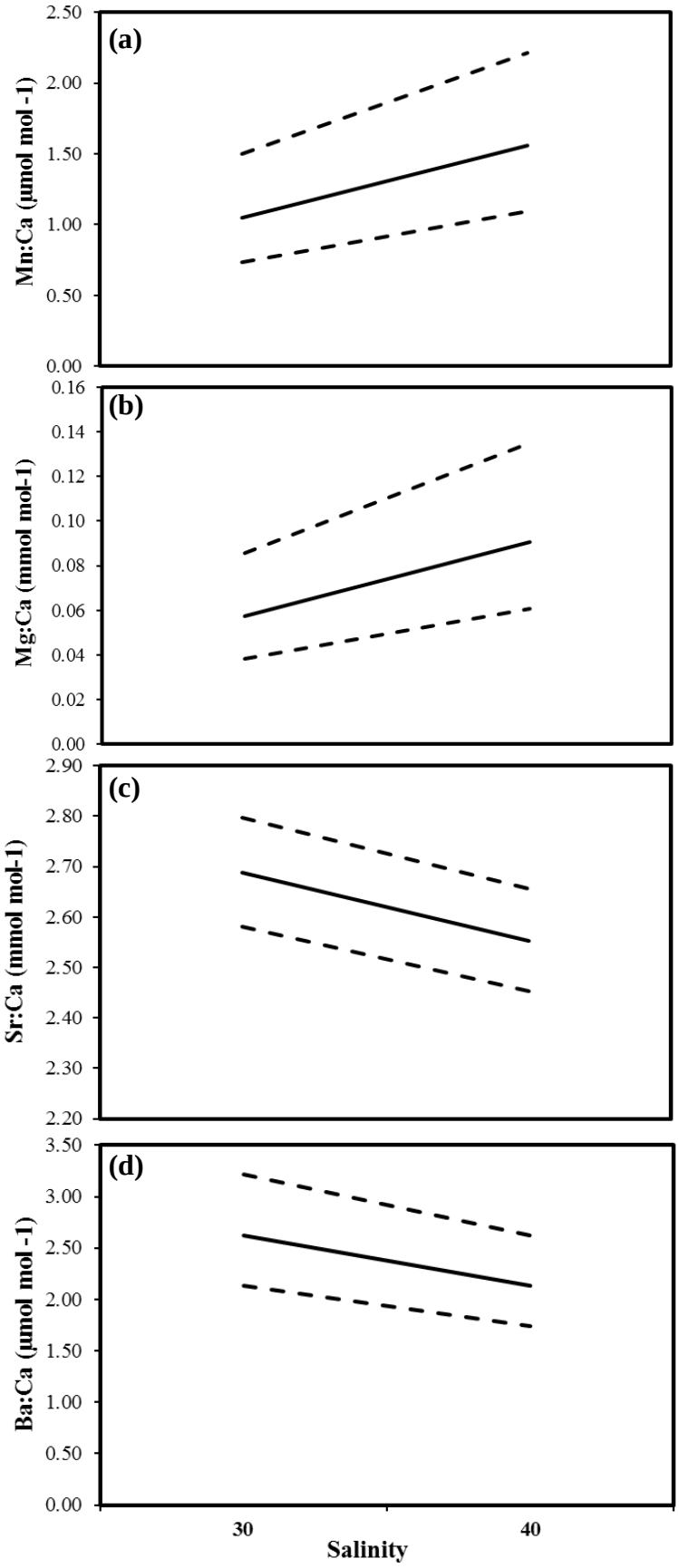

Fig. 4. Predicted effect (± 95% CI, dashed line) of salinity on (a) Mn:Ca, (b) Mg:Ca, (c) Sr:Ca, (d) Ba:Ca in otoliths of juvenile King George whiting based on the optimal model (see Table 4).

Relationship between water chemistry and otolith chemistry

There was no significant relationship between water elemental concentration and otolith elemental concentration for all elements ($p>0.01$) (Fig. 5, Table 6). Linear regression showed that water chemistry reflected 22%, 0%, 12%, and 10% of otolith Mn:Ca, Mg:Ca, Sr:Ca and Ba:Ca, respectively ($p>0.01$) (Table 6).

Table 6. Equations explaining the linear relationship between water chemistry and otolith chemistry of juvenile King George whiting. Also shown is the goodness of fit (R^2) and significance of the relationship (p value).

Element	**Equation**	**R^2**	**p value**
Mn:Ca (µmol mol^{-1})	Y = -0.10x + 1.91	0.22	0.06
Mg:Ca (mmol mol^{-1})	Y = -0.05x + 0.33	0.00	0.70
Sr:Ca (mmol mol^{-1})	Y = -0.08x + 3.21	0.12	0.10
Ba:Ca (µmol mol^{-1})	Y = 0.10x + 1.92	0.10	0.20

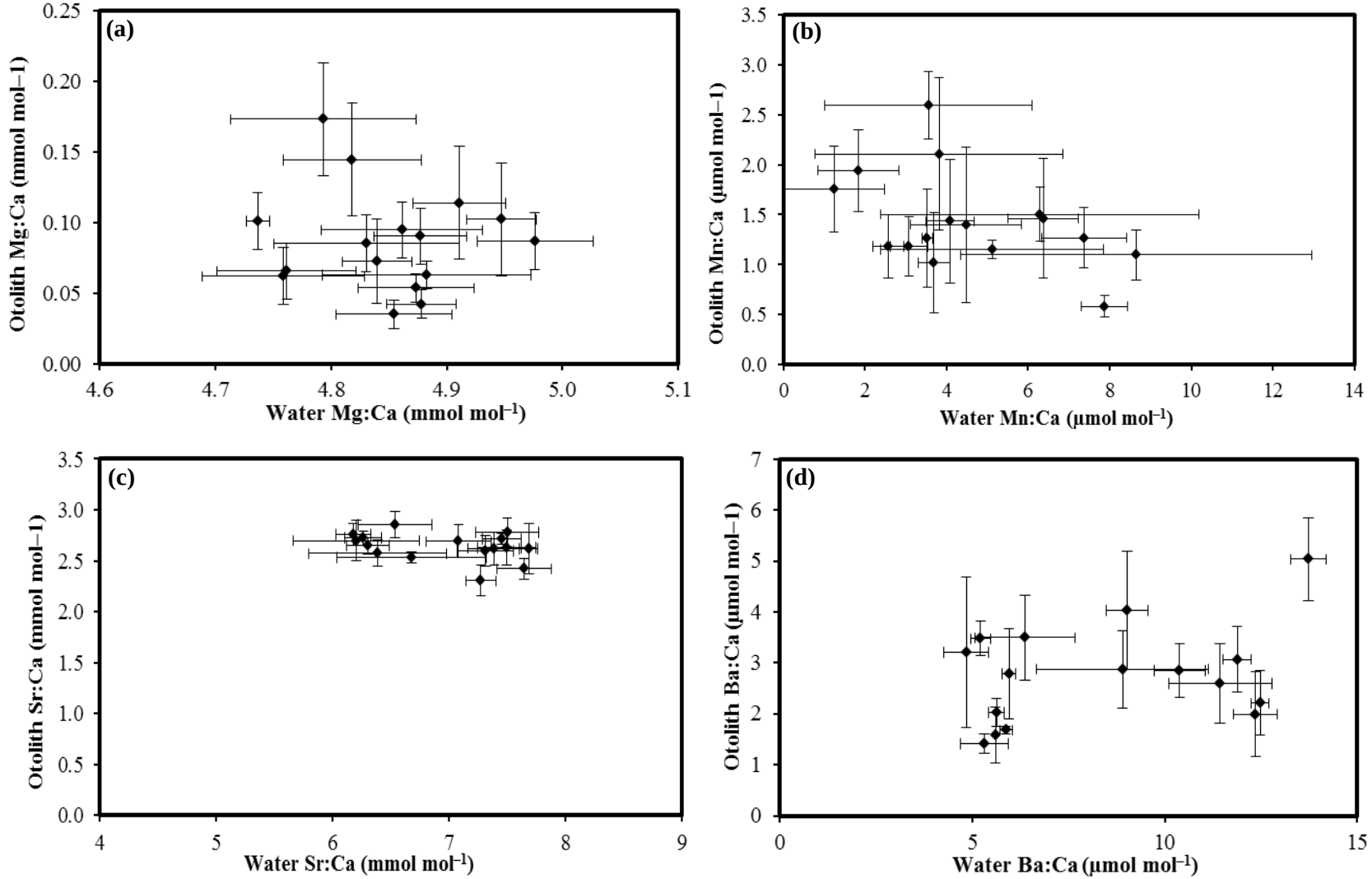

Fig. 5. Mean (±SE) otolith concentration of (a) Mg:Ca, (b) Mn:Ca, (c) Sr:Ca and (d) Ba:Ca plotted against mean (±SE) water elemental concentrations.

Discussion

Understanding how otolith elemental composition relates to environmental variability is essential to reconstruct the environmental histories of fish using otolith chemistry. The present study investigated the effects of temperature and salinity on otolith chemistry of juvenile King George whiting. Our findings showed that temperature had a minor influence on otolith chemistry relative to salinity. This suggests that otolith chemistry of King George whiting can provide useful information about the salinity history of the species.

Salinity effect

Otolith elemental chemistry displayed significant positive (Mn:Ca and Mg:Ca) and negative (Sr:Ca and Ba:Ca) relationships with salinity. Some previous studies have used Sr to track the movement of species across environments with salinity gradients (e.g. anadromous species) (Sturrock et al., 2012; Panfili et al., 2012). Likewise, Ba has been suggested as a tracer for salinity and generally shows negative relationships with ambient salinity (Dorval et al., 2007), which endorses our current findings (Table 7).

Although previous studies have suggested that there is a strong relationship between Sr:Ca and Ba:Ca and water salinity (Panfili et al., 2015; Dorval et al., 2007), individual species behave differently and incorporate elements into their otoliths in different ways. The negative effect of salinity on Sr:Ca and Ba:Ca in juvenile King George whiting might be due to osmoregulation and crystallisation processes (Elsdon and Gillanders, 2002; Campana, 1999). Among the key physiological barriers to elemental uptake (e.g. surrounding water, blood plasma and gills), the gills are assumed to be the biggest barriers (Campana, 1999). In this case, salinity interferes with Sr uptake from the gills and consequently its substitution with Ca in the otolith (Campana, 1999). In marine species the osmoregulation process is

energetically costly and this may regulate the amount of elemental uptake into the otolith via the gill's chloride cells (Ouattara et al., 2009). Chloride cells facilitate ion exchange between the blood plasma and the surrounding water (Evans et al., 2005). Their function in filtering Sr in the blood plasma varies in different species and is sensitive to osmoregulation (Sturrock et al., 2014). Thus, in hypersaline environments the chloride cells cannot filter elements in the blood cells and thereby the elements are blocked at the gill interface and are unlikely to be incorporated into the otolith (Panfili, 2015). This phenomenon might partially explain the lower Sr:Ca and Ba:Ca concentration at higher salinities (40ppt) in juvenile King George whiting otoliths. However, some studies have suggested that the concentration of some elements (e.g. Sr:Ca) might be controlled by hormones that regulate the growth and osmoregulation (Boeuf and Payan, 2001).

Most previous studies found no relationship between Mg:Ca and Mn:Ca and salinity (Gillanders and Munro, 2012; Martin and Wuenschel, 2006; Elsdon and Gillanders, 2002). However, Fowler et al. (1995) have found this relationship as negative. In our study, there was a positive correlation between Mn:Ca and Mg:Ca and salinity, which suggests that these elements reflect environmental salinity experienced by this species. The increased number of chloride cells at higher temperatures and salinities lead to increased metabolic rate of the fish (see Chapter 3, 4). The increased chloride cells facilitate ion exchange between the blood plasma and the surrounding water (Evans et al., 2005) and can explain the higher Mn:Ca and Mg:Ca at higher salinity (40ppt). However, differing results for different studies implies that the Mg:Ca and Mn:Ca relationship with salinity is likely species specific and might also be under physiological regulation (Walther et al., 2010). Factors such as growth might also regulate the Mg:Ca and Mn:Ca concentration in the otoliths (Martin and Thorrold, 2005). However in our study, fish size did not vary among treatments so growth was unlikely to influence results (see Table 3).

Table 7. A summary table showing the effects of temperature and salinity on otolith chemistry of marine/estuarine fish species from previously published literature.

Element: Ca	Relationship	Temperature	Salinity	Temperature×Salinity
Mg	Positive	Stanley et al., 2015, Barnes et al., 2013	Stanley et al., 2015, Rooker et al., 2004	Martin and Wuenschel, 2006
	Negative	Miller, 2009		
	No relation	DiMaria et al., 2010, Elsdon and Gillanders, 2002, Martin and Wuenschel, 2006	Gillanders and Munro, 2012, Elsdon and Gillanders, 2002; Martin and Wuenschel, 2006	
Mn	Positive	Stanley et al., 2015, 2009, Barnes et al., 2013	Rooker et al., 2004, Dorval et al., 2007; Forrester, 2005	Stanley et al., 2015, Martin and Wuenschel, 2006
	Negative	Miller, 2009, Barnes et al., 2013		
	No relation	Miller, 2009, DiMaria et al., 2010, Martin et al., 2004, Martin and Wuenschel, 2006	Gillanders and Munro, 2012, Elsdon and Gillanders, 2002; Martin and Wuenschel, 2006, Miller, 2009	
Sr	Positive	Bath et al., 2000, Martin and Wuenschel, 2006, Reis-Santos et al., 2013, Miller, 2009	Stanley et al., 2015, Panfili et al., 2012, Sturrock et al., 2012	Elsdon and Gillanders, 2002, Martin and Wuenschel, 2006, Barnes et al., 2013
	Negative	Radtke et al., 1990, DiMaria et al., 2010, Townsend et al., 1995	Campana, 1999, Elsdon and Gillanders, 2002	
	No relation	Chesney et al., 1998, Gallahar and Kingsford, 1996, Martin and Thorrold, 2005	Gillanders and Munro, 2012, Elsdon and Gillanders, 2005	
Ba	Positive	Miller, 2009, Reis-Santos et al., 2013, Martin and Wuenschel, 2006	Panfili et al., 2015, Dorval et al., 2007	Stanley et al., 2015, Elsdon and Gillanders, 2002, Martin and Wuenschel, 2006, Barnes et al., 2013
	Negative	DiMaria et al., 2010, Townsend et al., 1995	Stanley et al., 2015, Gillanders and Munro, 2012, Dorval et al., 2007, Reis-Santos et al., 2013, Elsdon and Gillanders, 2005	
	No relation	Martin and Thorrold, 2005, Gallahar and Kingsford, 1996		

Temperature effect

Relative to salinity, temperature had little effect on otolith chemistry, except for Ba:Ca (ΔAIC< 2, Table 4). Previous studies have shown mixed relationships between element concentration and temperature (see Table 7). Some studies have reported that the effect of temperature on otolith Ba:Ca and Sr:Ca is positive [e.g. black rockfish (*Sebastes melanops*) (Miller, 2009); European sea bass (*Dicentrarchus labrax*) (Reis-Santos et al., 2013); grey snapper (*Lutjanus griseus*) (Martin and Wuenschel, 2006)], whilst some found it was negative [e.g. Pacific cod (*Gadus microcephalus*) (DiMaria et al., 2010); Atlantic cod (*Gadus morhua*) (Townsend et al., 1995)]. Some others did not find any relationship [e.g. juvenile spot (*Leiostomus xanthurus*) (Martin and Thorrold, 2005) and rock black fish (*Girella elevata*) (Gallahar and Kingsford, 1996)], which was similar to our findings.

The non-significant relationships between temperature and Mg:Ca and Mn:Ca in otoliths of different species [e.g. Pacific cod (*Gadus microcephalus*) (DiMaria et al., 2010), spot (*Leiostomus xanthurus*) and juvenile grey snapper (*Lutjanus griseus*) (Martin and Wuenschel, 2006)] were in agreement with our findings. However, both negative and positive effects of temperature on Mg:Ca and Mn:Ca have been observed in other studies [e.g. black rockfish (*Sebastes melanops*) (Miller, 2009) and mulloway (*Argyrosomus japonicus*) (Barnes et al., 2013)].

The varying results indicate that the relationship between otolith chemistry and environmental conditions is complex. In addition, otolith chemistry relationship with temperature and salinity is different in different species, families and life histories. Several reasons may explain this complexity. Firstly, elements such as Mg, Mn, Ba and Sr in both seawater and blood, represent complexes that are hydrated ions (Sturrock et al., 2012). The concentration of hydrated ions in seawater is relatively constant and mostly changes with a change in salinity (Sturrock et al., 2012). Secondly, physiological processes (e.g. growth and

metabolism) might indirectly affect otolith chemistry (Walther et al., 2010). The kinetic growth effect is associated with the calcification process in the otolith, which facilitates the elemental precipitation and substitution with calcium (Sinclair, 2005). Further, protein synthesis during somatic growth can affect the protein composition of endolymphatic fluid (Kalish, 1991). Changes in chemical composition of the endolymphatic fluid can ultimately affect the ion uptake into the otolith and alter the elemental concentration (Kalish, 1991). For example, several studies have indicated a negative relationship between growth rate and otolith Sr:Ca and Ba:Ca (Miller et al., 2010; Walther et al., 2010). Finally, changes in otolith elemental concentration can likely be attributed to the biology of the species (i.e. thermal tolerance) and the selected temperatures (Elsdon and Gillanders, 2003). Different relationships between temperature and elemental concentration in otoliths is possibly due to not using broad ranges of experimental rearing temperatures (Elsdon and Gillanders, 2002).

Relationship between water chemistry and otolith chemistry

In the present study no significant correlation was found between otolith chemistry and water chemistry. This relationship has been found to be mixed in previous studies. For example, the relationship between otolith Sr:Ca and Ba:Ca and water chemistry can be linear (Macdonald and Crook, 2010; Martin and Wuenschel, 2006), exponential (Dorval et al., 2007) or show no relationship (Elsdon and Gillanders, 2005). Further, the relationship between otolith Mg:Ca and Mn:Ca and rearing water has also been found to be positive [e.g. juvenile mudsuckers (*Gillichthys mirabilis*) and spotted seatrout (*Cynoscion nebulosus*) (Dorval et al., 2007; Forrester, 2005)] or not significant [e.g. for juvenile black rockfish (S*ebastes melanops*) (Miller, 2009) and juvenile grey snapper (*Lutjanus griseus*) (Martin and Wuenschel, 2006)]. Different results indicate that most of the elemental uptake occurs during the otolith crystallisation process (Dorval et al., 2005). Additionally, the otolith elemental uptake from

the surrounding water is species-specific (Melancon et al., 2009) and can be influenced by other factors such as ontogeny (de Pontual et al., 2003), physiology (Kalish, 1991), diet (Kennedy et al., 2000) and genetics (Barnes et al., 2013; Clarke et al., 2011).

Conclusion

The results from this study suggest that salinity can be used as a potential marker for estimating the movement of juvenile King George whiting from shallow coastal areas to their spawning grounds in deeper water. Further, a weak temperature influence on otolith chemistry suggests that partitioning of elements in this species is fairly constant over the 9°C temperature range examined. Thus, the temperature history of fish could not be reconstructed from elemental chemistry data. However, other elements (Mn, Mg and Ba) might be mostly regulated by the physiology of the fish relative to temperature. Evaluating the effects of salinity on otolith chemistry of King George whiting will enable us to assign fish to specific habitats and reconstruct their salinity history. These outcomes are suitable for guiding the collection and interpretation of data about salinity change effects on temperate fish species as well as managing the fish stocks.

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Chapter 6: General Discussion

Amir and I (first trial) were collecting juvenile King George whiting form Barker Inlet, Adelaide (Photo credit: Nastaran Mazloumi and Amir Forghani)

General Discussion

Environmental variability influences aquatic organism biology and physiology (Lee et al., 2003). Throughout this thesis, different approaches were applied to examine the influences of environmental variability on growth, swimming performance, metabolic rate and otolith chemistry of King George whiting (*Sillaginodes punctatus*). Chapter 2 revealed that increasing winter sea surface temperature (SST) was associated with a decrease in otolith growth (a proxy of somatic growth). However, overall temperature and collection region had no significant influence on growth. Likewise, growth did not vary with recruitment or El-Niño Southern oscillation events. In chapters 3 and 4, the influence of temperature and salinity on aerobic metabolic rate and swimming performance of adult and juvenile King George whiting were assessed. In chapter 3, I examined the critical swimming performance (U_{crit}) and aerobic metabolic rate of the adult fish, as well as their potential to recover after a prolonged swimming performance at two temperatures (16°C and 26°C), using a swim chamber and resting chamber. Results indicated that the adult fish swum faster and consumed more oxygen at the higher temperature. They also recovered quicker in warmer water. In chapter 4, I measured the effect of a range of temperatures (16, 19, 22 and 25ºC) and two levels of salinity (30 and 40ppt) on metabolic rate and U_{crit} of the juvenile fish using a swim chamber. A decrease in salinity led to an increase in U_{crit} and decrease in SMR of the fish respectively, whereas it did not affect MMR and aerobic scope. Temperature affected SMR, MMR and aerobic scope, but had a minor influence on U_{crit}. The optimal temperature for aerobic performance was similar to the juvenile's collection site. The effects of temperature and salinity (the same treatments as mentioned above) on otolith chemistry of the juvenile fish were examined in chapter 5. Outcomes revealed that, salinity had a more significant influence on the otolith chemistry of juvenile King George whiting relative to temperature.

Climate-growth relationships

Southern Australia is experiencing rapid climate change which affects the aquatic ecosystem productivity, species distribution and recruitment and their population connectivity (Lake, 2003). Temperate marine fish are particularly vulnerable to climate change (Last et al., 2011). The oxygen solubility in water decreases with increasing salinity and temperature. The lower oxygen content in water leads to an increase in metabolic demand at increasing temperatures and salinities. The growth responses of marine fish to temperature change are species specific (Morrongiello and Thresher, 2015). In chapter 2, a negative relationship between the winter SST and inter-annual growth variation in King George whiting contrasted with the positive growth-temperature relationship observed for other fish species from a range of environments (Morrongiello and Thresher, 2015; Coulson et al., 2014; Pörtner et al., 2001). However, temperature increase can benefit growth up to some optimal temperature after which further warming or cooling can cause a decline in growth (Pörtner and Farrell, 2008). The thermal tolerance of adult and juvenile King George whiting is reported to be from 18ºC to 28ºC and 18ºC to 30ºC respectively (Meakin et al. 2014). Hence, it can be concluded that a negative growth relationship with winter SST in King George whiting was because the winter SST was near the lower thermal limit of the species.

The recruitment term for commercial and recreational fish species refers to when the fish reaches a suitable size for exploitation (Sponaugle, 2010). Survival of larvae and their chance of successful recruitment to juvenile and adult habitats are related to water temperature (Houde, 1989). There are many factors that can affect the growth and recruitment success, such as the sea water temperature and ocean currents (Sponaugle, 2010). However, good growth of larvae does not always lead to high recruitment (Muhling et al., 2008). The recruitment of fish in Spencer Gulf and Gulf St Vincent has declined since 2000 to the present time (Fowler et al., 2014). Recruitment decline in the species natal habitat over the

recent years can be associated with the heavy exploitation and disruption in spawning as well as climate change (Fowler et al., 2014). No significant relationship between recruitment and growth rate of the King George whiting was due to their juvenile potential tolerance to temperature change and consequently successful recruitment to adults. Significant tolerance to temperature change in juvenile fish (<3 y) may relate to increased production of heat shock protein (HSP) in response to temperature shock (Meakin et al., 2014).

The SOI is reflective of ENSO events and is calculated based on the pressure difference between Tahiti and Darwin (Power and Kociuba, 2011) and has a complex relationship with climate change (Morrongiello et al., 2011). The complex relationship between SOI and other large scale climate phenomenon such as the Pacific Decadal Oscillation and Indian Ocean Dipole can also affect the growth of the species (McGowan et al., 2009). Additional, previous studies have found a link between the SOI and precipitation anomalies, SST and drought in Southern Australia (Pui et al., 2012). It has been shown that El-Niño events cause warmer SST in late summer and winter and La-Niña does not interfere significantly with SST anomalies, but it has been predicted that temperature will increase during ENSO events by up to ~1°C and >2°C by 2030 and 2100 respectively (Holbrook et al., 2009).

Metabolic rate

Temperature and salinity may exert stress on fish and affect their physiology and distribution (Evans and Claiborne, 2006). Organisms respond to the induced stresses by regulating their metabolic rate and locomotion (Pörtner, 2010). In chapter 3, adult King George whiting aerobic metabolic rate increased with increasing temperature from 16 to 26ºC. In contrast, the juvenile fish metabolic rate increased with increasing temperature up to 22 ºC and then decreased with further warming up to 25ºC. Adult fish could swim faster in warmer water,

but juvenile fish U_{crit} were not affected by temperature and responded, rather, to salinity. Juvenile fish swam faster at lower salinity (30ppt) and also had a lower SMR value at lower salinity. The increased metabolic rate and swimming performance of adult King George whiting in response to elevated temperature was related to variation in oxygen solubility in different water temperatures (Verberk et al., 2011). The solubility of oxygen decreases with increasing temperature of the water (Verberk et al., 2011). Therefore, the fish oxygen demand and SMR increases in response to elevated temperature (Farrell, 2011).

In chapter 4, juvenile King George whiting had a temperature tolerance window from 16ºC to 25ºC. The juvenile's aerobic scope for activity and SMR illustrated that the optimized oxygen supply to tissues was between low (16ºC) and high pejus temperatures (22ºC) (T_p: pejus temperature, pejus=getting worse) with the optimum of aerobic scope close to the upper pejus temperature. The MMR was between 19 and 22ºC which was just below the upper pejus temperature (25ºC). Higher metabolic rate (both SMR and MMR) and low oxygen content at higher temperatures cause a mismatch between the oxygen supply and demand in marine fish. A mismatch between oxygen supply and demand is the first sign of thermal tolerance limitation which leads to reduced aerobic scope at extreme temperatures (Pörtner, 2010). Insufficient oxygen supply to tissues at both sides of the thermal window can also be associated with the species cardiovascular capacity (Evans and Claiborne, 2006). The cardiovascular system cannot keep pace with the increased oxygen demand at elevated temperatures. Hence the oxygen would not be sufficiently supplied to tissues which ultimately cause loss of performance or anaerobic metabolism (Pörtner and Peck, 2010). However, the selected temperatures were matched to the King George whiting temperature tolerance window, but further increase or decrease in temperatures out of their tolerance limit would cause a loss of performance or anaerobic metabolism.

A relationship between species body size and their thermal tolerance window has previously been shown (Pörtner et al., 2008; Björnsson et al., 2001). For example, juvenile fish often have a wider thermal window compared with adults (Pörtner et al., 2008). In addition, metabolic rate may also be related to body size and is higher at smaller body size (Palstra and Planas, 2012). This is in agreement with our results which showed higher metabolic rate (SMR, MMR and aerobic scope) in the juvenile fish compared with adults.

Swimming performance

Temperature is one of the most important environmental factors affecting U_{crit} of the fish. In chapter 3, U_{crit} of the adult King George whiting increased with increasing temperature from 16 to 26ºC. The increased swimming speed at the warmer temperature suggests that rising temperature may increase the energetic cost of swimming (Hein and Keirsted, 2011). In addition, whilst the fish reaches speeds close to the U_{crit}, the level of stress hormones such as cortisol and catecholamine increase in their blood plasma (Farrell, 2011). Further, activity of the lipoprotein lipase in red muscles increases at critical swimming speeds to provide enough lipids (preferred fuel for locomotion) to the muscles to fuel exercise (Ozorio et al., 2010). However the lipid metabolism process in red muscle is species related (Palstra and Planas, 2012) and was not examined in the current thesis, but deserves further investigation.

A number of factors can affect U_{crit}, such as developmental stage, body size, methodology, temperature and salinity (Plaut, 2000b; Plaut, 2000a; Taylor et al., 1996; Nicoletto, 1991). The adult King George whiting had higher U_{crit} compared with juvenile fish (chapters 3 and 4), which was likely related to body size. A lower U_{crit} value in juvenile fish is a reflection of their small size and their early developmental stage compared with adults.

A lack of a strong relationship between the temperature and the juvenile U_{crit} in chapter 4 can be explained by some factors. Firstly, the temperature treatments might not be broad enough to show any significant changes in swimming behavior. Secondly, the selected temperatures were close to the fish natural habitat temperature. Finally, the King George whiting migrate from the shallow nursery areas to a deep sea water for spawning purposes (Fowler et al., 2014) and experience different temperature and salinity gradients which enables them to compensate for potential osmotic challenges in their habitat. It has been shown that the osmoregulatory abilities to overcome the hypo-saline situation are species related (Whitehead et al., 2013). The juvenile King George whiting consumed less energy for osmoregulation at lower salinity (30ppt) and thereby could swim faster at this salinity. This also can explain why the juvenile fish had lower SMR at lower salinity (chapter 4). Additionally, intermediate to lower salinities of seawater reduce the SMR and increase the food conversion efficiency which ultimately allocates more energy for the fish to swim (Boeuf and Payan, 2001; Lambert et al., 1994).

When species are exposed to hypo-osmotic environments several physiological reasons can possibly describe their U_{crit} variation including, 1) the sodium, chloride, potassium and osmolality all decrease in less saline water and thereby the fish allocate more energy for swimming (Whitehead et al., 2013), 2) Change in plasma ion concentration in response to salinity decrease in order to assist the fish in maintaining acid-based balance in hypo-osmotic environment (Claiborne et al., 1994) and 3) A change in blood plasma cortisol and catecholamine due to the induced stress of transferring to a low saline water cause the fish to swim faster (Farrell, 2011). Overall, the ability of the juvenile fish to tolerate a wide range of temperatures and salinities can help them to survive and grow in their nursery grounds and successfully swim to spawning areas.

Otolith chemistry in response to temperature and salinity

In chapter 5, I examined the effects of temperature and salinity (the same ranges as chapter 4) on elemental concentration in otoliths of juvenile King George whiting. Otoliths have been used as a tool to study the environmental histories of fish (Elsdon et al., 2008). The otolith chemical composition can be regulated by both environmental parameters (e.g. temperature, salinity and water chemistry) and physiology (Elsdon et al., 2008). The outcomes from chapter 5 revealed that salinity was the major factor influencing the otolith chemistry of juvenile King George whiting. Significant positive (Mn:Ca and Mg:Ca) and negative (Sr:Ca and Ba:Ca) relationships between otolith chemistry and salinity were found. The otolith chemistry of juvenile King George whiting was not influenced by temperature possibly due to physiological processes (Campana, 1999), crystallography (Nielsen and Christoffersen, 1982) and genetics (Halden et al., 2000). Physiological processes such as growth and metabolism can affect otolith elemental composition (Martin and Thorrold, 2005). In our study, fish size did not vary among treatments so growth was unlikely to influence results. However, the SMR of the juvenile fish was lower at low salinity (30ppt) (chapter 4). This suggests that low basal metabolic rate at low salinity might have affected the incorporation of elements in the aragonite matrix of the otolith. Further, the elemental uptake from the fish gills increases with increasing metabolic rate (Yang et al., 2000). The increased number of chloride cells in response to higher salinity has likely resulted in increased SMR of the fish which facilitates ion exchange between the blood plasma and the surrounding water. This might indicate why Mg:Ca and Mn:Ca concentrations were higher at a higher salinity. The negative relationship with Ba:Ca and Sr:Ca and salinity increase could be related to the chloride cells ability to filter these elements into the blood plasma and might not be regulated by metabolic rate.

Inclusion of parameters (e.g. salinity and temperature) within the natural environment of the species would enable us to determine a realistic reconstruction of fish environmental history. In the present study, however, the salinity gradient is likely to be unrealistic since juvenile King George whiting might not experience very low salinities (30ppt) in their natural habitat. Moreover, including other indicators such as otolith $\delta^{13}C$ and $\delta^{18}O$ (Dorval et al., 2011) might assist in better interpretation of the fish life history.

Future research directions

The present thesis has assessed the effects of environmental parameters on long-term growth trends, otolith chemistry, aerobic metabolic rate and swimming performance of King George whiting. However, there still remains a wealth of knowledge to be gained from studying King George whiting life history and physiology. In particular future research should focus on:

1) Assessing several additional environmental factors that might affect the growth of King George whiting. For example, the influence of salinity on growth, as well as the interaction between temperature and salinity warrants further attention. At present there are no long term salinity data, but through the integrated marine observing system (IMOS) data portal this is likely to change.
2) Additional sampling from other locations in Australia, such as Victoria and Western Australian, may also be beneficial. Although my growth chronology research found region was not an important variable, collecting fish from a range of latitudinal gradients could give an idea whether fish from different locations have different thermal tolerance windows.
3) Performing experiments based on a wider temperature and salinity range than what was tested in the current thesis which would potentially be outside the tolerance limit

for the species. This would allow the critical temperature and salinity in which they start to perform anaerobically to be estimated.

4) Examining the effects of wide ranges of salinity on U_{crit} followed by measuring the excess post-exercise oxygen cost (EPOC) to determine if the fish use anaerobic oxygen cost for exercise.
5) Swimming performance is fueled by energy derived from carbohydrates, lipids and proteins from the diet. Sustained swimming can enhance energy utilization and consequently improve growth. Hence, examining the effects of diet on swimming activity of the King George whiting may imply important advantages for the possible future fish farming industry.
6) Further investigation is still needed to assess the King George whiting osmoregulation response to acute and chronic exposure to salinity gradients.
7) Further research can be done using otolith stable isotopes (e.g. $\delta^{13}C$ and $\delta^{18}O$) to potentially reconstruct metabolic histories.

Conclusion

Throughout this thesis different approaches were used to investigate King George whiting physiology and biology under a changing environment. The otolith chronology study provided evidence that current temperature has not yet significantly affected the growth of the King George whiting for the period covered by the otolith chronology study. However, longer term temperature changes (e.g. over 50-100 years) may have altered growth of the fish. The otolith chemistry analysis indicated that otolith elemental concentration was not affected by the current temperature. The elevated metabolic rate (both SMR and MMR) and higher aerobic scope at warmer water for the King George whiting suggested that increased water temperature may increase the survivorship of the fish up to an optimal temperature

range. However they were quite tolerant across a range of temperatures. The juvenile fish had an optimal aerobic metabolism between 16 and 19ºC. This indicates that the current temperature is within the juvenile King George whiting thermal tolerance window. The salinity influence on otolith chemistry of the juvenile fish also highlights the fact that the juveniles are experiencing a range of salinity gradients during their estuarine life which affects the otolith elemental composition. These outcomes are suitable for guiding the collection and interpretation of data about temperature and salinity effects on temperate fish species. Further, the survivorship and physiological responses of a King George whiting to a changing environment (e.g. metabolic demand and swimming behaviour) can potentially help to assess the sustainability of this species in the face of climate change. This information will deliver greater understanding into the complex relationships between the environment and the biology/physiology of a key commercial fish species in southern Australia. Through assessing the vulnerability of the key fish species to climate change, research strategies and management policies will be developed to deal with potential climate change effects on fish stocks. Outcomes from the present study can be broadened to other ectoderms for further understanding the impacts of climate change on physiology and biology of the aquatic organisms.

Every finish line is the beginning of a whole new race ☺