Ecology and physiology of forage fish species in the Murray Estuary and Coorong, South Australia

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Summary

Estuaries and coastal lagoons are the dynamic transition between marine, freshwater and terrestrial environments. As a result of drought and low freshwater input in the last decade the Murray Estuary and Coorong have become the largest degraded hyper-saline lagoon in Australia. This thesis investigates the changes in life history and ecology of three key forage fish species associated with environmental deterioration in the Coorong. Four studies were performed to investigate (1) the variation in fish assemblage structure, (2) fish growth performance, (3) fish feeding ecology and diet selection, and (4) physiological response of forage fish to changes in salinity under laboratory conditions. In study 1, the forage fish assemblages were characterised by greater abundance and dominance of small-mouthed hardyhead (*Atherinosoma microstoma*) in the South Lagoon; low abundance of sandy sprat (*Hyperlophus vittatus*) and Tamar goby (*Afurcagobius tamarensis*) in the North Lagoon, but complete absence of sandy sprat and Tamar goby in the South Lagoon. The spatial variation in distribution of forage fish is attributed to elevated salinity levels (Murray Estuary 2–30; North Lagoon 11–75 and South Lagoon 40–85) in the Coorong. This study indicates that the change of forage fish assemblage is mainly driven by salinity variation in the Coorong. In study 2, the estimated growth rates were 0.019 cm day^{-1} for small-mouthed hardyhead, 0.038 cm day^{-1} for Tamar goby and 0. 016 cm day^{-1} for sandy sprat. The length-weight relationship showed the variation of regression slope in small-mouthed hardyhead (b = 2.96), Tamar goby (b = 3.06) and sandy sprat (b = 3.1). Growth performance was mainly influenced by chlorophyll *a*, water transparency and salinity but to less extent by other environmental factors. This study indicates that environmental factors can significantly impact growth parameters of forage fish. Study 3 addresses food selectivity among forage fishes and reveals the dominance of crustaceans (amphipods, ostracods and harpacticoids) and the presence of nematodes and acanthocephalans in the gut of all forage fishes. Sandy sprat and Tamar goby showed high dietary overlap in the Murray Estuary while the diets of all three forage fishes were potentially overlapped in the North Lagoon. Prey abundance was temporally variable and predominantly regulated by salinity, pH, dissolved oxygen, water transparency and chlorophyll *a* in the Coorong. Study 4 detects the induction of stress as measured by reactive oxygen species using a range of salinity gradients overtime in forage fish. Salinity was found to affect superoxide dismutase activity in Tamar goby but not in small-mouthed hardyhead. Conversely, salinity altered catalase activity in small-mouthed hardyhead but not in Tamar goby. The study reveals that salinity stress occurs in Tamar goby but not in small-mouthed hardyhead. The current study indicates that the antioxidant response to stress varies with fish species. The overall findings of this thesis add new knowledge to improve our understanding of the impact of environmental variation on the population distribution, feeding ecology, growth performance and adaptation to physiological stress of small-bodied forage fish in the Coorong. This thesis contributes to the strategy of ecosystem management in an estuarine system of gradual environmental deterioration due to low freshwater input and protracted drought in southern Australia.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Md Afzal Hossain
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Chapter 1

General Introduction

1.1 Estuaries

Estuaries are the dynamic interface and complex transition between marine, freshwater and terrestrial environments (Mann 1982; Whitfield 1988). An estuary is defined as a semi-enclosed coastal body of water, with or without a free connection to the open sea, where sea water is diluted by freshwater from land drainage (Pritchard 1967). Typically, estuarine habitats are highly productive due to a continuous mixing of saltwater and a freshwater flow from land and sea (Gillanders *et al.* 2011), providing breeding, feeding and nursing habitats for a wide range of resident and migratory fauna from marine and fresh ecosystems (Whitfield 1988).

The ecological function of estuaries is mainly regulated by environmental and hydrological variability (Abrantes and Sheaves 2010). For example, shallow estuarine habitats are influenced by tidal action, freshwater inflow, water and air temperature, wind, wave action and rainfall (James *et al.* 2013). The seasonal and annual variations in those environmental variables cause frequent changes in primary and secondary productivity (Kennish *et al.* 2008). In particular, freshwater inflow transports sediments and nutrients to estuaries, which enhance phytoplankton and zooplankton production, thereby increasing biological and ecological productivity of estuaries (Sun *et al.* 2015). This high biological productivity attracts a numerous species of fish and birds of commercial, recreational and ecological significance to the estuarine system (Simier *et al.* 2004).

Despite their ecological and commercial significance, most estuaries around the world suffer natural and anthropogenic stresses (Blaber 2002; Edgar *et al.* 2000), including climate change, hydrological variation, river regulation and excessive sediment input, resulting in habitat loss (Gillanders *et al.* 2011). Salinity changes associated with low freshwater flow, due to drought and increasing human demand on scarce water resources, can heavily impact estuarine wetlands (Abrantes and Sheaves 2010; Michener *et al.* 1997). The resultant hydrological variation and salinity fluctuation deleteriously influences abundance, distribution and physiological tolerance of biotic communities in estuaries (Kennish 2002; Lester and Fairweather 2009). Hyper-salinity due to low freshwater flow, and other associated environmental variability, can affect the recruitment and development of fish, and thus influence the life history strategy of an estuarine fish population (Gillanders *et al.* 2011). To improve management of the dynamic estuarine system, it is therefore necessary to understand how environmental factors influence fish populations. Currently, most studies are focused on effects on fish populations with direct economic value (Alder *et al.* 2008; Ferguson *et al.* 2013; Brookes *et al.* 2015), but our understanding of the dynamic between environmental change and forage fish populations is poor.

1.2 Fish in Estuaries

Estuaries often provide a more diverse assemblage of fish species than nearby riverine and coastal wetlands (Elliott and Hemingway 2002). However, different fish species utilize estuaries in different ways. Some species are common in an estuary at an early life stage (i.e., juveniles) while some migrate from adjacent marine and riverine wetlands to the estuary, but others complete their entire life cycle within an estuary (Potter *et al.* 1990). Fish in estuaries are categorized into different functional groups based on the modes of feeding, reproductive strategies, salinity tolerance, and the life-history stages (Potter and Hyndes 1999; Whitfield 1999; Thiel *et al.* 2003; McLusky and Elliot 2004; Elliott *et al.* 2007). Among the common estuarine fish, Elliott *et al.* (2007)

described the most extensive functional guild for estuarine related fish species based on their spatial and temporal occurrence in marine, freshwater and estuarine environments and their biological attributes (Table 1.1). For instance, estuarine residents represent the fish species that complete their entire lifecycle within the estuarine habitats while estuarine migrants usually complete their larval stages outside the estuary.

The functional guilds of estuary-associated fishes can be related to greater food availability, due to high primary and secondary productivity and/or protection from predation in estuarine habitats (Beck *et al.* 2001; Kennish 2008). Typically, the biological productivity in an estuarine system is spatiotemporally variable due to environmental variation (Whitfield 1999), and primary production is greatly regulated by physical and biological factors within the dynamic system (Day *et al.* 1989; Wilson 2002). Therefore, the food variability associated with environmental changes can influence the ontogeny, survival and growth of fish in an estuary, affecting both abundance and distribution (Eriksson *et al.* 2005; Taylor *et al.* 2006). Thus, describing the interaction between environmental variability and the trophic relationships of fish and other estuarine organisms is of importance to ensure the sustainability of fish populations in the environmentally-complex estuarine systems.

However, our current understanding of how the life-histories of estuarine fish respond to habitat degradation resulting from environmental change is limited, especially in a reverse estuary where salinity can far exceed seawater salinity. Therefore, there is a need to examine changes in fish populations and the degree of physiological adaptation to varying salinity gradients.

Table 1.1 Functional groups of fishes in estuaries described by Elliot *et al.* 2007.

Functional group	Definition
Estuarine species	This category can be subdivided into: 1. Estuarine residents: estuarine species capable of completing their entire lifecycle within the estuarine environment. 2. Estuarine migrants: Estuarine species that have larval stages of their life cycle completed outside the estuary or are also represented by discrete marine or freshwater populations.
Marine migrants	Species that spawn at sea and often enter estuaries in large numbers and particularly as juveniles. Some of these species are highly euryhaline and move throughout the full length of the estuary. This category can be subdivided into: 1. Marine estuarine-opportunist: marine species that regularly enter estuaries insubstantial numbers, particularly as juveniles, but use, to varying degrees, nearshore marine waters as an alternative habitat. 2. Marine estuarine dependent: marine species that require sheltered estuarine habitats as juveniles but live along coasts where there are no such habitats and these species are thus dependent on the habitats of that type that are present in estuaries.
Marine stragglers	Species that spawn at sea and typically enter estuaries only in low numbers and occur most frequently in the lower reaches where salinities are approximately 35 PSU. These species are often stenohaline and associated with coastal marine waters.
Freshwater migrants	Freshwater species found regularly and in moderate numbers in estuaries and whose distribution can extend beyond the oligohaline sections of these systems.
Freshwater stragglers	Freshwater species found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries.
Anadromous	Species that undergo their greatest growth at sea and which, prior to the attainment of maturity, migrate into rivers where spawning subsequently occurs.
Semi-anadromous	Species whose spawning migration from the sea extends only as far as the upper estuary rather than going into freshwater.
Catadromous	Species that spend all of their trophic life in freshwater and which subsequently migrate out to sea to spawn.
Semi-catadromous	Species whose spawning run extends only to estuarine areas rather than the marine environment.
Amphidromous	Species which migrate between the sea and freshwater and in which the migration in neither direction is related to reproduction.

1.3 Estuary fisheries

Fisheries are an important part of human activities in coastal lagoons and estuarine wetlands (Pérez-Ruzafa and Marcos 2012) as estuaries around the world support great assemblages of commercially and recreationally important finfish and shellfish species (Kennish 2002). Estuary fisheries provide the highest productivity per hectare, although estuaries comprise a relatively small area compared to marine resources in the world ecosystem (Costanza *et al.* 1997). Many marine fish and shellfish species typically occur in estuarine habitats because of rich food resources (Kennish *et al.* 2008), and most of the prominent species from both marine and freshwater environments utilize estuaries for feeding, breeding, and migration purposes in at least some of their life stages (Wilson 2002). However, assemblages of estuarine fish and shellfish commercial species differ depending on the geographical position and ecological condition of each estuary (Blaber *et al.* 2000). For instance, tropical and subtropical estuaries in Australia comprise commercial fisheries of penaeid prawns, finfish, crab, oysters and octopus, while many of those species are not found in temperate regions of Australia (Robins *et al.* 2005). In Australian temperate estuaries, the high diversity of habitats with differing hydrological and physico-chemical attributes maintains the highly diverse fish assemblages (Gillanders *et al.* 2011).

In Australia, ~75% of commercial fisheries are known to use estuarine habitats to complete their life cycles (Creighton 2013), and most of these fisheries are partially or fully over-exploited because of overfishing (Lenanton and Potter 1987; Blaber *et al.* 2000). Overfishing is widely considered to be the major cause of over-exploitation of estuary-dependent fisheries around the world (Houde and Rutherford 1993), and may be responsible for the imbalance between biotic community structure and ecosystem function in the estuarine system (Jennings and Kaiser 1998). However, since environmental stressors such as drought and low freshwater flow can reduce food resources and cause variations in the assemblage structure of estuarine and migratory fish populations by affecting the growth and development of fish species (Zampatti *et al.* 2010), it is clear that such stressors have a significant impact on fishery productivity (Ferguson *et al.* 2013). Therefore, it is necessary to increase understanding of the impact of such environmental factors for sustainable management of common fisheries in a dynamic estuarine system.

1.4 Environmental impacts on estuaries

Estuaries and coastal lagoons experience both anthropogenic and natural stresses (Bachman and Rand 2008; Najjar *et al.* 2010). The former includes pollution, excess sedimentation, dredging, river water diversion, dam construction and deforestation (Schlacher and Wooldridge 1996; Edgar *et al.* 2000), while the latter includes environmental variables such as climate, geomorphology, freshwater inflows and salinity variation (Day *et al.* 1989; Edgar *et al.* 2000). All of these stresses can alter habitat availability and complexity in estuaries, leading to large scale alterations in the natural communities of estuaries (Day *et al.* 1989; Schlacher and Wooldridge 1996; Edgar *et al.* 2000).

Of the environmental factors, freshwater inflow is widely regarded as the “master variable” for the biological productivity and ecological health of estuaries (Power *et al.* 1995), functioning to maintain salinity gradients, sediment and nutrient transport, and provision of habitats for estuarine species (Sun *et al.* 2015). Freshwater flow determines the salinity regime, which impacts biotic communities via influencing the physiological adaptation of estuarine organisms (Grimes and Kingsford 1996; Roy *et al.* 2001). Thus, the magnitude of freshwater flow determines the ecological health of an estuary (Whitfield and Wooldridge 1994).

Likewise, salinity is the driving factor affecting the abundance, distribution and diversity of fish and other organisms in estuaries (Colburn 1988; Bachman and Rand 2008). In most cases, salinity plays a significant role in biological processes and regulates the structure of aquatic communities along with other abiotic factors such as temperature, turbidity and dissolved oxygen (Telesh and Khlebovich 2010; Taylor *et al.* 2014). In particular, increased salinity may lead to reduction of prey abundance and thus reduce growth and survival of fish and other organisms (Tsuzuki *et al.* 2003; Varsamos *et al.* 2005; Nordlie 2006), while fluctuation in salinity can cause stress and reduce the tolerance of fish and invertebrates to other water quality variables, ultimately limiting the distribution of fishes along the estuarine system. Such impacts can be reflected in reduction of species richness and diversity and changes in both physiological and ecological processes in estuaries (Gillanders *et al.* 2011). Therefore, exploration of the effects of environmental variability on the life cycle of fish is essential for conservation and management of estuarine systems.

1.5 The Murray Estuary and Coorong

The Murray Estuary and Coorong is the terminal estuary of the largest river system in Australia, the Murray–Darling River, and is located in the lower reaches of the River Murray in the Murray-Darling Basin (MDB). The catchment and tributary system of the MDB covers an area of 1,073,000 km^2. The Murray River falls into an extensive shallow lake system that includes Lake Alexandrina and Lake Albert (the lower lakes), prior to flowing into the Coorong and connecting to the Southern Ocean via the Murray Mouth (Fig. 1.1). The Coorong is a long (>100 km), narrow (<4 km wide) and shallow (mean depth ≈2 m) estuarine lagoonal system that connects to the Southern Ocean by a narrow channel at the Murray Mouth, with a narrow north-south strip of peninsular sand-dune separating the Murray Estuary and Coorong from the Southern Ocean. The Murray Estuary and Coorong is typically divided into three distinct regions: the Murray Estuary near the River mouth, the North Lagoon and the South Lagoon (Fig. 1.1). A narrow and shallow channel near the Parnka Point splits the Coorong into the North and South Lagoons.

In the late 1930s, five barrages were constructed between the Lakes and the Coorong to avoid saltwater intrusion into the Lakes and to retain stable freshwater storage in the lower Murray River for agricultural use and urban development (Ferguson *et al.* 2013). The freshwater flow through the barrages regulates the physical, hydrological and biological connectivity between the Coorong and the ocean (Webster 2010). Due to the recent Millennium Drought (1997-2009; Kämpf and Bell 2014), freshwater inflow to the Murray Estuary and Coorong significantly declined between 2002 and 2010, with no flow from 2007 to 2010 (Fig. 1.2). Consequently, the Coorong became an extremely hyper-saline system in the late 2000’s, with salinity over four times that of seawater (Brookes *et al.* 2009). As a result, the Murray Estuary and Coorong exhibits a typical inverse estuarine system with a north-south gradient of increasing salinity from fresh/brackish in the Murray Estuary near the Murray Mouth to marine/hyper-saline in the North and South Lagoons of the Coorong.

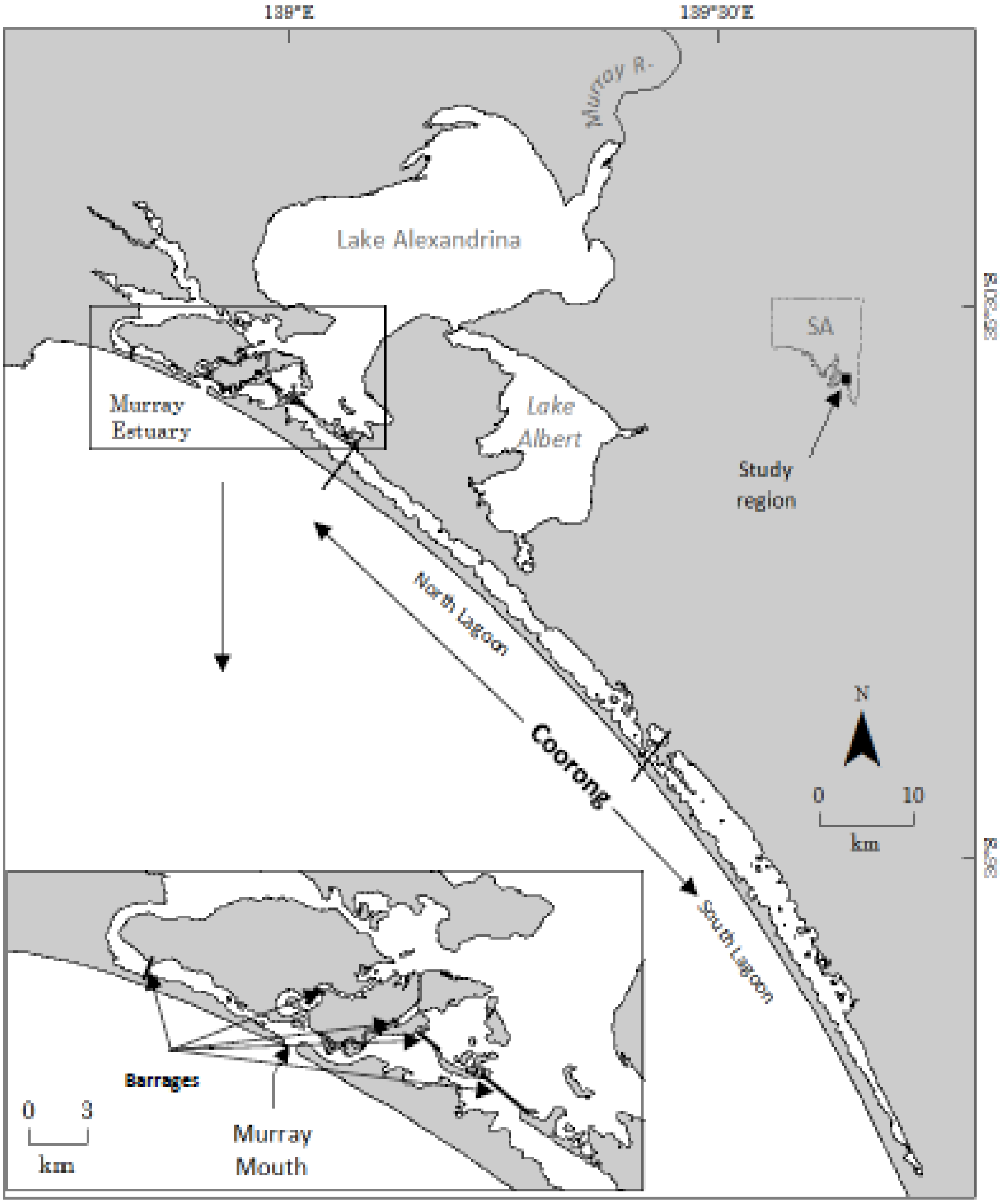

Fig. 1.1 Map of the Murray Estuary and Coorong including Lakes Alexandrina and Albert, South Australia. Inset shows the Murray Mouth and the five barrages (black lines) between the estuary, Lake Alexandrina and the Murray River.

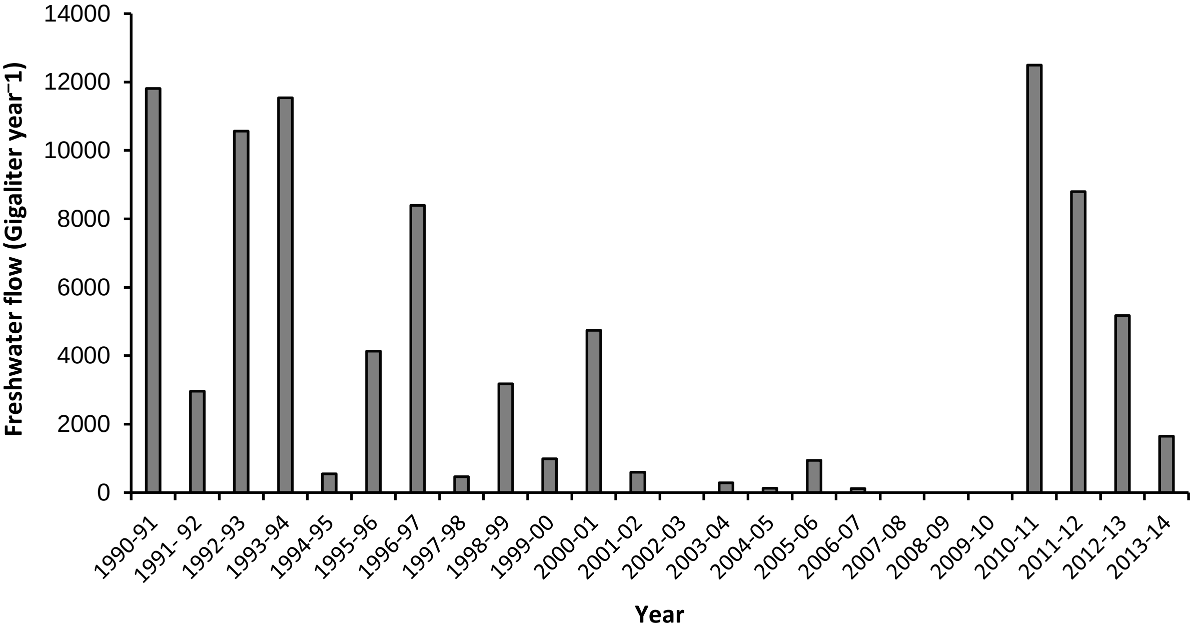

Fig. 1.2 Estimates of total annual freshwater flow (Gigaliter year^{-1}) through the barrages into the Murray Estuary and Coorong from 1990 to 2014 (data source: Murray-Darling Basin Authority 2014).

The Murray Estuary and Coorong form the largest Australian temperate estuary, with high economic and environmental significance, providing important breeding and feeding habitats for waterbirds and migratory and estuarine fish species (Paton 2010). It was listed as a Wetland of International Importance under the Ramsar Convention in 1985, but the Murray Estuary and Coorong is ecologically significant due to diverse assemblages of fish species (Higham *et al.* 2002), migratory shorebirds (Phillips and Muller 2006) and invertebrates (Dittmann *et al.* 2015). A number of commercial and recreational fishes such as mulloway (*Argyrosomus japonicus*), yelloweye mullet (*Aldrichetta forsteri*), black bream (*Acanthopagrus butcheri*) and greenback flounder (*Rhombosolea tapirina*) are common in the Murray Estuary and Coorong (Ferguson 2012). The Murray Estuary and Coorong also provide abundant assemblages of small-bodied forage fishes as the common food resources for piscivorous fish, birds and mammals in this aquatic ecosystem (Paton 2010; Brookes *et al.* 2015). However, although forage fish populations can be severely impacted by the salinity fluctuation and low freshwater flow associated with environmental variability in the Murray Estuary and Coorong, our understanding of growth, composition and life-history of forage fish is limited.

1.6 Forage fish in the Coorong

Forage fish are small-bodied species that are commonly consumed by piscivorous fish, birds and mammals in the aquatic ecosystem (Engelhard *et al.* 2014). Forage fishes play a significant role in food webs by transferring energy from primary producers like algae and/or primary consumers such as zooplankton to higher trophic levels; seabirds, marine mammals and carnivorous fishes (Springer and Speckman 1997, Pikitch *et al.* 2012). Among the small-bodied fish, small-mouthed hardyhead (*Atherinosoma microstoma*), sandy sprat (*Hyperlophus vittatus)* and Tamar goby (*Afurcagobius tamarensis*) are the most common forage fish species in the Murray Estuary and Coorong (Paton 2010).

Small-mouthed hardyheads are widely distributed in the temperate streams, inland lakes, estuaries and adjacent marine areas of south-eastern Australia, Tasmania and Victoria and the Coorong lagoon in South Australia (Thompson and Bray 2011). In the Coorong, this species is abundant in the South Lagoon and also common in the North Lagoon and the Murray Estuary (Eckert and Robinson 1990; Noell *et al.* 2009). Tamar goby are common in Victoria, New South Wales, eastern South Australia and northern Tasmania (Lintermans 2007), and are distributed in the Murray Estuary and part of the

North lagoon in the Coorong (Wedderburn *et al.* 2016). On the other hand, sandy sprats are distributed from southern Queensland to southern Western Australia (Rowling *et al.* 2010) and are common in estuaries and inshore waters of South Australia, migrating between the sea and the Murray Estuary and Coorong. In the Coorong, these small-bodied fish are common prey for piscivorous fishes and birds (Paton 2010). Thus, forage fish play a pivotal role in food web trophic dynamics and are indirectly of commercial significance in the Murray Estuary and Coorong (Brookes *et al.* 2015).

Recently, the Murray Estuary and Coorong have been recognized as ecologically degraded estuaries due to the adverse effects of the historical drought in the MDB in the last decade (Phillips *et al.* 2006). The elevated salinity and low freshwater flow have significantly affected the abundance, distribution and recruitment success of estuarine and migratory fish species in the Murray Estuary and Coorong (Zampatti *et al.* 2010; Kingsford *et al.* 2011; Bucater *et al.* 2013) and have reduced the abundance of food resources such as phytoplankton (Jendyk *et al.* 2014), picophytoplankton (Schapira *et al.* 2010) and zooplankton (Geddes *et al.* 2016). Geddes and Francis (2008) reported that the change in prey abundance has greatly affected the overall trophic ecology and food web structure in the Coorong, but these factors also influence the abundance, distribution, ontogeny and physiological tolerance of forage fish, hindering fish growth, development and reproduction (Gillanders *et al.* 2011). Knowledge of how the growth performance of forage fish is impacted by reduced prey abundance due to environmental variability in the Murray Estuary and Coorong is still limited, thus limiting the capacity to make management decisions regarding the Coorong estuary.

Salinity fluctuation appears to be a key factor affecting species distribution and life history traits in estuarine ecosystems (Telesh and Khlebovich 2010; Williams *et al.* 1990).

Although estuarine organisms can develop physiological and biochemical mechanisms to adapt to long-term yearly salinity change (Williams *et al.* 1990), the wide seasonal salinity variation and hyper-salinity in the Murray Estuary and Coorong can induce stress in fish species and limit the distribution of forage fish (Webster 2010; McNeil *et al.* 2013). However, the processes of biochemical and physiological responses to stress due to rapid salinity fluctuation are not fully understood, suggesting a need for research on the physiological responses of estuarine forage fish species under a varying salinity gradient.

1.7 Study objectives

The aim of this study is to understand the population ecology and physiology of key forage fish species in the Coorong estuary, South Australia, by describing the factors and processes that regulate population structure and abundance. The specific objectives are:

1. to investigate the spatial and temporal changes in assemblage structure of key forage fish species in relation to environmental variability in the Murray Estuary and Coorong;
2. to determine the growth pattern and environmental effects on growth performance of forage fish in the Murray Estuary and Coorong;
3. to investigate prey selection and diet overlap among three key forage fish species in the Murray Estuary and Coorong; and
4. to investigate the physiological response to salinity variation of two key estuarine forage fish species in the Murray Estuary and Coorong.

The present thesis consists of four studies to address these objectives. Three key forage fish species, small-mouthed hardyhead, sandy sprat and Tamar goby, were selected as these are commonly found in the Murray Estuary and Coorong.

1.8 Thesis organisation

1.8.1 Study 1: Spatial and temporal changes of three prey fish assemblage structure in a hypersaline lagoon: the Coorong, South Australia

The aim of this study is to investigate the spatial and temporal changes in the abundance and distribution of three key prey fish species in the Murray Estuary and Coorong. This study determines the changes in key environmental variables and the variations of selected prey fish assemblage structure in the Coorong. The results of this study improve understanding of the spatiotemporal variability of prey fish assemblage with respect to the salinity gradients in the Murray Estuary and Coorong, South Australia, leading to further investigation of the effect of environmental factors on the growth of major forage species.

1.8.2 Study 2: Environmental effects on growth performance of three forage fish in a hyper-saline lagoon: the Coorong, South Australia

The aim of this study is to determine the age-dependent growth pattern of three forage fish with different habitat preferences in the Murray Estuary and Coorong. This study explores the growth response of forage fish to environmental variability. The results of this study improve understanding of the impact of salinity and other environmental factors on the growth performance of small-bodied forage fishes in the dynamic Coorong estuary.

1.8.3 Study 3: Diet overlap and resource partitioning among three forage fish species in Coorong, the largest inverse estuary in Australia

The aim of this study is to investigate prey selection and diet overlap among three key forage fish species in the Murray Estuary and Coorong. This study determines the dietary overlap among the forage fish species and prey variability in relation to environmental changes in the Coorong. This study improves our knowledge of prey selection and changes in prey abundance in response to environmental variability in the Murray Estuary and Coorong.

1.8.4 Study 4: Salinity stress response in estuarine fishes from the Murray Estuary and Coorong, South Australia

The previous studies investigated the impact of environmental fluctuation on fish population structure, fish growth, food and feeding. This study aims to investigate physiological response to salinity variation in two key estuarine forage fish species in the Murray Estuary and Coorong. The small-mouthed hardyhead (*A. microstoma*) and Tamar goby (*A. tamarensis*) were used in this study as they have contrasting salinity tolerance where the former lives in super-saline water but the latter lives in fresh and brackish water. This study investigates the stress response of estuarine forage fishes to a variable salinity regime, using metabolic enzymes including superoxide dismutase and catalase. The results of this study provide new insights into our understanding of the physiological responses and resilience of estuarine forage fish under salinity stress.

The present thesis consists of six chapters describing the research objectives, methods and results ultimately contributing to the understanding of the biology and ecology of forage fish species in the Murray Estuary and Coorong. Chapter 1 provides a general introduction to estuaries, estuarine fish and fisheries, the influence of environmental factors, identifies a gap in knowledge and presents the study objectives of the present thesis. The results section of this thesis is organised as four independent data chapters (Chapter 2 - 5), representing four major experiments, each of which is presented as a manuscript for journal publication. Therefore, some repetition between chapters may be observed in the background and methods. Although independent objectives are presented in each chapter, each contributes towards the overall thesis objectives stated in this introduction chapter. Within each chapter, the word "study" refers solely to each respective experiment. All the studies were performed by the author of the present thesis under the supervision of the supervisors. However, all supervisors are listed as a co-author on each manuscript for publication in peer reviewed journals to acknowledge their scientific inputs towards completion of this thesis research. Chapter 6 is a general discussion reviewing the main results observed in each study and suggestions for future research are also included in this thesis.

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Chapter 2

Spatial and temporal changes of three prey-fish assemblage structure in a hypersaline lagoon: the Coorong, South Australia

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2.1 Abstract

Estuaries and coastal lagoons are the dynamic interface between marine, freshwater and terrestrial environments. The Coorong, an Australian wetland, has been ecologically degraded by protracted drought and subsequent low freshwater flow and transformed into a hyper-saline lagoon system. The Coorong consists of the North and South lagoons and connects to the Southern Ocean through a narrow channel at Murray Estuary. The present study investigates spatiotemporal variation of three primary prey-fish assemblage in the Murray Estuary and Coorong. Spatial change in prey-fish assemblage was detected, but temporal variation was not significant. Prey-fish assemblage was dominated by greater abundance of small-mouth hardyhead (*Atherinosoma microstoma*) in the South Lagoon. There was low abundance of sandy sprat (*Hyperlophus vittatus*) and Tamar goby (*Afurcagobius tamarensis*) in North Lagoon, and complete absence of both species in South Lagoon. The spatial variation in distribution of prey-fish assemblage was attributed to elevated salinity gradients (Murray Estuary 2–30; North Lagoon 11–75 and South Lagoon 40–85). The change of prey-fish assemblage is mainly driven by the salinity variation in the Murray Estuary and Coorong. This study improves our understanding on the dynamics of small-bodied prey fish assemblage and key environmental factors regulating fish distribution in the Murray Estuary and Coorong.

Additional keywords: estuary, Murray Estuary, North Lagoon, salinity.

2.2 Introduction

Estuaries and coastal lagoons are the dynamic interface and transition zone between marine, freshwater and terrestrial environments (Whitfield 1988, Edgar *et al.* 1999, Zampatti *et al.* 2010). Estuarine habitats are spatially and temporally complex due to the continuous mixing of saltwater and fresh water and the fluctuations in freshwater flow influencing salinity levels (Gillanders *et al.* 2011). Estuarine ecosystems are also characterised by a high diversity of physical habitats accommodating aquatic flora and fauna (Day *et al.* 1989). Naturally, estuaries are highly productive and often provide breeding, feeding and nursery habitats for a wide range of fish species (Whitfield 1999; Elliott and McLusky 2002; Bucater *et al.* 2013). High productivity in estuaries attracts a wide range of predator species to consume prey species. The natural variability within the estuarine system can impact both spatial and temporal changes in distribution and abundance of fish, other vertebrates and invertebrates (Whitfield 2005). Estuarine habitats also provide migratory pathways for fish and invertebrates, including species with commercial importance (Payne and Gillanders 2009).

Spatial and temporal changes in physicochemical and environmental factors strongly influence the abundance and assemblage structure of fish in estuaries (Pessanha and Araújo 2003). Physicochemical variability plays a significant role in biological processes and regulates the structure of aquatic communities along with other abiotic factors (e.g., salinity, temperature, turbidity and dissolved oxygen; de Moura *et al.* 2012; Taylor *et al.* 2014). Among abiotic factors, salinity is widely recognised as the main factor influencing the abundance, distribution and assemblage structure of aquatic species in estuarine systems (Day *et al.* 1989; Martino and Able 2003; de Moura *et al.* 2012). The increased salinity in estuaries may lead to the reduction of species richness, diversity of estuarine fishes and low abundance of freshwater and diadromous species (Gillanders *et al.* 2011).

The Murray Estuary and Coorong is the terminus estuary of the Australia's largest river system (i.e., Murray–Darling River). The Murray Estuary and Coorong are separated from the freshwater system of the Murray River by a series of low-head dam constructed between 1935 and 1940 to prevent saline intrusion up to the Murray River as the level of water extraction increases (Ferguson *et al.* 2013). From 2001–2010, there was a significant reduction in freshwater flow to the Murray Estuary and Coorong due the impact by the decadal drought in the Murray–Darling Basin (MDB) and the Coorong became an extremely hyper-saline system with salinity over 4 times of seawater in late 2000's (Brookes *et al.* 2009). Recently, the Murray Estuary and Coorong were identified as ecologically degraded estuaries due to the negative effects of the prolonged drought in the MDB in the last decades (Phillips *et al.* 2006). Consequently, the abundance, distribution and recruitment of biota and their composition have been significantly impacted by the hyper-saline conditions across the Murray Estuary and Coorong (Kingsford *et al.* 2011; Bucater *et al.* 2013; Leterme *et al.* 2013; Jendyk *et al.* 2014). The increased salinity associated with low freshwater flow can affect fish abundance, spatial distribution and assemblages across regions within the estuary (Pollard 1994; Wedderburn *et al.* 2008; Wedderburn *et al.* 2012).

Prey fish are the most abundant fish species in the aquatic ecosystem and play important functions by transferring energy from primary producers (e.g., algae) and/or consumers (e.g., zooplankton) to higher trophic levels (e.g., seabirds, marine mammals and carnivorous fishes) (Pikitch *et al.* 2012; Engelhard *et al.* 2014). In the Coorong, small-bodied fishes such as sandy sprat (*Hyperlophus vittatus)*, Tamar goby (*Afurcagobius *tamarensis*) and small-mouthed hardyhead (*Atherinosoma microstoma*) are the most common prey-fish species for piscivorous fishes and birds (Geddes and Francis 2008; Brookes *et al.* 2009; Deegan *et al*. 2010; Paton 2010). Sandy sprat and Tamar goby are distributed across the Murray Estuary and North Lagoon while estuarine small-mouthed hardyhead are exclusively present in the South Lagoon (Brookes *et al.* 2009). The abundance and species composition of prey-fish assemblages are indicative of productivity and ecological health of an estuarine system (Engelhard *et al.* 2014).

In Australia, many studies have been conducted on spatial and temporal changes of fish assemblages in the temperate estuaries and lagoons (Potter and Hyndes 1994; Jackson and Jones 1999; Hoeksema and Potter 2006; Zampatti *et al.* 2010; Bucater *et al.* 2013; Ferguson *et al.* 2013). For instance, Ferguson *et al.* (2013) reported that low freshwater flow and prolonged drought along with fishing have contributed to the reduction of species richness and diversity in the past 25 years in the lower Murray River system. Zampatti *et al.* (2010) reported that low freshwater flow through the barrages affected recruitment success and fish assemblages with decreased relative abundance of catadromous and freshwater fishes in the Murray Estuary and Coorong. However, little is known on the variation of abundance, distribution and assemblage structure of prey fish communities in relation to salinity changes associated with low freshwater flow to the Murray Estuary and Coorong. Typically, the salinity impact on fish distribution is more intensified in a dry summer as salinity can reach extreme values when freshwater flow from the Murray River to the Coorong is low. This study was conducted in a dry season from November to March and the results would represent a period to reflect the distribution of forage fish species under a high salinity stress in the Murray Estuary and Coorong.

The aim of this study was to investigate the spatial and temporal changes in the abundance and distribution of three key prey-fish species i.e., sandy sprat, Tamar goby and small-mouthed hardyhead in the Murray Estuary and Coorong as well as changes in key environmental variables that regulate the variations of selected prey-fish assemblage. We hypothesise that the elevated salinity associated with increased distance from the Murray Mouth and the prolonged dry period in summer would lead to changes in assemblage structure of prey fish in the Murray Estuary and Coorong. The composition and relative abundance of small-bodied prey fish species would be altered due to the variation of salinity together with temperature, dissolved oxygen, water transparency and pH. The results of this study will advance our understanding of the spatiotemporal variability of prey-fish assemblage along salinity gradients in the Murray Estuary and Coorong, South Australia.

2.3 Materials and methods

2.3.1 Study region

The study was conducted in the Murray Estuary and Coorong, South Australia (Fig. 1) including three regions: the Murray Estuary, the North Lagoon and the South Lagoon. The Coorong is a long (>100 km), narrow (<4 km wide) and shallow (mean depth ≈2 m) estuarine lagoon with a strong north–south salinity gradient. The Coorong is separated from the Southern Ocean by a narrow sand-dune strip at the end of the largest river in Australia, the Murray–Darling River. The Murray Estuary is characterised by connecting the Murray River and the Southern Ocean through a narrow channel at the Murray Mouth in Goolwa.

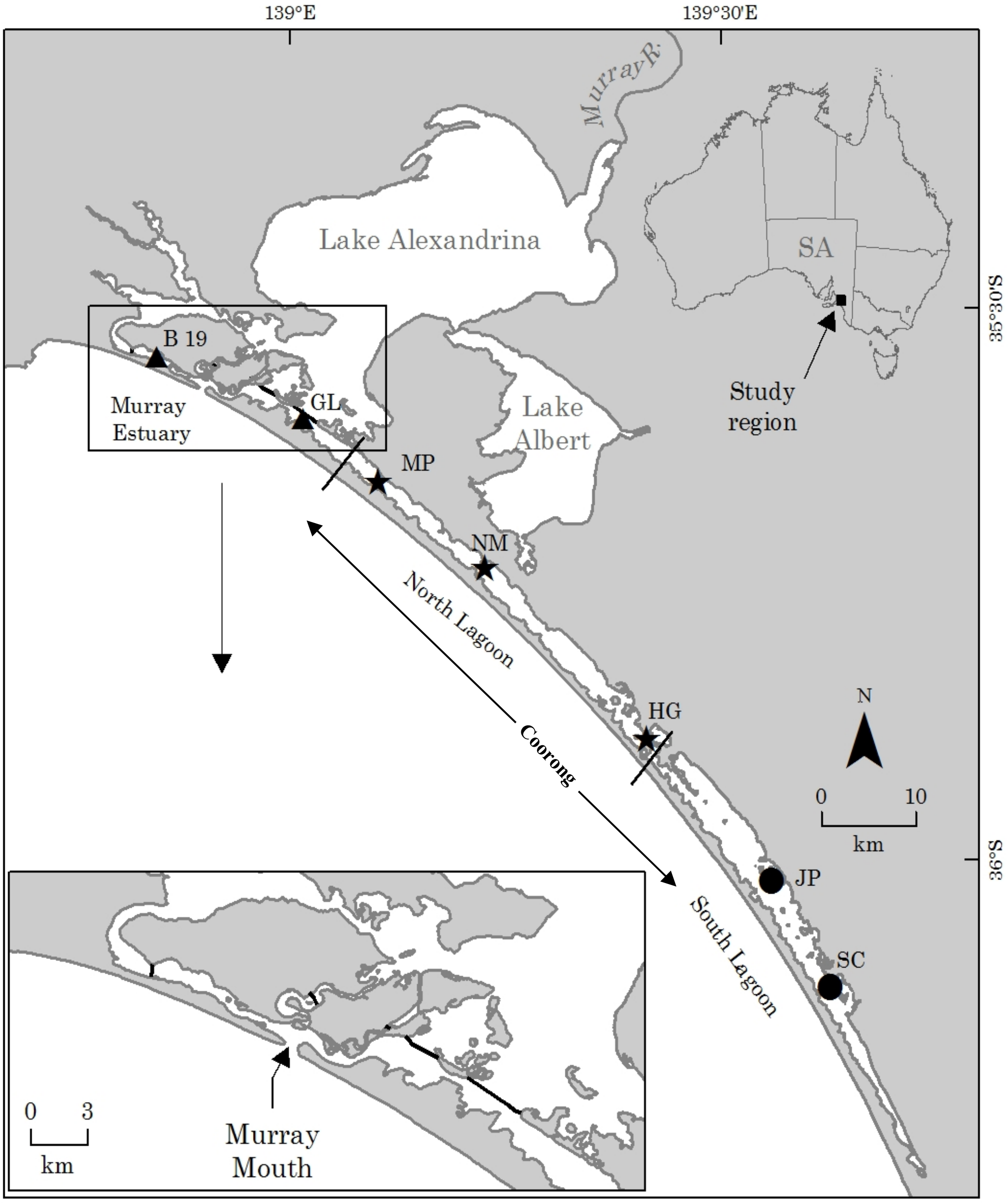


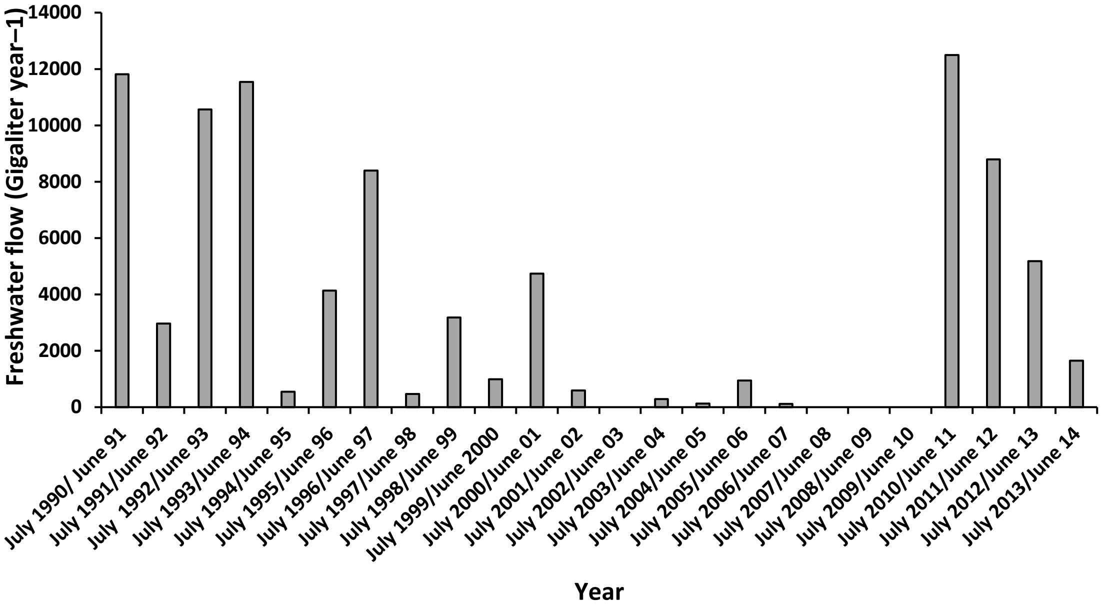
Fig. 2.1 Map of Murray Estuary, North Lagoon and South Lagoon showing the sampling sites; Beacon 19 (B19), Godfry's landing (GL), Mark point (MP), Noonameena (NM), Hells Gate (HG), Jack point (JP) and Salt Creek (SL) in the Coorong, South Australia.

The Coorong naturally splits into North and South Lagoons at Hells Gate near the Parnka Point. The Murray Estuary and Coorong is an inverse estuary influenced by the freshwater inflow through the Murray barrages, located in the vicinity of the Murray Mouth. The freshwater flow through the barrages facilitates the physical, hydrological and biological connectivity between the Coorong and the ocean. As a result, salinity typically increases from fresh/brackish in the Murray Estuary near the Murray Mouth to marine/hyper-saline in the North Lagoon and South Lagoon of the Coorong.

2.3.2 Data Collection

Monthly sampling was conducted at each site over a five-month period (November 2013 to March 2014) during a moderate water flow year (Fig. 2.2). Two sites from the Murray Estuary, three sites from the North Lagoon and two sites from the South Lagoon were selected for sampling to cover a broad salinity gradient. At each site, prey-fish were sampled with a seine net, 61 m long, 29 m wing length (22 mm mesh), and 3 m bunt length (8 mm mesh). The seine net was deployed in a semi–circle, sampled to a maximum depth of 2.0 m and swept over an area ~600 m^2. This allowed quantitative sampling to estimate the relative abundance of fish. Three replicates of a seine net shot were conducted at each site. On each sampling occasion, all captured fish species were transferred to an aerated holding tank and identified to species. The selected prey species, sandy sprat, Tamar goby and small-mouthed hardyhead were numerated for abundance and all other captured fish species were released back to water. Relative abundance was calculated as the number of fish per seine net shot. Two of three species are pelagic and Tamar goby is demersal but this sampling method does not make any bias for collection of these three species. During fish collection at each site, physicochemical variables including salinity, water temperature, dissolved oxygen (DO) and pH were measured in triplicate at 30 cm below the water surface using a water quality meter (TPS, model 90FL). Water transparency was measured using a Secchi disk at each site on each occasion of fish sampling. All samplings were conducted from a boat in the Murray Estuary and North Lagoon and by wading in South Lagoon.

a)



b)

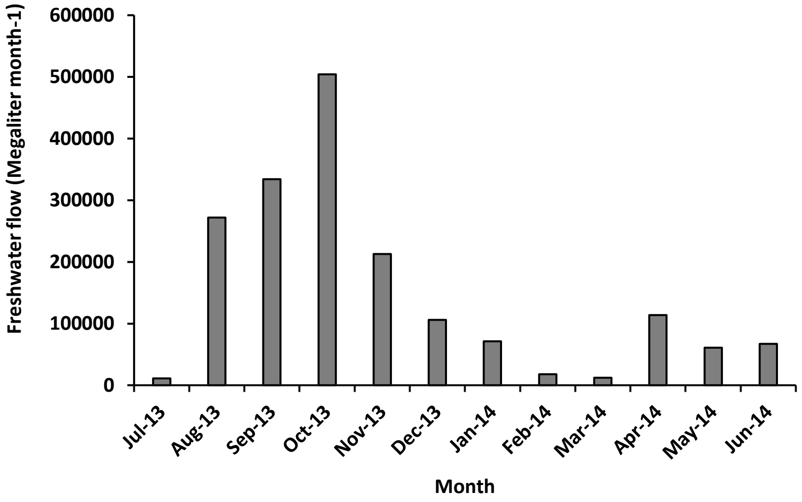


Fig. 2.2 a) Annual freshwater flow (Gigaliter year^{-1}) from 1990 to 2014, and b) Monthly freshwater flow (Megaliter month^{-1}) from July 2013 to June 2014 across the dams into the Murray Estuary and Coorong.

2.3.3 Statistical analysis

Relative abundance data of each prey fish were transformed into log(x+1) to down-weight undue influence of highly abundant species (Anderson *et al.* 2008) and a dummy species was added to adjust for samples with no catch (Ye *et al.* 2012). The log(x+1) transformed data of prey-fish relative abundance were used to construct a Bray-Curtis resemblance matrices (Anderson 2001). The environmental variables were normalised and used to construct a Euclidean distance resemblance matrices. Permutational analysis of variance (PERMANOVA) was run using the resemblance matrices to test the difference of each environmental variable (univariate) and prey fish relative abundance (multivariate) among months, regions and sites. The analysis consisted of three factors, including months (random, 5 levels), regions (fixed, 3 levels) and sites nested within the region (random, 7 levels). When the PERMANOVA showed a significant main effect or interaction between treatments, pairwise comparisons (pseudo-t test) were used to identify the specific level of difference. In this study, the differences between sites within a region were not focused as the site effects were unlikely to reveal much meaningful information of biological significance due to closer locations. Further test of the effect of sites nested within a region can lead to statistical artefact due to the strong treatment effect of the region. Therefore, the interaction effects of sites nested within region of this PERMANOVA design were not given in-depth interpretation (Anderson *et al.* 2008). Unrestricted permutation was accomplished for each factor and interaction with 999 permutations to detect differences at $\alpha = 0.05$ (Anderson 2001). One-way similarity percentage (SIMPER) analysis was also performed on the factors with significant impact to identify the fish species contributing the most to differences between levels. Then, the analysis of principal coordinates (PCO) on prey-fish samples was carried out using the Bray–Curtis resemblance matrix of prey fish relative abundance for the ordination. Vector overlays were used to indicate the species Spearman rank correlation with the ordination axes where the length of the vector indicated the strength of the relationship. To identify the effect of environmental variables on prey-fish assemblage, a distance-based linear model (DistLM) was performed where the log(x+1) transformed prey fish relative abundance and normalised environmental data were used (Anderson *et al.* 2008). A distance based redundancy analysis (dbRDA) was then plotted to give a visual representation of the influence of environmental variables on the changes in prey-fish assemblage structure. All tests were performed using PRIMER v6 (Clarke and Gorley 2006) with the PERMANOVA+ add–on (Anderson *et al.* 2008).

2.4 Results

2.4.1 Water quality

PERMANOVA results showed significant differences in salinity among months (PERMANOVA, P = 0.04) and regions (PERMANOVA, P = 0.003, Fig. 2.3a). In particular, an increasing trend in salinity gradient was observed along with the north–south axis of the Coorong lagoon. Salinity was highly varied during the study period and ranged 2–30 in Murray Estuary, 11–75 in North Lagoon, and 40–85 in South Lagoon. In the Murray Estuary region, the highest salinity (~31 pus) was observed at the Godfrey's landing site in March 2014 while the Beacon 19 site had the lowest salinity (~2) in November 2013 (Fig. 2.3a). There was a substantial change in salinity in the North Lagoon region with the highest salinity (~75) at the Hells Gate site in February 2014 and lowest salinity (11) at the Mark Point in January 2014 (Fig. 2.3a). Similarly, the highest salinity (~85) at the

Jack Point in March 2014 and the lowest (~40) at the Salt Creek site in January 2014 were recorded in the South Lagoon (Fig. 2.3a). For pH comparison, there was a significant spatiotemporal variation among months (PERMANOVA, *P* = 0.001) and regions (PERMANOVA, *P* = 0.036, Fig. 2.3b). pH was variable among the regions (Murray Estuary: 8.13–8.42; North Lagoon: 6.82–8.59 and South Lagoon: 6.11–8.27) during the study period (Fig. 2.3b). The highest pH (8.59) at the Noonameena site in the North Lagoon in February 2014 and the lowest (6.11) at the Jack Point in the South Lagoon in November 2013 were observed (Fig. 2.3b). In addition, water temperature was temporally variable (PERMANOVA, *P* = 0.001) and ranged (17.50–22.73 ºC) in the Murray Estuary, (17.37–22.87 ºC) in the North Lagoon and (15.27–23.07 ºC) in the South Lagoon with higher water temperatures in January 2014 and February 2014 compared to other sampling months at Murray Estuary and North Lagoon (Fig. 2.3c). However, sampling in March 2014 showed comparatively high water temperature at South Lagoon and the Hells Gate of North Lagoon (Fig. 2.3c). Similarly, the water transparency also exhibited significant temporal (months; PERMANOVA, *P* = 0.038) and spatial variations (regions; PERMANOVA, *P* = 0.006) over the study period. In the Murray Estuary region the highest water transparency (200 cm) occurred at Godfrey's landing site in March 2014 and the lowest water transparency (25 cm) was observed at the Beacon 19 site in November 2014. Water transparency reached maximum (50 cm) in March 2014 and minimum (12 cm) at the Mark Point site in the North Lagoon in November 2013. In the South Lagoon region, the highest water transparency (80 cm) was measured at the Salt Creek site in November 2013 while the lowest water transparency (20 cm) was recorded at Jack point and Salt Creek sites in December 2013 (Fig. 2.3e). However, DO did not show any spatial and temporal variation during the study period.

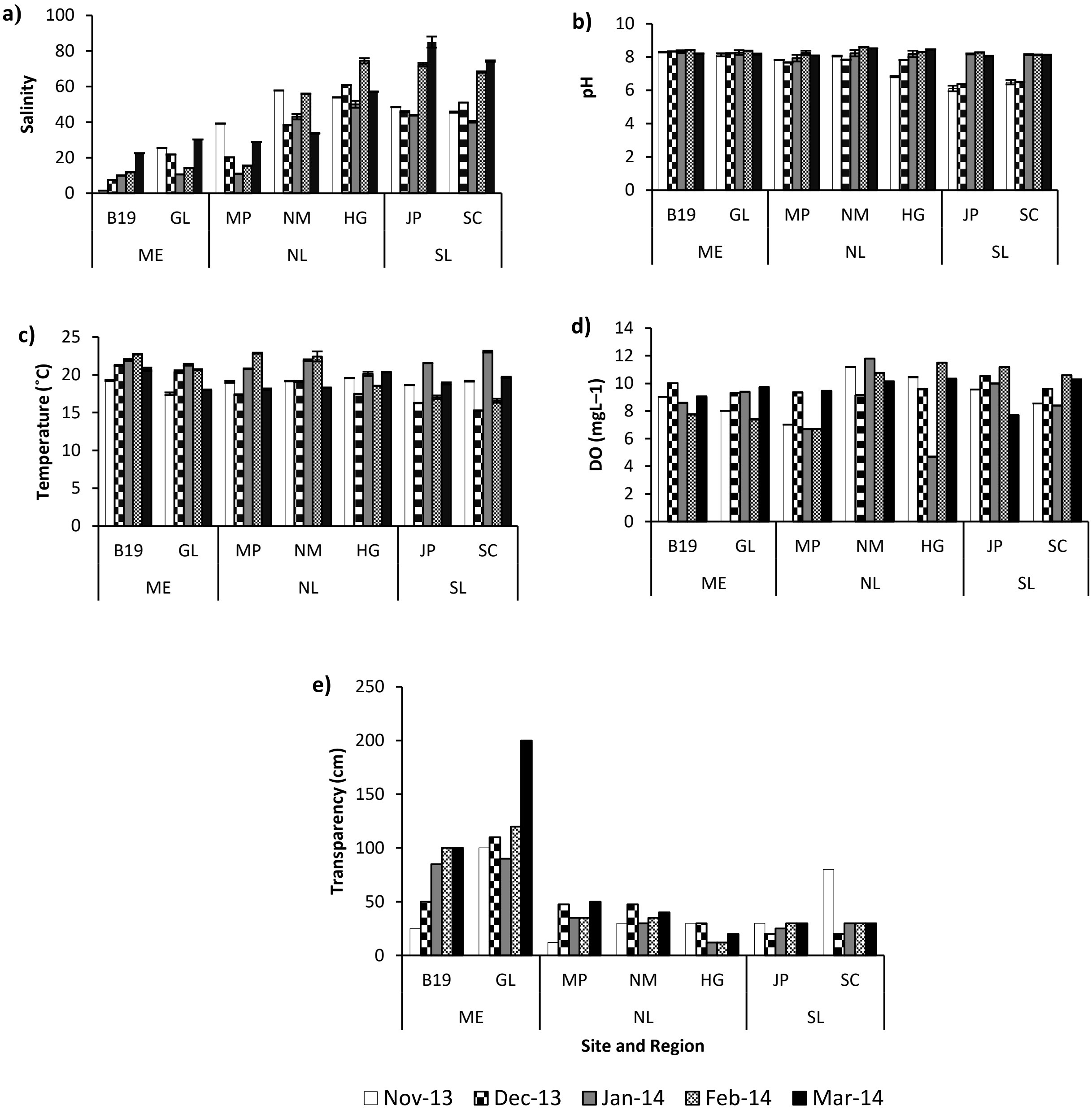


Fig. 2.3 Mean values ± S. E. of (a) salinity, (b) pH, (c) temperature (^{o}C) (d) water transparency (cm) and (e) dissolved oxygen (DO; mgL^{-1}) of water at each site in three regions (ME: Murray Estuary, NL: North Lagoon and SL: South Lagoon) in the Murray Estuary and Coorong form November 2013 to March 2014.

2.4.2 Fish catch

A total of 73,344 individuals of three prey fish species (small-mouthed hardyhead, sandy sprat and Tamar goby) were collected at seven sites in the Murray Estuary and Coorong from November 2013 to March 2014 (Table 2.1). The most abundant species, small-mouthed hardyhead represented ~67% of the total catch (Table 2.1). Sandy sprat and Tamar goby contributed to ~32% and ~1.3% of the total catch, respectively (Table 2.1). Most of the small-mouthed hardyhead were confined to the South Lagoon (abundance >1000) and part of the North Lagoon, but only a small number of this species was found in the Murray Estuary (~70) during the sampling period (Fig. 2.4). Noticeably, the small-mouthed hardyhead showed a temporal increase in abundance with extended distribution from south to north across the Murray Estuary and Coorong from November 2013 to March 2014 (Fig. 2.4). In contrast, the sandy sprat was highly abundant and distributed across the Murray Estuary and northern part of the North Lagoon but was completely absent in the South Lagoon during the study period (Fig. 2.4). Conversely, the relative abundance of Tamar goby was very low over the study period. The distribution of Tamar goby was also limited to the Murray Estuary and North Lagoon (Fig. 2.4).

Table 2.1 Catch summary of small-mouthed hardyhead, sandy sprat and Tamar goby with percentage to total number of fish collected in the Murray Estuary and Coorong during the study period.

			Monthly fish catch (Number)						
Common name	**Family**	**Species**	**Nov–13**	**Dec–13**	**Jan–14**	**Feb–14**	**Mar–14**	**Total**	**Species %**
Small-mouthed hardyhead	Atherinidae	*Atherinosoma microstoma*	1574	2762	7596	10053	26928	48913	66.7
Sandy sprat	Clupeidae	*Hyperlophus vittatus*	2274	13567	3385	3715	555	23496	32.0
Tamar goby	Gobiidae	*Afurcagobius tamarensis*	391	58	120	247	119	935	1.3
Total			4239	16387	11101	14015	27602	73344	100.0

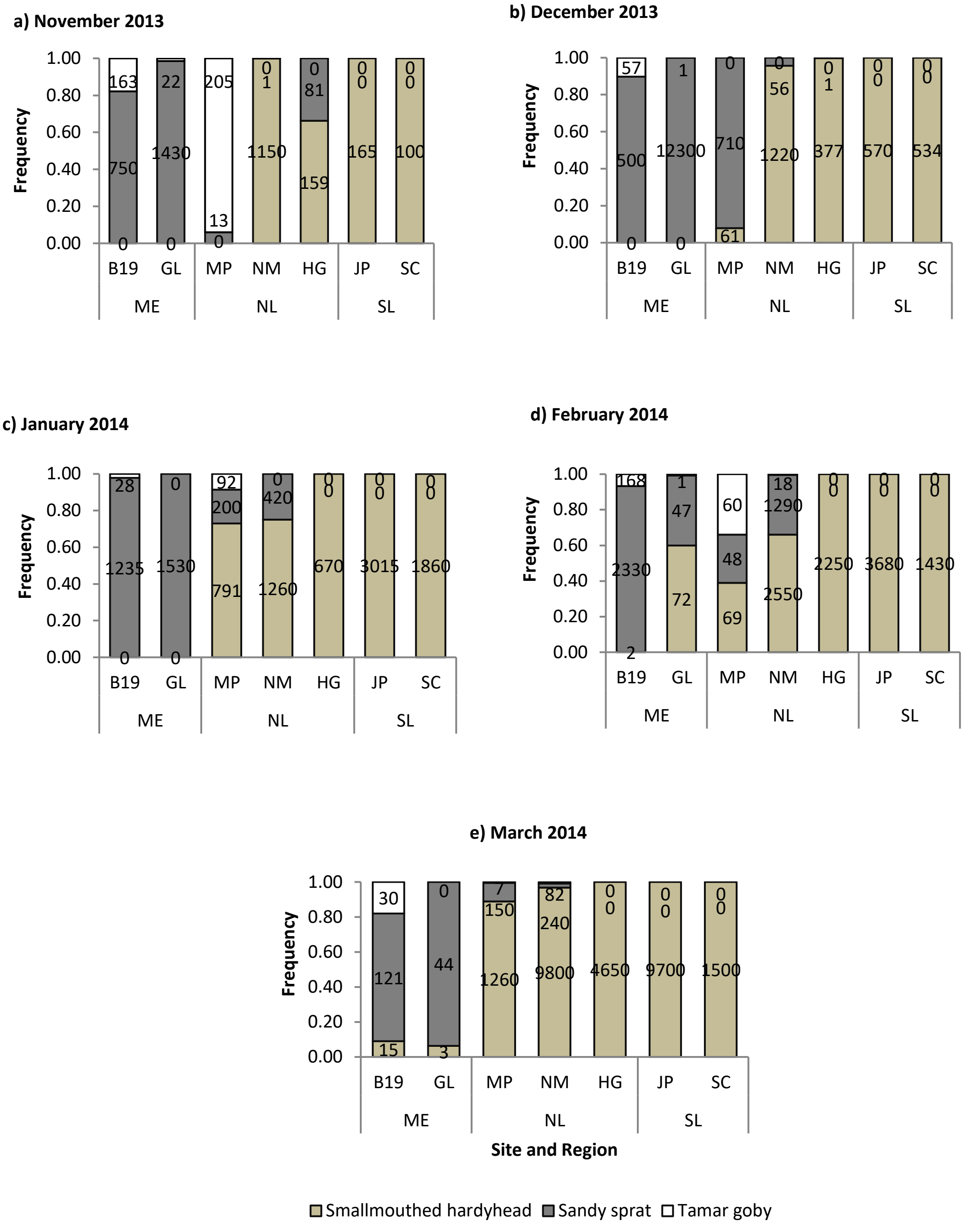


Fig. 2.4 Species composition of three prey fish species at each site in three regions in the Murray Estuary and Coorong. The number in the bar indicates the number of individuals collected at each site.

2.4.3 Spatiotemporal variation in prey-fish assemblage

PERMANOVA showed a significant spatial difference in relative abundance of prey-fish assemblage among the regions (PERMANOVA, *P* = 0.002), but temporal variation was not significant (PERMANOVA, *P* = 0.239, Table. 2.2). Pairwise test indicated significant differences in relative abundance of prey-fish assemblages between the Murray Estuary and the North Lagoon (PERMANOVA, *P* = 0.03) and between the South Lagoon and the Murray Estuary (PERMANOVA, *P* = 0.001).

Table 2.2 PERMANOVA results for prey fish relative abundance collected during November 2013 to March 2014. This PERMANOVA table includes fixed factors contributing to the changes of prey-fish assemblage during this study. Significant difference was set at *P* < 0.05.

	Assemblage structure				
Source of variation	df	SS	MS	Pseudo–F	P (perm)
Month	4	5254	1313.5	1.42	0.239
Region	2	80365	40182	5.726	0.002
Site (Region)	4	24258	6064.5	6.556	0.001
Month × Region	8	8911.9	1114	1.204	0.33
Month × Site (Region)	16	14800	925.02	3.947	0.001
Residuals	70	16402	234.31		

The greatest dissimilarity in prey-fish assemblage structure occurred between Murray Estuary and South Lagoon (SIMPER, 91% in dissimilarity; Table 2.3). The variation in prey-fish assemblage was attributed to the high relative abundance of small-mouthed hardyhead (mean abundance 5.74) and the absence of sandy sprat and Tamar goby in the South Lagoon. A similar variation in the prey-fish assemblage was detected between the North Lagoon and the South Lagoon (SIMPER, 37% in dissimilarity; Table 2.3). However, the variation in prey-fish assemblage between Murray Estuary and North Lagoon (SIMPER, 66% in dissimilarity) was attributed partially to the high relative abundance of sandy sprat and the presence of Tamar goby in Murray Estuary, whereas small-mouthed hardyhead contributed 49% to the differences between these two regions. Overall, there was an increase in abundance of small-mouthed hardyhead from the Murray Estuary to the North Lagoon and South Lagoon and a complete absence of sandy sprat and Tamar goby in the South Lagoon.

Principal coordinate ordination (PCO) of the prey-fish assemblage explained 94% of total variation in the first two axes (i.e. PCO1 and PCO2) and resulted in a clear separation of fish samples among three regions of the Murray Estuary and Coorong (Fig. 2.5). Separation along PCO1 in the samples among the Murray Estuary (triangle), North Lagoon (asterisks) and South Lagoon (squares) indicated the increasing abundance of small-mouthed hardyhead and the decreasing abundance of sandy sprat and Tamar goby in the Murray Estuary and Coorong (Fig. 2.5).

Table 2.3 One-way SIMPER analysis results among the regions based on the log transformed data of relative abundance indicating species contribution to the overall dissimilarity between regions with significant difference for the prey-fish assemblage from November 2013 to March 2014. Mean abundance is measured by the number of fish per net. Consistency ratio (CR) indicates the consistency of difference in abundance between regions with larger values indicating greater consistency. A cumulative cut-off of 90% was applied. Mean dissimilarities are expressed as a percentage ranging from 0 (identical) and 100 (totally dissimilar).

Species name	Mean Abundance		CR	Contribution (%)	Cumulative (%)
Region					
Murray Estuary vs North lagoon:	Mean dissimilarity = 66.92%				
	Murray Estuary	North lagoon			
Small-mouthed hardyhead	0.43	5.03	1.85	49.09	49.09
Sandy sprat	4.6	2.43	1.39	33.86	82.96
Tamar goby	1.55	1.05	1.01	17.04	100
Region					
Murray Estuary vs South lagoon:	Mean dissimilarity = 91.90%				
	Murray Estuary	South lagoon			
Small-mouthed hardyhead	0.43	5.74	2.63	49.42	49.42
Sandy sprat	4.6	0	2.07	38.34	87.75
Tamar goby	1.55	0	0.95	12.25	100
Region					
North lagoon vs South lagoon:	Mean dissimilarity = 37.92%				
	North Lagoon	South lagoon			
Sandy sprat	2.43	0	1.16	40.81	40.81
Small-mouthed hardyhead	5.03	5.74	1.1	40.68	81.49
Tamar goby	1.05	0	0.64	18.51	100

In contrast, PCO2 represented the increasing abundance of sandy sprat and Tamar goby and low abundance small-mouthed hardyhead in the Murray Estuary and Coorong. Consequently, South Lagoon had more small-mouthed hardyhead and Murray Estuary had more sandy sprat and Tamar goby. North Lagoon was characterised by a mix of the three prey species (Fig. 2.4).

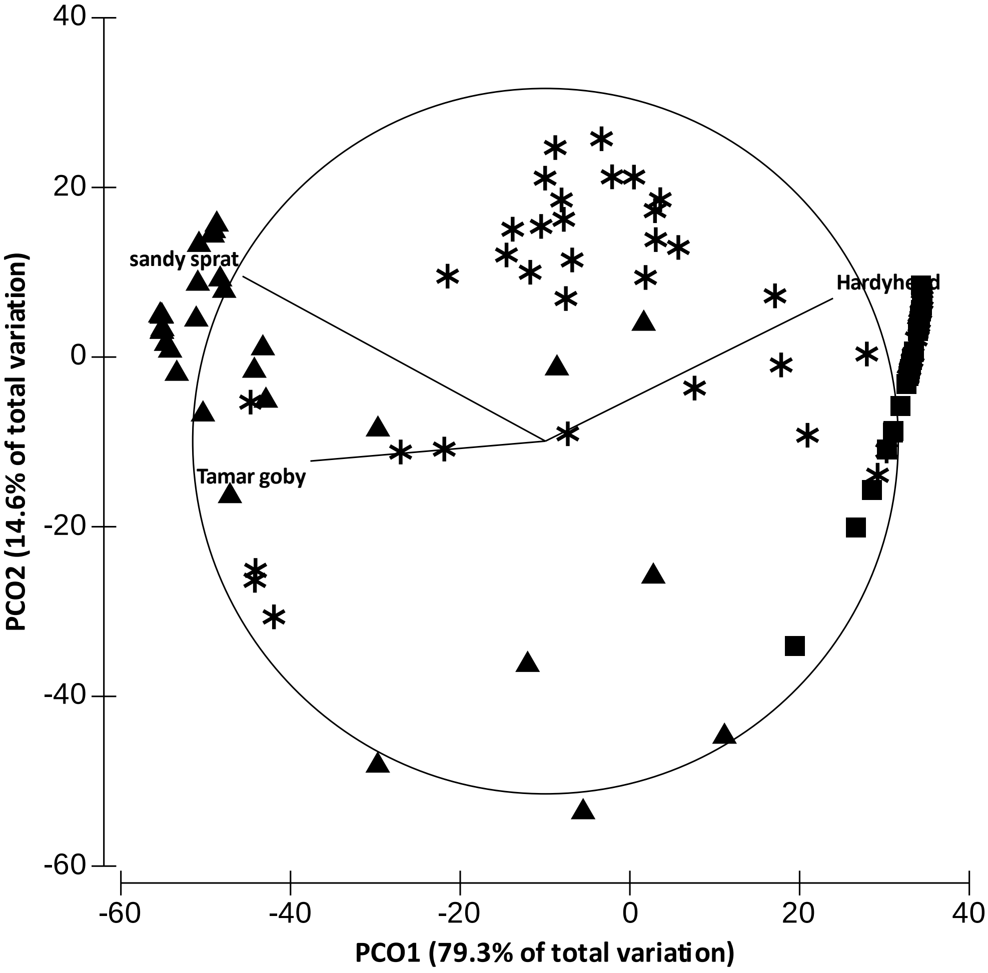


Fig. 2.5 Principal Coordinates (PCO) of samples on the basis of the Bray-Curtis measure of log transformed abundance of prey-fish species collected by seine net at each site from three regions: i.e., Murray Estuary (triangle), North Lagoon (asterisks) and South Lagoon (squares). The vector overlay indicates Spearman rank correlations between species and PCO axes 1 and 2.

2.4.4 Environmental effect on prey-fish assemblage

Salinity, pH and water transparency were the most influential variables predicting the spatial variation of prey-fish assemblage (DistLM, *P* = 0.001, Table 2.4).

Table 2.4 DistLM sequential results of environmental variables on prey-fish assemblage (SS=Sum of Square; Prop=Proportion of the variation; Cumul=Cumulative variation).

Variable	**SS**	**Pseudo–F**	**DistLM*, P***	**Prop**	**Cumul.**
Salinity	71021.0	92.2	0.001	0.472	0.472
pH	8077.9	11.6	0.001	0.053	0.525
Temperature	1257.0	1.8	0.183	0.008	0.534
Dissolved oxygen	1703.2	2.5	0.099	0.013	0.545
Water transparency	8670.2	14.4	0.001	0.057	0.603

Ninety-six percent of the variation in prey-fish assemblage could be explained by the first two axes and salinity was the key driving factor for the abundance and distribution of these prey-fish species (Fig. 2.6). Separation along dbRDA1 in the samples from the Murray Estuary (triangle), North Lagoon (asterisks) and South Lagoon (squares) could indicate a positive relationship with salinity (Fig. 2.6). However, the separation along dbRDA2 in the samples was driven by a positive relationship with high water transparency and negative relationship with pH in the Murray Estuary and Coorong (Fig. 2.6).

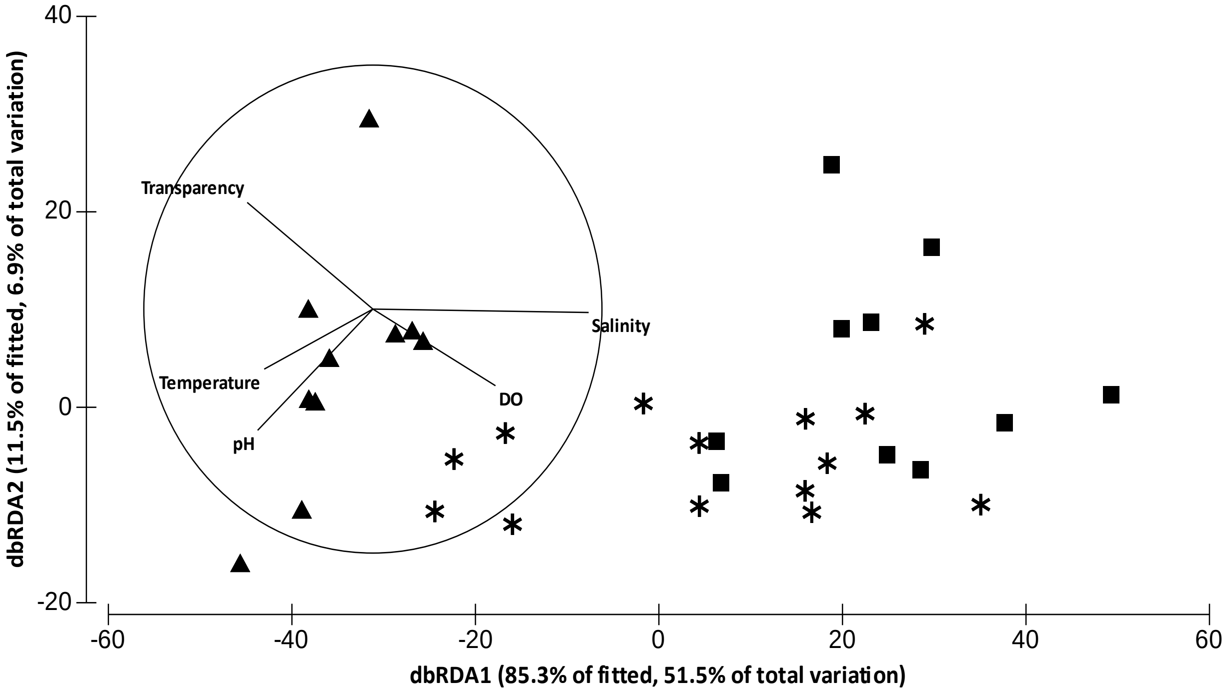


Fig. 2.6 dbRDA ordination of species-abundance data collected from different sites and regions of the Murray Estuary and Coorong *versus* the predictor variables: salinity, water transparency, temperature, pH and DO. Data points are displayed as Murray Estuary (triangle), North Lagoon (asterisks) and South Lagoon (squares).

2.5 Discussion

Prey-fish assemblage in the Murray Estuary and Coorong was mainly driven by the increased abundance of small-mouthed hardyhead and decreased abundance of sandy sprat and Tamar goby from Murray Estuary, North Lagoon to South Lagoon. Estuarine atherinids including small-mouthed hardyhead are abundant in the Coorong and other Australian temperate estuaries (Potter and Hyndes 1994; Young and Potter 2002). The dominance of small-mouthed hardyhead in the current study is most likely due to its ability to tolerate high level and a broad range of salinity (LD_{50}:

lower 3.3 – upper 108; Lui 1969; Molsher *et al.* 1994). During this study in November 2013, the small-mouthed hardyhead was sampled exclusively in the South Lagoon and North Lagoon. With increasing salinity towards summer/early autumn, small-mouthed hardyhead appeared to be distributed towards the Murray Estuary, The high abundance of sandy sprat in the Murray Estuary and North Lagoon is possibly due to recruitment of juveniles and migration to the Murray Estuary and North Lagoon (Rogers and Ward 2007; Zampatti *et al.* 2010). The limited distribution and low abundance of Tamar goby in the Murray Estuary and North Lagoon are likely to be associated with the environmental factors and habitat suitability, such as salinity tolerance (Gill and Potter 1993). Nevertheless, the LC_{50} salinity tolerance of Tamar goby from the Coorong is 73.2 at 14 ºC (winter) and 71.4 at 23 ºC (summer) in the laboratory conditions (McNeil *et al.* 2013). Tamar goby was reported to occur in the North Lagoon and absent in the South Lagoon during 2012-13 (Livore *et al.* 2013). In the current study, the salinity gradient of Murray Estuary (2–14) and North Lagoon (11–75) in the Coorong was within the range of the salinity tolerance of Tamar goby, which explains the occurrence and higher abundance of Tamar goby in these regions whereas they are completely absent in the South Lagoon (salinity 85).

The distributional pattern of these three forage fish is mainly dependent upon the salinity regulated by freshwater flow to the Murray Estuary and Coorong. If the study would continue in other seasons (i.e, during high water flow seasons), the hyper-salinity in the South Lagoon might be expected to decrease and to allow southward distribution of sandy sprat and Tamar goby. However, it is unlikely to decrease the hyper-salinity of the South Lagoon to the marine/brackish salinity even during the highest water flow to the Coorong (Livore *et al.* 2013). Therefore, the sandy sprat and Tamar goby would probably still occupy in the area within the Murray Estuary and the North Lagoon during a higher water flow season. Similarly, the population of euryhaline small-mouthed hardyead would predominantly occur in the South Lagoon. Thus the salinity gradient would be the main controlling factor for the distribution of these forage fish in the Coorong. Therefore, this distributional pattern of sandy sprat and Tamar goby might be persistent in other months (July-October) as the salinity in the South Lagoon would not drastically change from hypersaline to marine/brackish to accommodate sandy sprat and Tamar goby while the small-mouthed hardyhead would be distributed throughout the Murray Estuary and Coorong even during high water flow seasons.

Salinity is a key environmental determinant, varying spatially and temporally in the Murray Estuary and Coorong (Brookes *et al.* 2009). Salinity in the Murray Estuary and Coorong is mainly driven by freshwater flow through the dams and from upstream. The salinity ranged 30−43 at the Murray Estuary, 61−86 in the North Lagoon and 105−164 in the South lagoon during 2006−07 (Ye *et al.* 2013). Salinity in the South Lagoon remains hypersaline at 59−98 in 2010−11, 86−94 in 2011−12 and 76−79 in 2012−13 (Livore *et al.* 2013). Hyper-salinity is typical in the Coorong and persistent even during high fresh water flow into the Murray Estuary (Ye *et al.* 2012). The results of the current study show wide salinity variation from fresh−brackish in Murray Estuary, saline in North Lagoon, to hyper-saline in South Lagoon. Spatiotemporal variation of salinity in the current study is mainly due to low freshwater flow through the dams. In the Murray Estuary, low salinity (fresh to brackish) is mainly regulated by the freshwater flow through the dams from the Murray River. Increased water transparency in the Murray Estuary and North Lagoon from spring to summer might be related to the low input of freshwater flow into the system from the Murray River throughout the season. Variations of DO concentration in Murray Estuary (7–10 mg L^{-1}), North Lagoon (4–11 mg L^{-1}) and South Lagoon (7–11 mg L^{-1}) over the study period also indicate optimal range for the distribution of fish in an estuarine habitat (Williams 1998). In estuaries, pH levels are highly correlated to salinity, photosynthesis and DO variations (Ringwood and Keppler 2002). In the current study, the increased water temperature in March 2014 is likely due to shallow water depth in the South Lagoon and the Hells Gate of the North Lagoon. Spatial and temporal variation of pH in the current study is presumably due to salinity variation across the Murray Estuary and Coorong. However, pH value of current study in the Murray Estuary and Coorong is within the optimal level for estuarine fish species (Noell *et al.* 2009).

Spatial variation in prey-fish assemblage structure in this study was mainly regulated by salinity and water transparency in the Murray Estuary and Coorong. The spatial and temporal variation of pelagic prey-fish assemblage was shown to have influenced the abundance and distribution of other predators in a temperate estuary in New Jersey, USA (Hagan and Able 2003). The small-mouthed hardyhead can tolerate extreme salinity and able to access a broad food source in the Murray Estuary and Coorong (Boeuf and Payan 2001; Vega–Cendejas and Hernández de Santillana 2004).Therefore, the spatiotemporal variation in the distribution of euryhaline small-mouthed hardyhead in this study could potentially influence the overall trophic ecology of the Murray Estuary and Coorong. However, the high abundance and wide distribution of small-mouthed hardyhead are possibly intensified due to suitable habitat such as hyper-marine salinity and aquatic vegetation (i.e. sea grass) in South Lagoon (Potter and Hyndes 1994). The sandy sprat is reported to spawn in nearby the in-shore marine habitat and commonly migrates to the estuaries for development in early life stages (Rogers and Ward 2007). The greater abundance of sandy sprat in the Murray Estuary and North Lagoon is probably related to its tolerance of marine salinity in a nearby marine habitat for feeding and reproduction (Zampatti *et al.* 2010). The abundance and distribution of Tamar goby in the Murray Estuary and North Lagoon may be limited by suitable habitats, especially the ambient salinity (Gill and Potter 1993). These probably explain the absence of Tamar goby and sandy sprat in the South Lagoon. However, as very little information is available on the habitat use and migratory behaviour of the benthic prey-fish species such as Tamar goby, further research is required to understand the abundance change of benthic fishes in the Murray Estuary and Coorong.

Along with salinity, water transparency showed significant influence on abundance and assemblage structure of prey fish in the present study. In particular, water transparency influences primary productivity through photosynthesis and regulates the overall biological productivity in an ecosystem (Herman and Heip 1999). Water transparency in the estuarine system can be predominantly regulated by suspended solids transported by the freshwater flow into the system. During the study period (November 2013- March 2014), there was a significant reduced freshwater flow to the Murray Estuary and Coorong through the dams. In this study, the variation of water transparency among the regions are most likely due to plankton abundance rather than suspended solids transported by freshwater inflow. Water transparency can also affect fish behaviour, distribution and abundance as water clarity influences the feeding process of visual feeders (Gray *et al.* 2012). Thus, the increased abundance and distribution of small-mouthed hardyhead from south to north of the Murray Estuary and Coorong may be partially due to improved food availability regulated by water transparency.

In addition to salinity and transparency, pH may be a limiting factor in the estuarine system that reflects the combined effects of salinity, DO, nutrients, transparency and temperature on water quality. The pH variation to extreme values can negatively impact the physiological response of estuarine organisms (Ringwood and Keppler 2002). The spatial and temporal variation of pH in the current study is likely due to the variation of salinity along with water transparency and photosynthetic activities in the Murray Estuary and Coorong, but the pH change is within the suitable range for most fish species in the Coorong.

Likewise, water temperature and dissolved oxygen can affect fish distribution, growth and metabolism in estuaries (Harrison and Whitfield 2006). In saline water, DO and salinity are correlated depending upon ionic composition and concentration, temperature and air pressure (Williams 1998). Fish at the early life stage are usually more vulnerable to low oxygen (Levin *et al.* 2009). Typically, estuarine hypoxia (i.e., low oxygen) occurs due to water stratification and lack of water exchange from surface and bottom during extremely high salinity and temperature (Levin *et al.*, 2009). Hypoxia in an estuarine and marine ecosystem is also related to habitat structure and water depth and further influences prey-fish abundance and overall fish assemblages (Zhang *et al.* 2014). However, the influence of DO on prey-fish assemblage in the Murray Estuary and Coorong should be interpreted with caution as DO can co-vary with many other factors thus complicating the effect of hypoxia (Ye *et al.* 2012).

The variation in distribution of prey-fish assemblage could be associated with the environmental variability. Species-specific responses to these environmental factors are likely to reflect the physiological tolerance and adaptation of each fish species to the environmental conditions. Such variation may be ecologically important, allowing prey species to maximise the exploration for habitat use and food resources in dynamic estuary-hypersaline systems, and subsequently supporting higher level organisms through trophic links. This study improves our understanding of the dynamics of small-bodied prey species and key environmental drivers in a temperate estuary of a heavily regulated large river system in Australia. Such knowledge forms a basis for further developing our understanding of food web and trophic ecology in similar estuarine systems.

2.6 Acknowledgement

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Chapter 3

Environmental effects on growth performance of three forage fish in a hyper-saline lagoon: the Coorong, South Australia

This chapter has been submitted to the Journal of Applied Ichthyology as:

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3.1 Abstract

The present study investigates environmental impacts on growth performance of three forage fish small-mouthed hardyhead (*Atherinosoma microstoma*), Tamar goby (*Afurcagobius tamarensis*) and sandy sprat (*Hyperlophus vittatus*) in the Murray Estuary and Coorong. Fish were sampled using a seine net and fish age was estimated using the daily increment of sagittal otoliths to determine growth patterns of these three forage fishes. The estimated growth rates were 0.019 cm day^{-1} (r^2 = 0.98) for small-mouthed hardyhead, 0.038 cm day^{-1} (r^2 = 0.95) for Tamar goby and 0. 016 cm day^{-1} (r^2 = 0.94) for sandy sprat. The length-weight relationship indicated the slope (b = 2.96; r^2 = 0.97) in small-mouthed hardyhead, (b = 3.06; r^2 = 0.98) in Tamar goby and (b = 3.1; r^2 = 0.88) in sandy sprat. Spatiotemporal variation in the condition factor was observed in all three forage fish across the salinity gradients. The growth of forage fish was predominantly influenced by chlorophyll-*a*, water transparency, salinity and to a less extent by temperature and oxygen. This study suggests that environmental factors can significantly impact growth parameters of forage fish. The results provide new knowledge to explain growth variations of small-bodied forage fish in a reserve estuary with a wide gradient of salinity.

Additional keywords: salinity, chlorophyll, estuary, growth, forage fish,

3.2 Introduction

Estuaries are naturally dynamic environments with varying salinity, high nutrient input from runoff and high biological productivity. Estuaries support a large biological assemblages of multi-species including fish, waterbirds and invertebrates (Beck *et al.* 2001; Ferguson *et al.* 2008). Globally, estuaries are often subjected to the impacts of anthropogenic development, resource exploitation and river regulation (Morrongiello *et al.* 2014). In estuaries, environmental factors frequently vary and can influence the overall biological productivity (Gillanders and Munro 2012). Therefore, growth and development of estuarine organisms are likely to be affected by salinity change, hydrological alterations and temperature variation (Gillanders *et al.* 2011; Gillanders and Munro 2012; Madeira *et al.* 2013). Salinity (Boeuf and Payan, 2001; Panfili *et al.* 2004), temperature (Jenkins and King 2006) and food availability (Admassu and Ahlgren 2000; Massou *et al.* 2002) are commonly recognised as limiting factors for growth and development of fish. Thus, the ontogeny and life history traits of fish and other organisms can be influenced by natural variability of environmental factors in an estuarine system (Rogers and Ward 2007).

The Murray Estuary and Coorong are an inverse estuary and lagoon located at the terminus of the Australia’s largest river system, i.e., the Murray–Darling Basin. The Murray Estuary and Coorong are important habitats for large-bodied commercial and recreational fishes and small-bodied forage fish species (Brookes *et al.* 2015). In 1940’s, a series of dams were built across the Murray Mouth at the Murray Estuary to avoid saline water incursion to the Murray River and lower Lakes. From 2001 to 2010 the Murray Estuary and Coorong experienced the worst drought in history and low freshwater inflow from up streams (Ferguson *et al.* 2013). As a result, water salinity has increased throughout the system, generally with marine condition in the Murray

Estuary, marine to hyper-saline at North Lagoon and extremely hyper-saline (>100) at South Lagoon in the Coorong. Hyper-salinity is typical in the Coorong and persists even during the period of high freshwater flow into the Murray Estuary (Ye *et al.* 2012). During drought and low freshwater inflows, hyper-salinity condition exacerbated and the extent increased in the Coorong. Consequently, the ecological condition further degraded throughout the system (Ferguson *et al.* 2013; Leterme *et al.* 2015). Salinity is widely considered an overwhelming factor driving the ecological and physiological adaption of fish and other organisms in the Murray Estuary and Coorong (Webster 2010; Hossain *et al.* 2016). Hyper-salinity has adversely impacted the abundance and distribution of vertebrates and invertebrates (Noell *et al.* 2009; Dittman *et al.* 2015) in the Coorong. Salinity influences the abundance of food resources such as phytoplankton (Jendyk *et al.* 2014), picophytoplankton (Schapira *et al.* 2010) and zooplankton (Geddes *et al.* 2016) in the Coorong. Therefore, hyper-salinity associated with low freshwater flow can influence the growth and development of estuarine resident and migratory fish species in the Coorong (Gillanders *et al.* 2015).

Forage fish are small-bodied species and commonly fed on by piscivorous fish, birds and mammals in the aquatic ecosystem (Engelhard *et al.* 2014). Forage fish typically play an important role by transferring energy from low to high trophic levels (e.g., seabirds, marine mammals and carnivorous fishes) in estuarine and marine food webs (Pikitch *et al.* 2012). In the Coorong, small-mouthed hardyhead (*Atherinosoma microstoma*), sandy sprat (*Hyperlophus vittatus)* and Tamar goby (*Afurcagobius tamarensis*) are small-bodied fish that are important prey for piscivorous fish (Giatas and Ye 2016) and birds (Paton 2010). Thus, these forage fish are significant players in food webs and are ecologically important to the Coorong commercial fishery (Brookes *et al.* 2015). Small-mouthed hardyhead are widespread to temperate streams, inland lakes, estuaries and adjacent marine areas in south-eastern Australia, Tasmania and Victoria and the Coorong lagoon in South Australia (Thompson and Bray 2011). In the Coorong, this species is dominant in the South Lagoon and also found in the North Lagoon and the Murray Estuary (Eckert and Robinson 1990; Noell *et al.* 2009; Hossain *et al.* 2016). Tamar goby is commonly found in Victoria, New South Wales, eastern South Australia and northern Tasmania in Australia (Lintermans 2007). However, Tamar goby is mainly distributed in the Murray Estuary and part of North Lagoon in the Coorong (Hossain *et al.* 2016; Wedderburn *et al.* 2016). On the other hand, sandy sprat is common in estuaries and inshore waters in South Australia and are distributed from southern Queensland to southern Western Australia (Rowling *et al.* 2010). Sandy sprat migrate from sea to the Murray Estuary and, North Lagoon of the Coorong. However, Tamar goby and sandy sprats are completely absent at the South Lagoon in the Coorong (Hossain *et al.* 2016).

Fluctuation of environmental factors can affect fish growth, development and reproduction in estuaries (Gillanders *et al.* 2011). At the Bemm River estuary in Australia, freshwater inflow can influence the growth and spawning of the estuary perch *Percalates colonorum* (Morrongiello *et al.* 2014). At the Mundau lagoon in Brazil, salinity has impacted the growth and ontogeny of mullets *Mugil liza* (Sousa *et al.* 2015). In the Coorong, the elevated salinity and low freshwater flow have caused spatial and temporal variation in abundance, distribution and assemblage of forage fish (Hossain *et al.* 2016) and reduced the fish species diversity (Zampatti *et al.* 2010). The growth rate of sandy sprat larvae is reduced by salinity variation due to irregular freshwater flow in the lower reaches of the Murray River in the Coorong (Rogers and Ward 2007). Molsher *et al.* (1994) suggested that the changes of life history and reproductive ecology of small-mouthed hardyhead are attributed to food variability associated with salinity change.

However, our knowledge on growth performance of small forage fish species under extreme environmental conditions (e.g. salinity) is still limited.

Hyper-salinity usually occurs in the Coorong in dry summer when freshwater flow from the Murray River is low. This study covered the dry season from November to March when forage fish would experience high salinity stress in Coorong. The aim of this study was to determine the age-dependent growth pattern of three forage fish that have different habitat preference. We hypothesised that environmental variability could affect the growth of forage fish in the Murray Estuary and Coorong. The results of study would improve our understanding on the impact of salinity and other environmental factors on the growth and body condition of small-bodied fishes that greatly contribute to the forage of commercially important fishes.

3.3 Materials and methods

3.3.1 Study area

The Murray Estuary and Coorong are an inverse estuary and saline lagoon located 70 km south of Adelaide, South Australia (Fig. 3.1). The Murray Estuary is the terminal of the Murray River and connects the estuary and Coorong lagoon with the Southern Ocean by a narrow channel at the Murray Mouth. The Coorong is stretched by >100 km in length, ≈2 m mean depth and <4 km width and separated from the Southern Ocean by a narrow strip of peninsular sand-dune. Typically, the Murray this

Estuary and Coorong split into three distinct regions: Murray Estuary in the vicinity of the Murray River mouth (salinity 7–21), North Lagoon (salinity 20–76) and the South Lagoon (salinity 76–79; Livore *et al.* 2013). The Coorong is divided into two main lagoons, the North Lagoon and the South Lagoon. The North Lagoon is separated from the South Lagoon by a narrow and shallow channel at Parnka Point in the Coorong. Overall, the Murray Estuary and Coorong exhibits an inverse estuarine system with a north-south gradient of increasing salinity from 2 to ~80.

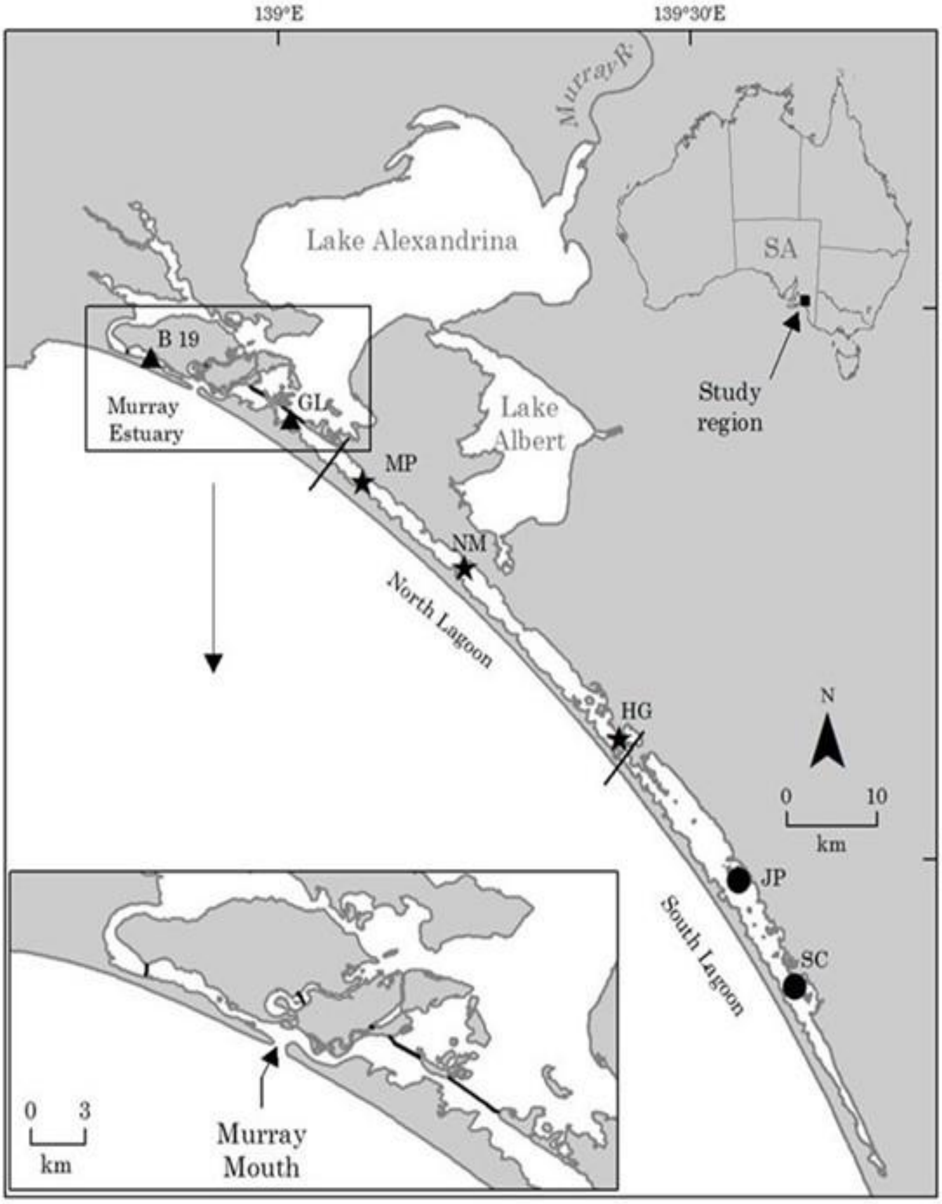

Fig. 3.1 Map of Murray Estuary, North Lagoon and South Lagoon showing the sampling sites; Beacon 19 (B19), Godfry's landing (GL), Mark point (MP), Noonameena (NM), Hells Gate (HG), Jack point (JP) and Salt Creek (SL) in the Coorong, South Australia.

3.3.2 Data collection

Sampling was performed at three regions: the Murray Estuary, the North Lagoon and the South Lagoon. Two sites in the Murray Estuary, three sites in the North Lagoon and two sites in the South Lagoon were selected for sampling to cover the existing typical broad salinity gradient in the Coorong. Fish were sampled using a seine net of 61 m long, 29 m wing length (22 mm mesh) and 3 m bunt length (8 mm mesh) at each site. Sampling was conducted on a monthly basis from November 2013 to March 2014 in a low flow year. The seine net was arrayed in a semi-circle and covered an area of ~ 600 m^2 to a maximum depth of 2 m at each site in the Murray Estuary and Coorong. Of the collected fish at each site, 20 individuals of each species of sandy sprat, Tamar goby and small-mouthed hardyhead were transferred to an aerated holding tank and euthanized using AQUI–S^{TM} (40 mg L^{-1}). The euthanized forage fish were preserved in 10% formalin for otolith collection in the laboratory. Naturally, the selected forage fishes are short-lived and perineal small-bodied species. Therefore, both the juveniles and adults of each species were collected to calculate the age of the forage fish from zero to one year. Length and weight of each individual fish species was recorded to the nearest mm of total length (TL) and weighed to the nearest 0.1 g (wet weight: WW). Zooplankton samples were taken in the vicinity of fish sampling sites using a modified 35-L Schindler-Patalas plankton trap with 50-µm mesh. Zooplankton collected in the cod-end were stored in a 250-mL plastic container and fixed in 5% formalin for identification and counting. Water samples were also collected and filtered to measure chlorophyll-*a* concentration on a spectrophotometer (Turner 450 Fluorometer). In addition, at each site three replicates of physicochemical variables including salinity, temperature, dissolved oxygen (DO) and pH were recorded at 30 cm below the water surface using a water quality meter (TPS, model 90FL) around mid-day. Water transparency was measured using a Secchi disk at each site on each sampling day. All samples were collected on a boat in the Murray Estuary and North Lagoon, and from the shore in the South Lagoon.

3.3.3 Laboratory analysis

3.3.3.1 Zooplankton identification

Zooplankton samples were poured onto a gridded Greiner square petri dish (12 × 12 cm) for identification and quantification. The individuals of zooplankton were identified and counted on an inverted microscope (Nikon Eclipse TS100F) to the lowest possible taxonomic level using identification keys (Hamond 1971; Hamond 1973; Smirnov and Timms 1983; Bayly 1992; Shiel 1995).

3.3.3.2 Otolith preparation

Sagittal otoliths were extracted from the small-mouthed hardyhead (n = 135), Tamar goby (n = 60) and sandy sprat (n = 95) using a pair of fine forceps (Dumont AA-Epoxy coated Forceps) on a dissecting microscope (Olympus SZ30) in the laboratory. Otoliths were then cleaned, dried, labelled and stored in plastic vials. As the otoliths of forage fish are very small in size, the grinding and polishing technique was used in order to obtain a thin transverse section (Pannella 1971; Ye *et al.* 2002). Otolith was mounted on a glass slide using thermoplastic resin (Crystalbond 509) in a manner that the anterior half of the otolith extended beyond the edge of the slide. Holding the slide to adjust otolith orientation, the anterior half was hand-ground away using 600 grit wet/dry sand paper. Then the ground face of the otolith was finely polished using three different grades of imperial lapping film (15 µm, 9 µm and 3 µm) based on the otolith's primordium. The slide was then heated and the remaining half of the otolith was removed and remounted in the centre on another glass slide with polished face down. The posterior half of the otolith was ground and polished until a transverse section of otolith was 250 µm thick and contained the otolith primordium. Immersion oil was used during the reading of the irregular surface for clear visualisation.

3.3.3.3 Age determination

The polished otolith mounted on slide was read and counted for the opaque rings on compound microscope (Olympus CX40) for daily age determination (Fig. 3.2). To assess ageing precision, three independent counts of daily increments for each otolith were performed without the prior knowledge of fish length or other data. The average of three readings was considered the age of fish. In addition, the average percent of error (APE) was used to measure the precision of the estimated age. The otoliths that showed >5% APE were rejected for age estimation (O’Sullivan 2007).

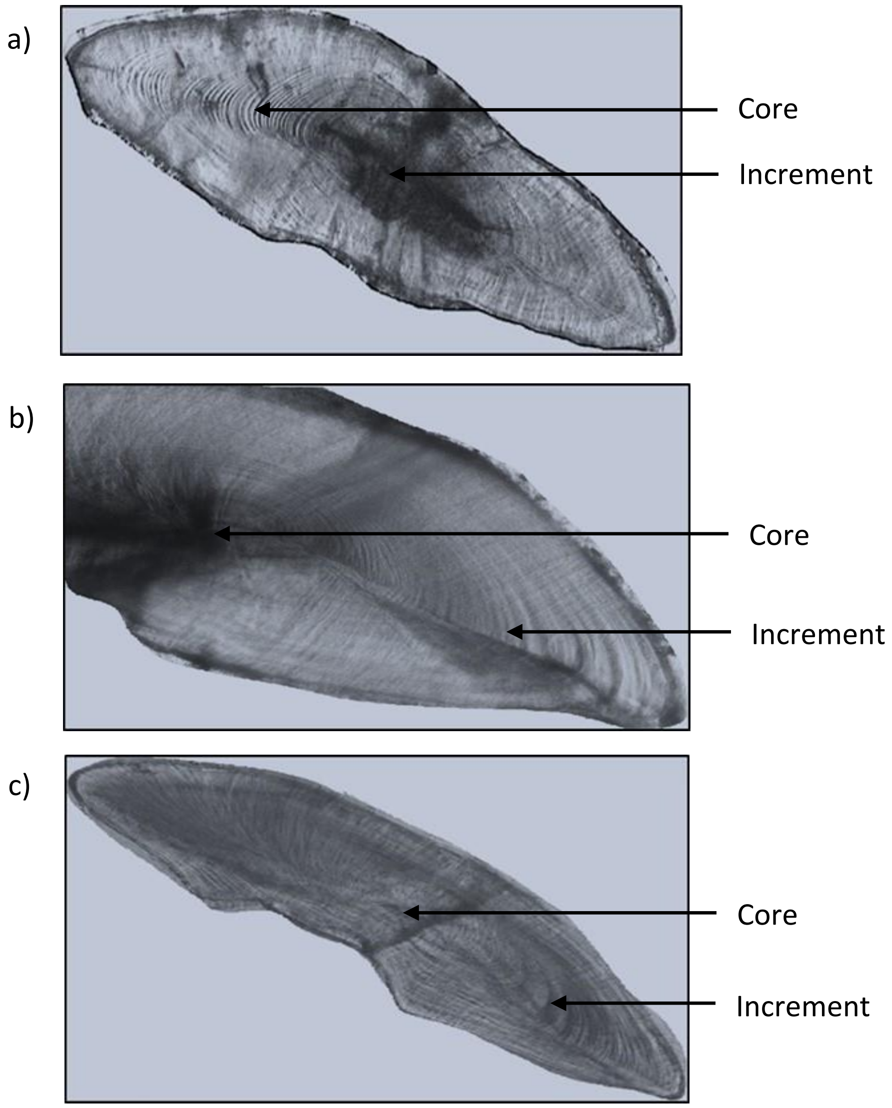

Fig. 3.2 Polished section of sagittal otolith of a) small-mouthed hardyhead, b) Tamar goby and c) sandy sprat showing daily growth increments (opaque zone; scale bar = 100 µm) from the Murray Estuary and Coorong.

3.3.4 Data analysis

Average percent error (APE) of counts were calculated as:

$$APE_j = 100 \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - X_j\right|}{X_j}$$

where R is the number of times the fish are aged, X_{ij} is the *i*th age determination of the *j*th fish, and X_j is the mean age estimate for the *j*th fish (Beamish and Fournier, 1981). Growth parameters were estimated by fitting the estimated age-at-lengths to the von Bertalanffy growth equation: $L_t = L_\infty [\ 1\text{-}e^{K(t\text{-}to)}]$, where L_t is total length (TL) at age t, L_∞ the theoretical asymptotic length, k the body growth coefficient and t_0 the theoretical age when fish length is equal to 0. Length-weight relationship of fish was calculated for each forage fish population using the power equation $W = qL^b$, where W is the total weight of the fish (g); L is the total length of fish (cm); q and b are the regression parameters. The 95% confidence limits of b were calculated to estimate differences between the individuals of each forage fish collected at different regions in the Murray Estuary and Coorong (Zar 1999; Aschenbrenner and Ferreira 2015). The condition factor of each forage fish was estimated to determine the growth performance of each species at different regions in the Murray Estuary and Coorong during the study period. The condition factor (q) of an individual was calculated using the transformed power equation $q = W/L^b$ (King 1995). The estimated b value from the power equation $W = qL^b$ was applied in estimation of condition factor.

3.3.5 Statistical analysis

The fourth root transformation of the condition factor data of all three forage fish was performed prior to analysis. The fourth root transformed data of the condition factor were used to construct a Bray-Curtis resemblance matrix (Anderson *et al.* 2008).

The environmental variables were normalised and used to construct a Euclidean distance resemblance matrices. Permutational analysis of variance (PERMANOVA; pseudo-P > 0.05) was run using the resemblance matrices to test the difference of each environmental variable (univariate) and growth performance of all three forage fish among months and regions in the Murray Estuary and Coorong (Clarke and Warwick 2001). In case of growth performance analysis, the model was designed with two factors including five sampling months as random five levels and three sampling regions as fixed three levels. For analysis of environmental variables, the design consisted of three factors, including months (random, 5 levels), regions (fixed, 3 levels) and sites nested within the region (random, 7 levels). Pairwise post-hoc comparisons using the multivariate analog of the *t*-test (pseudo-*t*) were performed at each level to identify significant difference. Unrestricted permutation was performed for each factor and interaction with 999 permutations to detect differences at $\alpha = 0.05$ (Anderson 2001). A distance-based linear model (DistLM) was performed to identify the effect of environmental and biological variables on condition factor of forage fish. Normalised environmental data, Shannon-Weaver index (*H'*) of zooplankton diversity and fourth root transformed condition factors of forage fish were used in DistLM analysis (Anderson *et al.* 2008). A distance based redundancy analysis (dbRDA) was then plotted during DistLM analysis to give a visual representation of the influence of environmental variables on the variation of condition factors. All tests were performed using PRIMER v6 (Clarke & Gorley, 2006) with the PERMANOVA+ add–on (Anderson *et al.* 2008).

3.4 Results

3.4.1 Environmental variables

Salinity was significantly different among months (*P* = 0.04) and regions (*P* = 0.003, Fig. 3.3a). In particular, a north-south increasing trend in salinity gradient was observed in the Coorong lagoon. Salinity was highly variable during the study period and ranged 2–30 in Murray Estuary, 11–75 in North Lagoon, and 40–85 in South Lagoon. In the Murray Estuary region, the highest salinity (~31) was measured at the Godfrey's landing site in March 2014 while the Beacon 19 site showed the lowest salinity (~2) in November 2013 (Fig. 3.3a). There was a remarkable variation in salinity in the North Lagoon with the highest salinity (~75) at the Hells Gate site in February 2014 and lowest salinity (11) at the Mark Point in January 2014 (Fig. 3.3a). Similarly, the highest salinity (~85) at the Jack Point in March 2014 and the lowest (~40) at the Salt Creek site in January 2014 were measured in the South Lagoon (Fig. 3.3a). In contrast, pH showed the significant spatiotemporal variation among months (*P* = 0.001) and regions (*P* = 0.036, Fig. 3.3b). The pH ranged 8.13–8.42 at Murray Estuary; 6.82–8.59 at North Lagoon and 6.11–8.27 at South Lagoon during the study period (Fig. 3.3b). The highest pH (8.59) was recorded at the Noonameena site in the North Lagoon in February 2014 and the lowest (6.11) was observed at the Jack Point in the South Lagoon in November 2013 (Fig. 3.3b). Water temperature showed temporal variation (*P* = 0.001) and ranged 17.50–22.73 ºC in the Murray Estuary, 17.37–22.87 ºC in the North Lagoon and 15.27–23.07 ºC in the South Lagoon. Water temperatures were higher in January 2014 and February 2014 compared to other sampling months in the Murray Estuary and North Lagoon (Fig. 3.3c). However, sampling in March 2014 demonstrated comparatively high water temperature in the South Lagoon and at Hells Gate of North Lagoon (Fig. 3.3c). In addition, the water transparency exhibited significant temporal (months; *P* = 0.038) and spatial variations (regions; *P* = 0.006) over the study period. The highest water transparency (200 cm) was measured at Godfrey's landing site in the Murray Estuary region in March 2014 and the lowest (25 cm) was observed at the Beacon 19 site in November 2014.

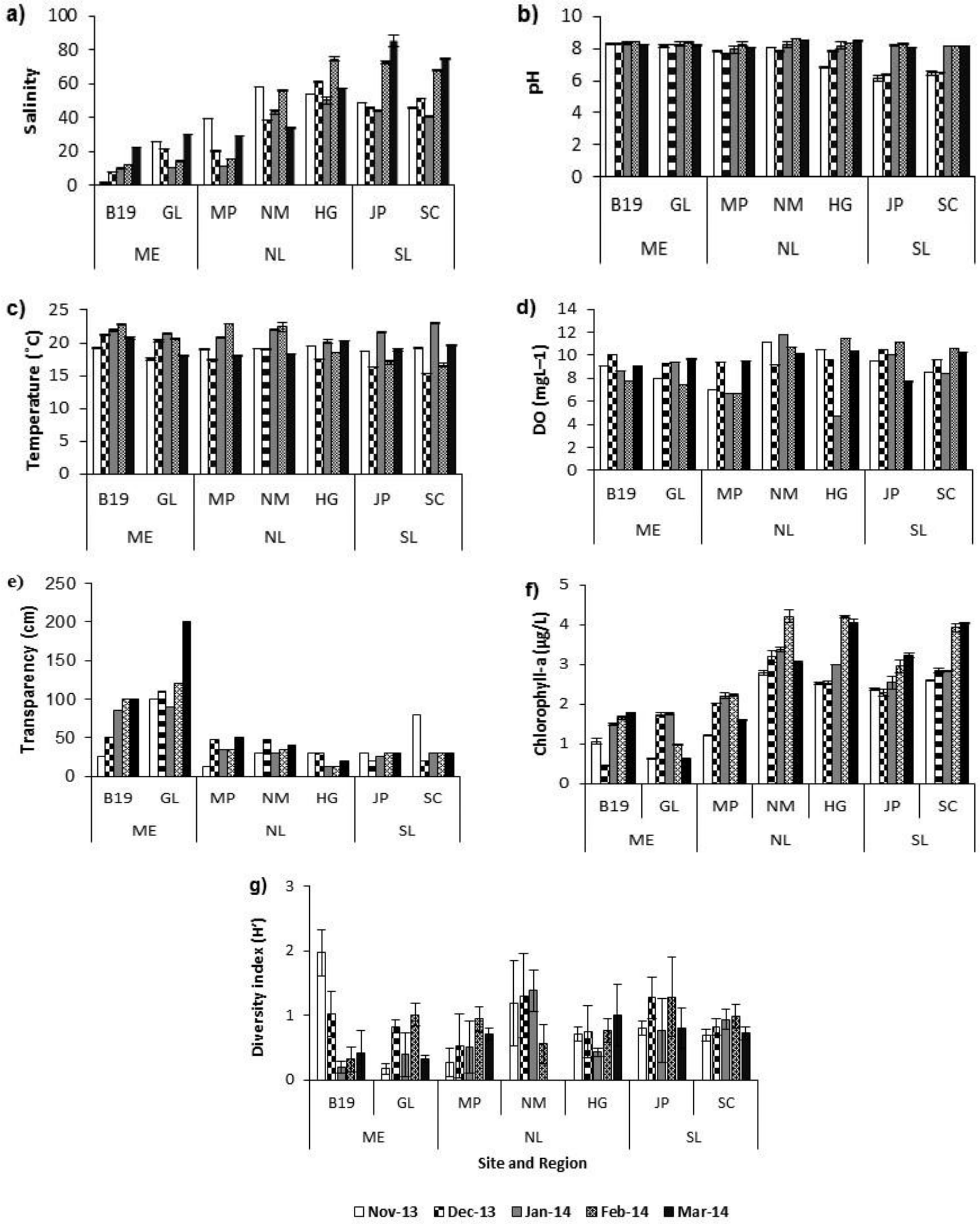

Fig. 3.3 Mean values ± S. E. of (a) salinity, (b) pH, (c) temperature (°C), (d) water transparency (cm), (e) dissolved oxygen (DO; mgL^{-1}), (f) chlorophyll-a (µg/L), (g) zooplankton diversity (*H'*) at each site in three regions (ME: Murray Estuary, NL: North Lagoon and SL: South Lagoon) in the Murray Estuary and Coorong from November 2013 to March 2014.

Water transparency exhibited maximum (50 cm) in March 2014 and minimum (12 cm) at the Mark Point site in the North Lagoon in November 2013. In the South Lagoon, the highest water transparency (80 cm) was detected at the Salt Creek site in November 2013 while the lowest water transparency (20 cm) was recorded at Jack point and Salt Creek sites in December 2013 (Fig. 3.3e). Similarly, chlorophyll-a showed significant temporal (months; $P = 0.024$) and spatial (regions; $P = 0.012$) variations and ranged 0.44–1.79 µg/L in the Murray Estuary, 1.21–4.21 µg/L in the North Lagoon and 2.27–4.03 µg/L in the South Lagoon. The highest chlorophyll-a (4.21 µg/L) was recorded at the Noonameena site in the North Lagoon in February 2014 and the lowest (0.44 µg/L) was observed at the Beacon 19 site in the Murray Estuary in December 2013 (Fig. 3.3f). However, DO and zooplankton diversity did not show any spatial and temporal variation during the study period.

3.4.2 Growth

Estimated age-at-length data of each individual of each forage fish were fitted to the von Bertalanffy model. In this study, the maximum total length of collected fish (small-mouthed hardyhead = 8.7 cm; Tamar goby = 8.9 cm and sandy sprat = 7.0 cm) were used as L_α and fitted to the von Bertalanffy model of each forage fish species. The von Bertalanffy model detected the growth rates (K = 0. 019 cm day^{-1}; $r^2 = 0.98$, Fig. 3.4a) in small-mouthed hardyhead, (K = 0.038 cm day^{-1}; $r^2 = 0.95$, Fig. 3.4b) in Tamar goby and (K = 0. 016 cm day^{-1}; $r^2 = 0.94$, Fig. 3.4c) in sandy sprat in the Murray Estuary and Coorong during the study period. Length-weight relationships were calculated using the data of 512 small-mouthed hardyhead, 226 Tamar goby and 344 sandy sprat. The estimated length and weight relationship was $W = 0.01 \times L^{2.96}$ with $r^2 = 0.97$ and 95% confidence limits; 2.88–3.04, Fig. 3.5a) in small-mouthed hardyhead. In case of Tamar goby, the relationship showed $W = 0.01 \times L^{3.06}$ ($r^2 = 0.98$ and 95% confidence limits; 2.88–3.24, Fig. 3.5b). Similarly, the length-weight relationship of sandy sprat was determined as $W = 0.01 \times L^{3.1}$ with 95% confidence limits 2.98–3.22 and $r^2 = 0.88$ (Fig. 3.5c).

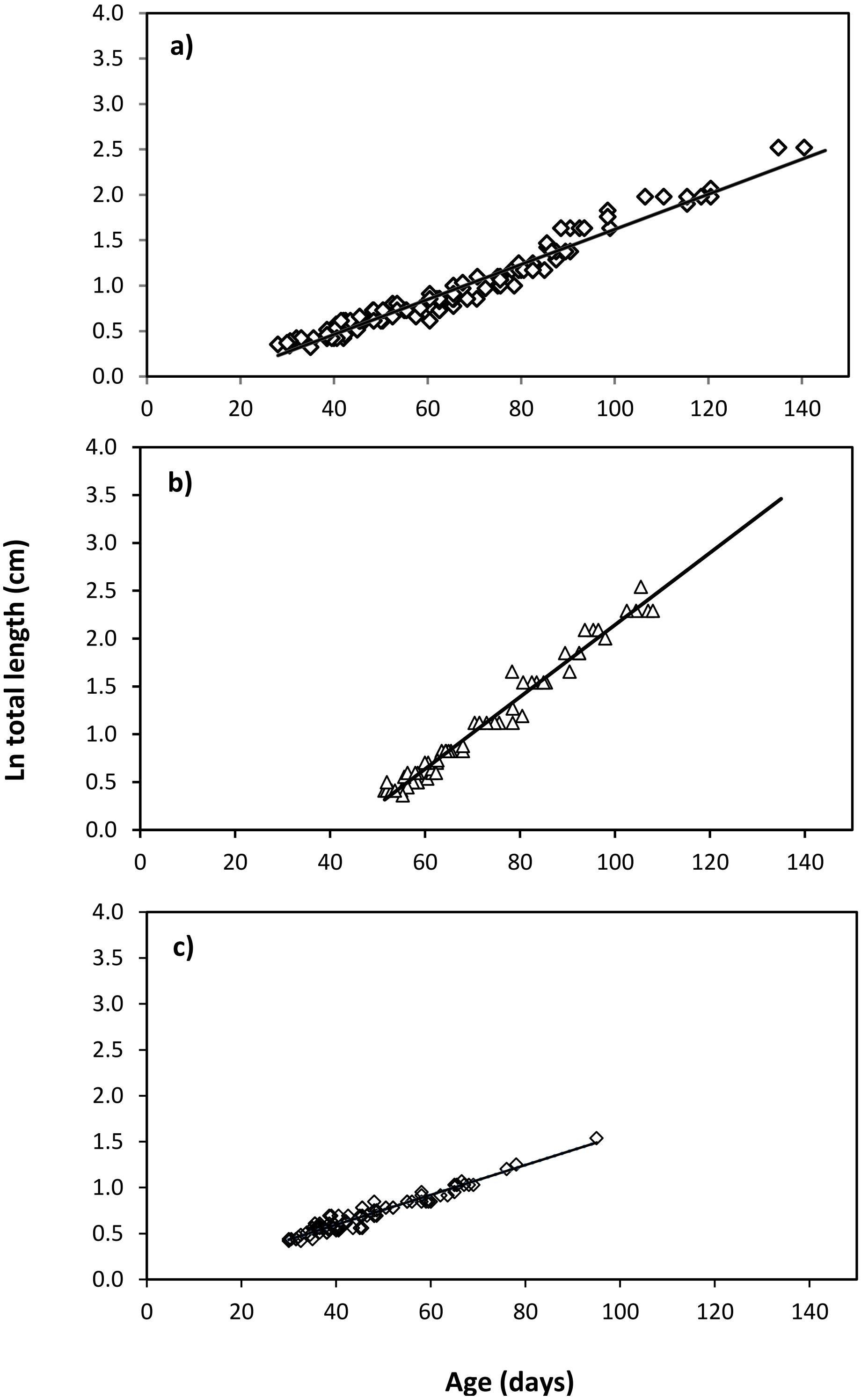

Fig. 3.4 The von Bertalanffy model of a) small-mouthed hardyhead, b) Tamar goby and c) sandy sprat. Natural logarithmic of length at age data were fitted to the model in growth rate estimation of each forage fish species.

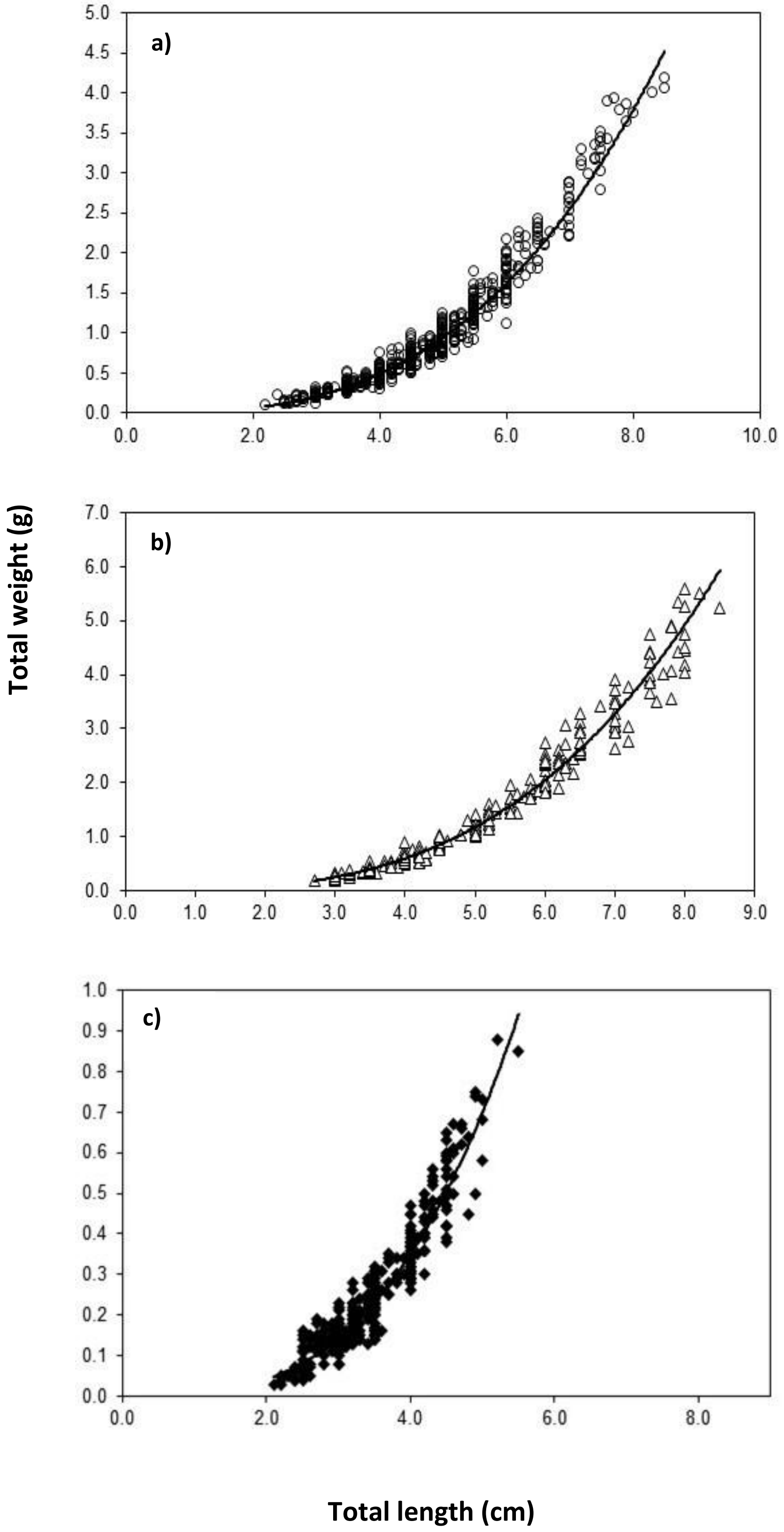

Fig. 3.5 Length-weight relationship of a) small-mouthed hardyhead, b) Tamar goby and c) sandy sprat from the Murray Estuary and Coorong.

3.4.3 Variation in condition factor

PERMANOVA showed a significant spatial (*P* = 0.004) and temporal (*P* = 0.001) variation in condition factors of all three forage fish in the Murray Estuary and Coorong during the study period (Table 3.1).

Table 3.1 PERMANOVA results of condition factors of all three forage fish at different regions in the Murray Estuary and Coorong. The data of fish condition factor were combined together as the forage fish are small-bodied and short-lived species. This PERMANOVA table includes fixed factors contributing to the changes of condition factor during this study. Significant difference was set at *P* < 0.05.

Source	**df**	**SS**	**MS**	**Pseudo-F**	***P* (perm)**
Month	4	586.22	146.55	10.375	0.001
Region	2	2015.50	1007.80	17.255	0.004
Month × Region	8	468.79	58.60	4.148	0.002
Residuals	1099	15524	14.136		

However, a month by region interactions (*P* = 0.002) were detected in condition factors of all three forage fish, suggesting that the pattern of variations were not consistent between months and regions. Pairwise test indicated significant differences in condition factors among the months except December 2013 *vs* January 2014, December-2013 *vs* March 2014 and January-14 *vs* March-14 (Table 3.2). Similarly, condition factors of forage fish were significantly variable among the regions except between the South Lagoon and the North Lagoon of the Coorong.

Table 3.2 PERMANOVA results of pair-wise comparison between the months and regions of condition factor of all three forage fish species in the Murray Estuary and Coorong.

Groups	**pseudo-*t***	***P* (perm)**
Nov-13 *vs* Dec-13	5.216	0.001
Nov-13 *vs* Jan-14	4.771	0.001
Nov-13 *vs* Feb-14	3.626	0.002
Nov-13 *vs* Mar-14	5.632	0.001
Dec-13 *vs* Jan-14	0.228	0.929
Dec-13 *vs* Feb-14	2.592	0.003
Dec-13 *vs* Mar-14	0.326	0.755
Jan-14 *vs* Feb-14	2.468	0.013
Jan-14 *vs* Mar-14	0.514	0.636
Feb-14 *vs* Mar-14	2.586	0.014
South Lagoon *vs* North Lagoon	1.710	0.164
South Lagoon *vs* Murray Estuary	3.968	0.036
North Lagoon *vs* Murray Estuary	5.694	0.005

3.4.4 Environmental effects on growth performance

Salinity (DistLM, P = 0.001), water transparency (DistLM, P = 0.001) and chlorophyll-a (DistLM, P = 0.001) were the most influential variables to predict the spatial and temporal variations in condition factor of all three forage fish in the Murray Estuary and Coorong (Table 3.3). These three variables were the best combination of predictors on the variation in condition factor, which together contributed 36% (proportion: 0.36) to the variation. However, water temperature and DO were also significant in the model (DistLM, *P* = 0.001) but these variables together explained only 0.8% (proportion: 0.008) variation of condition factor (Table 3.3). Similarly, in the dbRDA analysis, the first two axes (i.e. dbRDA1 and dbRDA2) explained 100% of the variability in forage fish condition factor while chlorophyll-a, salinity and water transparency were the main driving factors of that variability (Fig. 3.6).

Table 3.3 DistLM sequential results of environmental and biological variables on the condition factor of all three forage fish species at different regions in the Murray Estuary and Coorong over the study period (SS=Sum of Square; Prop = Proportion of the variation; Cumul= Cumulative variation).

Variable	**SS(trace)**	**Pseudo-F**	**DistLM *P***	**Prop.**	**Cumul.**
Salinity	1109.70	70.56	0.001	0.060	0.060
pH	27.42	1.74	0.180	0.001	0.061
Temperature	97.93	6.26	0.008	0.005	0.066
Dissolved oxygen (DO)	54.91	3.52	0.049	0.003	0.069
Water transparency	1373.00	95.47	0.001	0.074	0.143
Chlorophyll-a	4241.40	401.50	0.001	0.228	0.371
Zooplankton diversity	4.21	0.40	0.562	0.001	0.371

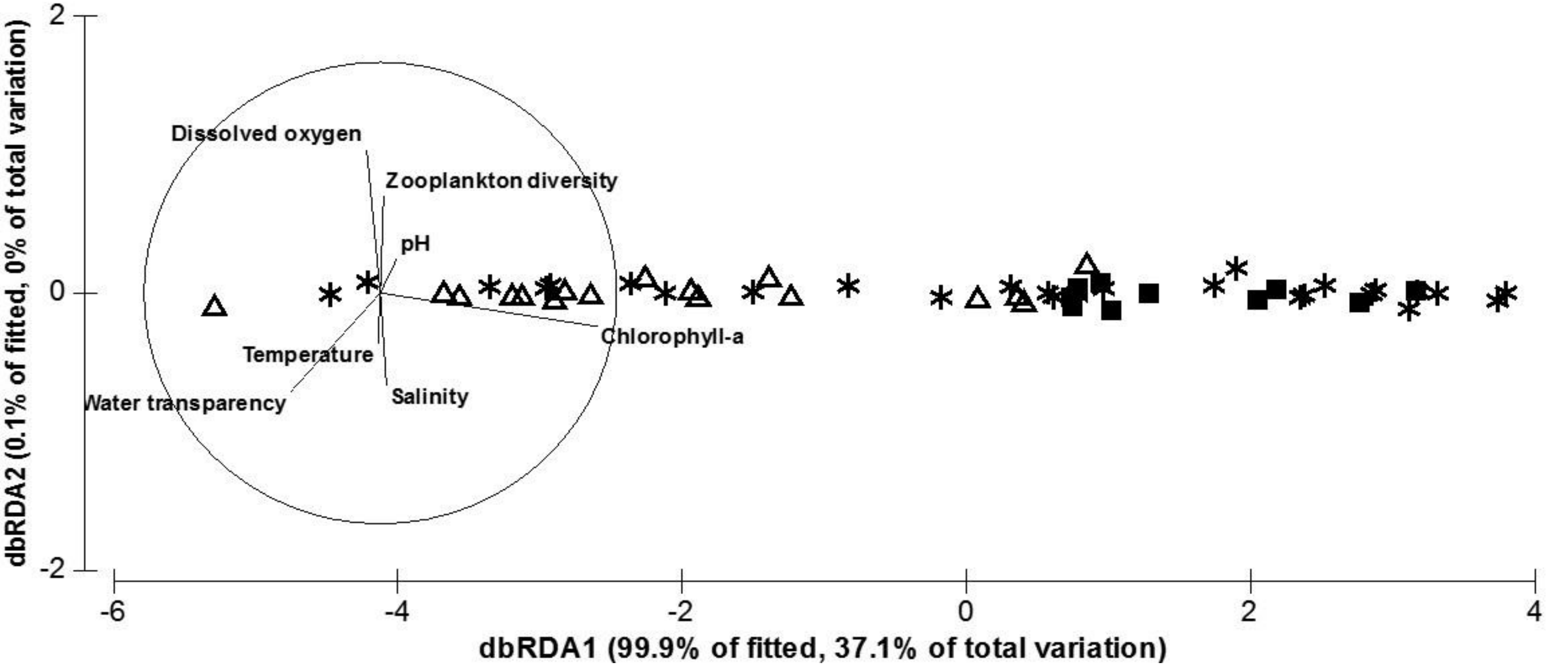

Fig. 3.6 dbRDA ordination of fourth root transformed condition factor of forage fish at three regions in the Murray Estuary and Coorong *versus* predictor variables: chlorophyll-a, water transparency, salinity, pH, water temperature, DO and zooplankton diversity. Data points are displayed as Murray Estuary (triangle), North Lagoon (asterisk) and South Lagoon (square).

3.5 Discussion

The growth coefficient and L_∞ derived from the von Bertalanffy model indicate rapid growth of small-mouthed hardyhead and Tamar goby. In the current study, the estimated growth rate was 0.19 mm day^{-1} and L_∞= 8.7 cm for small-mouthed hardyhead. This result is similar to the growth pattern of small-mouthed hardyhead found in a previous study in the Coorong (Molsher *et al.* 1994) and other estuaries in Australia (Prince and Potter 1983; Potter *et al.* 1986). Small-mouthed hardyhead is a multiple spawner with protracted breeding season from September to December in the Coorong and exhibits post-breeding mortality at the end of the first year spawning (Molsher *et al.* 1994; Potter *et al.* 1986). In October fish larvae usually recruit to the spawning population and most adults die in November after spawning (Molsher *et al.* 1994). It is therefore likely that the fast growth is an adaptation for early recruiting to the spawning population.

Likewise, Tamar goby has a short lifespan of 1-2 years (Lintermans 2007). The Tamar goby is a ubiquitous spawner and spawns exclusively during spring (October–December) but spawning lasts over 5 months (Cheshire *et al.* 2013). In the current study, the growth rate of Tamar goby was 0.38 mm day^{-1} with the largest size of 8.9 cm in the Coorong. Of the goby species, Tamar goby is usually most abundant in the lower reaches of the Murray River Estuary and the North lagoon in the Coorong (Noell *et al.* 2009). The extended spawning season with greater recruitment of Tamar goby is usually accomplished during spring and summer in the Coorong (Cheshire *et al.* 2013). Therefore, current growth pattern of Tamar goby is likely accorded with the recruitment of the young in the dry season.

In the present study, sandy sprat showed the growth rate 0.16 mm day^{-1} with L_∞= 7 cm in the Murray Estuary and Coorong. Rogers and Ward (2007) reported the average growth rate 0.12 mm day^{-1} for the 20.1–27.6 mm length juvenile sandy sprat in the Coorong. Despite slow growth of larval sandy sprat in previous studies in the Coorong (Rogers and Ward 2007) and in the coast of south-western Australia (Gaughan *et al.* 1996), this species showed more rapid development in the Murray Estuary and Coorong in this study. In particular, the growth and abundance of sandy sprat are strongly related to the freshwater inflow and salinity regime in the Coorong (Brookes *et al.* 2015). Sandy prat requires marine conditions for spawning though this species uses estuaries as feeding and nursery habitats (Gaughan *et al.* 1996). In this study, marine salinity at the Murray Estuary and the North Lagoon might stimulate the spawning of sandy sprat during the study period, and the rapid growth of new recruits could potentially be mediated by the increased productivity associated with freshwater inflows to the Murray Estuary.

Length-weight relationship indicates a positive isometric growth (slope b = 3.04) for small-mouthed hardyhead in the Murray Estuary and Coorong. Our finding is similar to the growth (b = 2.79–3.13) of this species in the Coorong two decades ago (Molsher *et al.* 1994) and other atherinids such as sand smelt *Atherina boyeri* (b = 3.33) at Mellah Lagoon in Eastern Algeria (Boudinar *et al.* 2016). Growth variation (*b* values) in fish can be influenced by environmental variables including salinity and temperature in the estuary (Ricker 1975). Particularly, salinity variation is an overwhelming factor influencing fish growth through extra energy spending on osmoregulation in the estuary (Boeuf and Payan 2001). Small-mouthed hardyhead is a euryhaline estuarine fish and tolerates a wide range of salinity fluctuation (LD_{50}: 3.3 – 108; Lui 1969) both in the laboratory and field. The ability of salinity tolerannce enables the wide distribution of small-mouthed hardyhead across different regions in the Coorong (Wedderburn *et al.* 2016). In this study, current growth trend of small-mouthed hardyhead is most likely attributed to its wide salinity tolerance that allows this species to explore abroad food resources in the Coorong.

In contrast, Tamar goby (slope b = 3.06) showed positive allometric growth at the Murray Estuary and at the North Lagoon in the Coorong. The growth of Tamar goby is similar to other gobiids where the rock goby *Gobius paganellus* shows allometric growth (b = 3.163) in Azores, Portugal (Azevedo and Simas 2000). Tamar goby in the Coorong shows LC_{50} salinity tolerance of 73.2 at 14 ºC (winter) and 71.4 at 23 ºC (summer) in the laboratory condition (McNeil *et al.* 2013). Despite the hyper-marine salinity tolerance of Tamar goby, this species is completely absent in the South lagoon (salinity 40–85) in the Coorong (Ye *et al.* 2012; Hossain *et al.* 2016). The spawning and recruitment success of Tamar goby are inhibited by the varying salinity regime associated with low freshwater flow into the Coorong (Bice 2010). Thus, the wide salinity variation in the Coorong contributes to the discrepant growth of Tamar goby at the Murray Estuary and the North Lagoon in the Coorong.

Similarly, sandy sprat exhibited allometric growth (slope b = 3.1) at the Murray Estuary and at the North Lagoon in the Coorong. The length-weight relationship of sandy sprat in the present study is similar to other clupeoid species such as the anchovy *Engraulis encrasicolus* (slope b = 3.134) in Black Sea (Satilmis *et al.* 2014). Usually the length-weight relationship in fish is age-specific and varies with sex, gonad maturity and the spawning period (Wootton 1998). The marine sandy sprat frequently migrate to the nearby estuaries and wetlands for breeding and larval nursing (Gaughan *et al.* 1996; Rogers and Ward 2007). In the Coorong, the spawning of this species occurs from October to February (spring and summer) and peaks in November (Rogers and Ward 2007). It is possible that the sandy sprat would migrate to the Coorong for breeding and feeding during the study period.

The condition factor of fish depends on gonadal development, food availability and environmental variability in the estuarine system (Froese 2006; Morrongiello *et al.* 2014). Russell *et al.* (2015) reported that the growth performance of barramundi (*Lates calcarifer*) is better in Lake Tinaroo where the prey was more abundant than in the Johnstone River in Australia. In the current study, the spatiotemporal variation in forage fish is possibly related to the preferred food resources in the Coorong. Among forage fishes, small-mouthed hardyhead and sandy sprat feed on planktonic and epi-benthic prey in estuaries (Prince *et al.* 1982; Humphries and Potter 1993) while Tamar goby is an epibenthic feeder (Humphries and Potter 1993). Low freshwater flow during the drought period reduces the diversity of zooplankton (Geddes *et al.* 2016) and benthic organisms (Dittmann *et al.* 2015) in the Coorong. Thus, the ultimate low food variability is likely reflected on the variation of condition factors of these three forage fish species in the Coorong.

Growth performance of all three forage fishes is related to the changes in chlorophyll-a, water transparency and salinity. In this study, chlorophyll-a and transparency together explained ~30% variation of the condition factor. This result is supported by other studies where high chlorophyll-a and transparency are positively related to the growth of tilapia (*Oreochromis leucostictus*) in Ugandan crater lakes (Efitre *et al.* 2009). Typically, the production of chlorophyll-a can be related to the water transparency and can often be associated with plankton bloom. Hemraj *et al.* (2017) reported the interactions in plankton community associated due to the variation in water flow in the Murray Estuary and Coorong. Thus, the variation in chlorophyll-a in this study is possibly influenced by the changes in water transparency regulated by freshwater flow rather than plankton bloom in the Murray Estuary and Coorong. Leterme *et al.* (2015) reported a major shift of phytoplankton community from chlorophytes at Murray Estuary to diatoms and picophytoplankton at North lagoon and South lagoon, indicating variation in primary productivity across different salinity regions in the Murray Estuary and Coorong. It is likely therefore, the diversity and abundance of prey organisms (zooplankton and benthos) can be influenced by the variability of primary production that ultimately impacts the fish growth in the Coorong.

In addition to chlorophyll-a, water transparency significantly influenced the growth of forage fish in the present study. Water transparency usually regulates productivity by influencing primary productivity through photosynthesis (Herman and Heip 1999). The impact of water transparency on abundance, distribution and growth of fish and other organisms is widely reported in wetlands and estuaries (Manning *et al.* 2013; Rosso *et al.* 2010). The growth performance of yellow perch (*Perca flavescens*) is impacted by water transparency in Lake Erie, USA (Manning *et al.* 2013). Abundances of *Cnesterodon decemmaculatus*, *Jenynsia multidentata*, *Corydoras paleatus*, *Pimelodella laticeps* and *Odontesthes bonariensis* are significantly affected by water clarity at Mar Chiquita, Gómez, Carpincho and Rocha Lakes in Argentina (Rosso *et al.* 2010). In addition, water transparency can affect fish growth by interfering the feeding process of visual feeders (Gray *et al.* 2012). Thus, the difference of growth performance of forage fish among regions could be attributed to food availability and water transparency in the Murray Estuary and Coorong.

The changes in salinity can be often an overwhelming stressor regulating fish growth and productivity in estuaries (Boeuf and Payan 2001; Harrison and Whitfield 2006). In the present study, salinity explained 6% of fish condition factor. Salinity can be an ecological factor and physiological barrier limiting the function of aquatic organisms (Telesh and Khlebovich 2010). In particular, the growth of estuarine fishes is often affected by salinity tolerance because most energy is utilised in osmoregulation instead growth (Cardona 2000). Panfili *et al.* (2004) reported that hyper-salinity (>60 psu) affects the growth performance and reduces the size-at-maturity of Bonga shad *Ethmalosa fimbriata* at the inverse Saloum estuary in West Africa. In the Coorong, salinity variation is likely to influence growth through energy reallocation to osmoregulation between geographic regions.

Water temperature and DO only explained <1% of fish condition in forage fishes in the Coorong. Although temperature can influence the growth of estuarine fish species such as black bream (*Acanthopagrus butcheri*) in the Murray River estuary (Doubleday *et al.* 2015), the impact of temperature on growth performance of forage fish was not detected in this study. The possible reason is that the temperature range (15.27–23.07 ºC) during the study period is suitable for the growth of these forage fish species in the Coorong. Hypoxia can potentially impact the growth and ontogeny of estuarine fishes (Froeschke and Stunz 2012). However, estuarine hypoxia generally occurs due to water stratification and lack of water exchange from surface and bottom habitats (Levin *et al.* 2009). In this study, the oxygen level ranged from 4.70 to 11.80 mg/L, which is much higher than the hypoxia level and is unlike to affect the growth of forage fish in the Coorong (Williams 1998).

In summary, the von Bertalanffy model and length-weight relationship suggest a trend of fast growth of all three forage fish in the early life history, but fish growth performance varied among regions in the Coorong. Chlorophyll a is the most important single variable that explained ~23% of growth variation. Chlorophyll *a,* water transparency and salinity together explained ~36% of growth variations. However, temperature, dissolved oxygen and pH only contributed 0.9% towards growth variation. The results of this study improve our understanding on how environmental factors could contribute to the variation of growth performance of forage fishes in an estuarine-hypersaline lagoonal system. Such knowledge would improve our understanding on the environmental regulation on the growth performance of small-bodied forage fish in estuarine systems.

3.6 Acknowledgement

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Chapter 4

Diet overlap and resource partitioning among three forage fish species in Coorong, the largest inverse estuary in Australia

This chapter has been submitted to Environmental Biology of Fishes as:

Hossain, A., Hemraj, D., Ye, Q., Leterme, S., and Qin, J. (2016). Diet overlap and resource partitioning among three forage fish species in Coorong, the largest inverse estuary in Australia. (under review).

4.1 Abstract

As the largest inverse estuary in Australia, the Coorong has been degraded by protracted drought due to low river flows to the estuary. The present study investigates the gut content, prey composition and dietary overlap of three forage fishes small-mouthed hardyhead (*Atherinosoma microstoma*), Tamar goby (*Afurcagobius tamarensis*) and sandy sprat (*Hyperlophus vittatus*) influenced by environmental variation in the Murray Estuary and Coorong. The prey species identified in fish stomachs were dominated by crustaceans (amphipods, ostracods and harpacticoids), but nematodes and acanthocephalans were also common to all forage fishes. The diet of the sandy sprat and Tamar goby highly overlapped in the Murray Estuary and all three forage fishes showed potential diet overlap in the North Lagoon. Spatiotemporal variation of prey diversity was observed in small-mouthed hardyhead while temporal variation of prey diversity was observed in sandy sprat and Tamar goby. Overall, the prey abundance was temporally variable and pre-dominantly regulated by salinity, pH, dissolved oxygen, water transparency and chlorophyll *a* in the Murray Estuary and Coorong. This study adds to our knowledge on dietary overlap and resource partitioning among small-bodied forage fishes mediated by environmental factors in the Murray Estuary and Coorong.

Additional keywords: salinity, pH, chlorophyll, forage fish, zooplankton, gut content

4.2 Introduction

Estuaries are highly productive and often function as a habitat for migration, breeding and feeding ground for fish and other fauna (Costello *et al.* 2002; Jendyk *et al.* 2014). Thus, a productive estuary supports the abundance and diversity of fish species (Dolbeth *et al.* 2008; Whitfield 1999). The ontogeny, survival and growth of fish are largely influenced by habitat and food availability in an estuary (Taylor *et al.* 2006). Typically, the estuarine productivity is spatiotemporally variable due to environmental changes (Whitfield 1999) and primary production is greatly regulated by physical and biological factors within the dynamic system (Day *et al.* 1989). Therefore, the food variability in estuaries can affect predator–prey interactions (i.e., prey selection; Cantanhêde *et al.* 2009) and result in potential overlapping in diet due to species competition (Eriksson *et al.* 2005).

Prey selection by fishes can be regulated by relative abundance, distribution and presence of prey types in estuaries and other aquatic environments (Cantanhêde *et al.* 2009). In contrast, fish dietary overlap can be influenced by prey selection associated with intraspecific and interspecific food competition and resource partitioning (Abrantes *et al.* 2015). Dietary overlap in fish is mainly regulated by species competition for habitat use (Munday *et al.* 2001; Wedderburn *et al.* 2014), foraging behaviour (Higginson and Rusxton 2015) and developmental stage of fish (Nunn *et al.* 2012). For instance, variation in prey selection and overlapping in diets of redfin perch (*Perca fluviatilis*) and golden perch (*Macquaria ambigua ambigua*) were observed during drought and flood condition in terminating lakes of the Murray–Darling Basin in Australia (Wedderburn *et al.* 2014). In other study, Bachiller and Irigoien (2015) reported significant dietary overlap among the small pelagic fish species in the Bay of Biscay in Spain due to spatial variation in food availability. However, environmental variability may have a major effect on feeding ecology of fish through influencing the spatial and seasonal variation of food availability (Xie *et al.* 2000).

The Coorong is an inverse estuary at the terminus of the largest river system (Murray–Darling River) in Australia. In the 1930s, a series of barrages were constructed to separate the Murray Estuary and Coorong from the riverine freshwater system to prevent saline water intrusion up to the Murray River and adjacent lakes (Webster 2010). The Coorong is recognized as a habitat of significant importance to the native and migratory fish and bird species (Paton 2010). Naturally, the Murray Estuary and Coorong promotes the proliferation of small-bodied forage fish that are used as food for piscivorous fish to support commercial and recreational fisheries (Brookes *et al.* 2015). Since the European settlement, the Murray Estuary and Coorong have dramatically changed from their natural form due to river regulation, water extraction and the construction of tidal barrages that create a physical and ecological barrier between the marine and freshwater environments (Webster 2010). In the last decade, the Murray Estuary and Coorong were more severely impacted, leading to ecological degradation due to protracted drought and lack of freshwater inflow from up streams. Consequently, the water salinity became marine in the Murray Estuary, hypersaline in the North Lagoon and extremely hypersaline (>100) in the South Lagoon (Webster 2010; Leterme *et al.* 2015).

Salinity is the most driving factor influencing the variation in abundance and distribution of flora and fauna in the Murray Estuary and Coorong (Brookes *et al.* 2015). Elevation of salinity associated with drought and low freshwater flow has reduced the abundance and distribution of phytoplankton (Jendyk *et al.* 2014), zooplankton (Geddes *et al.* 2016) and benthos (Dittmann *et al.* 2015) in the Murray Estuary and Coorong. Hyper-salinity due to low freshwater flow has reduced fish species diversity in the

Coorong (Zampatti *et al.* 2010). Noell *et al.* (2009) investigated the flow related effects on fish ecology and found a declining trend in fish species diversity along the salinity gradient in the Murray Mouth and Coorong region. Consequently, in the South Lagoon, only one euryhaline species small-mouthed hardyhead persisted in extreme hyper salinity (Hossain *et al.* 2016). Recently, Geddes *et al.* (2016) reported the low diversity of zooplankton community in the Murray Estuary and Coorong compared to other estuaries in south-east Australia. Low diversity and availability of food resource may affect the overall food web and feeding ecology of small-bodied forage fishes in the Murray Estuary and Coorong region.

Small-bodied forage fishes are an important component in the marine and estuarine food webs and can transfer energy from primary producers to piscivorous fish, birds and mammals (Springer and Speckman 1997). In the last few decades, research in forage fish in marine and estuarine habitats have been globally active due to its ecological importance, commercial use for animal food and significant contribution to human food security (Alder *et al.* 2008). Forage fishes such as small-mouthed hardyhead (*Atherinosoma microstoma*), Tamar goby (*Afurcagobius tamarensis*) and sandy sprat (*Hyperlophus vittatus*) are the main prey species for a range of fish, birds and mammals in the Murray Estuary and Coorong (Deegan *et al.* 2010; Paton 2010). Small-mouthed hardyhead is mostly abundant at the southern part of the Coorong although is widely distributed throughout system (Noell *et al.* 2009; Hossain *et al.* 2016). On the other hand, Tamar goby and sandy sprat are abundant in the Murray Estuary and North Lagoon but usually absent in the South Lagoon of the Coorong (Hossain *et al.* 2016; Wedderburn *et al.* 2016). The abundance and distribution of forage fish are greatly influenced by the changing salinity in the Murray Estuary and Coorong (Zampatti *et al.* 2010; Hossain *et al.* 2016).

In this inverse estuary, recent studies have focussed on fish trophic ecology (Geddes and Francis 2008), trophic dynamics (Deegan *et al.* 2010) and diets of large estuarine fish species (Giatas and Ye 2015). Geddes and Francis (2008) reported that change in prey abundance has strongly affected the overall trophic ecology and food web structure in the Coorong. Deegan *et al.* (2010) found the length reduction of food chain among fish species is attributed to the decline of prey diversity along with the increasing salinity gradient in the Murray Estuary and Coorong. Very recently, Bice *et al.* (2016) reported that the freshwater discharge to the Murray Estuary could improve the productivity in the system. However, the complex food web in the Murray Estuary and Coorong is not well understood. Other than few emperical data, little is known on prey selection, dietary composition and resource partitioning among the small-bodied forage fish species in the Murray Estuary and Coorong where a great variation of salinity prevails in the environment.

The aim of this study was to investigate prey selection and diet overlap among three key forage fish species, small-mouthed hardyhead, Tamar goby and sandy sprat in the Murray Estuary and Coorong. We hypothesise that (i) the prey composition and abundance are affected by the variation of salinity and other environmental variables in the Murray Estuary and Coorong and (ii) the elevated salinity would lead to a shift in food resource and an increase of diet overlap among forage fish species.

4.3 Materials and methods

4.3.1 Study region

The Murray Estuary and Coorong is located 70 km south of Adelaide, South Australia. The Coorong is a long (>100 km), narrow (<4 km) and shallow (mean depth ≈2 m) estuarine lagoon and lies at the terminus of the largest Murray-Darling River in

Australia. The Coorong is a wetland of international importance for supporting numerous species of fish, invertebrates and birds (Paton 2010). Typically, the Murray Estuary and Coorong split into three distinct regions: Murray Estuary in the vicinity of the mouth of the Murray River, North Lagoon and South Lagoon (Fig. 4.1).

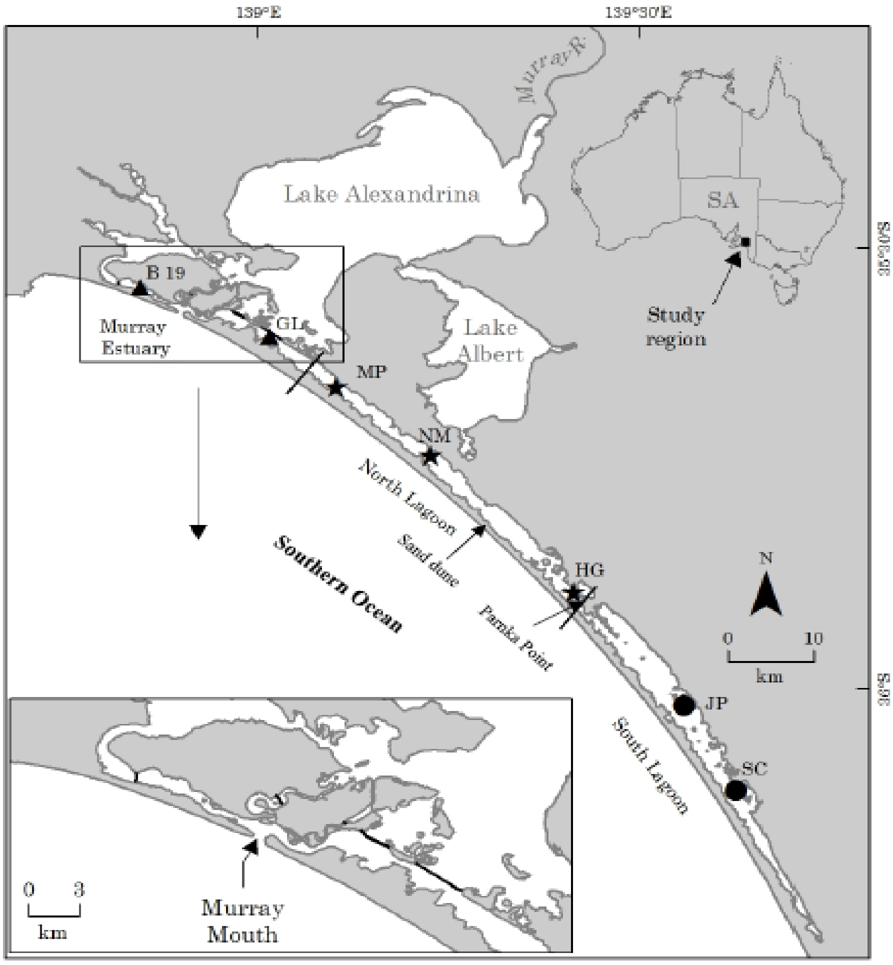

Fig. 4.1 Map of the Murray River estuary, North Lagoon and South Lagoon showing the 7 sites; Beacon 19 (B19), Godfry's landing (GL), Mark point (MP), Noonameena (NM), Hells Gate (HG), Jack point (JP) and Salt Creek (SC).

The Murray Estuary is connected to the Southern Ocean and Encounter Bay by a narrow channel at the Murray Mouth of the Murray River. The Coorong is protected from the Southern Ocean by a barrier of established fore sand-dune and is naturally divided into the North Lagoon and South Lagoon at the Parnka Point near the Hells Gate. A series of tidal barrages separate the Murray Estuary and Coorong from the Murray River and Lower Lakes (i.e., Lake Alexandrina and Lake Albert) and form distinctive environmental features in the system. As a result, the Murray Estuary is a dynamic system influenced by both tidal flushes and Murray River flows. While the Murray Estuary and the North Lagoon are more affected by freshwater releases from the Lower Lakes via the barrages, the South Lagoon also receives low inflows of South East Drainage Scheme (SEDS) from Salt Creek (Ye *et al.* 2011). Overall, the Murray Estuary and Coorong is characterised as a reverse estuary with strong north-south gradients of increasing salinity.

4.3.2 Field sampling

Sampling was conducted at three regions: the Murray Estuary, the North Lagoon and the South Lagoon from November 2013 to March 2014. Two sites in the Murray Estuary, three sites in the North Lagoon and two sites in the South Lagoon were selected for sampling to cover the broad salinity gradient typical of that environment. At each site, forage fishes were sampled using a seine net in the Murray Estuary and Coorong lagoons. A seine net of 61-m long, 29-m wing length (22-mm mesh) and 3-m bunt length (8 mm mesh) was used in a semi-circle and swept over an area ~ 600 m^2 to a maximum depth of 2 m at each site to catch both juvenile and adult forage fish. Among the fish collected, 20 individuals of each species of small-mouthed hardyhead, Tamar goby and sandy sprat were transferred to an aerated holding tank and euthanised using AQUI–S^{TM} (40 mg L^{-1}). The euthanised forage fish were preserved in 10% formalin for gut content analysis. Zooplankton samples were taken in the vicinity of fish sampling sites using a modified 35-L Schindler-Patalas plankton trap with 50-µm mesh from ≈1 meter water depth in the Murray Estuary and Coorong. Zooplankton collected in the cod-end were stored in a 250-mL plastic container and fixed in 5% formalin for identification and counting. Water samples were also collected and filtered to measure chlorophyll-*a* concentration on a spectrophotometer (Turner 450 Fluorometer).

Along with forage fish and zooplankton sampling at each site, physicochemical variables including salinity, water temperature, dissolved oxygen (DO) and pH were measured at 30 cm below water surface using a water quality meter (TPS, model 90FL) around mid-day. Water transparency was also measured using a Secchi disk. Three replicates were used at each sampling site. All samples were collected on a boat in the Murray Estuary and North Lagoon, and from the shore in the South Lagoon.

4.3.3 Laboratory analysis

4.3.3.1 Gut content

To assess the fish gut content, a small incision was made through the abdomen of each fish and the entire gut was removed and transferred to a petri dish. The contents of the gut were then removed using a fine forceps and all the contents (prey) were identified up to the lowest taxon and counted under a dissecting microscope (Olympus SZ30). In some cases, a compound microscope (Olympus CX 40) was used for species identification.

4.3.3.2 Zooplankton identification

Collected samples (250 ml) were allowed to settle the organisms at the bottom of the sampling jar and excess formalin solution was carefully removed with a 60 mL syringe to avoid resuspension of the organisms. Zooplankton samples were poured onto a gridded Greiner square petri dish (12 × 12 cm). An inverted microscope (Nikon Eclipse TS100F) was then used to identify the prey individuals to the lowest possible taxonomic level using several identification keys and count the number (Hamond 1971; Hamond 1973; Smirnov and Timms 1983; Bayly 1992; Shiel 1995).

4.3.4 Data analysis

4.3.4.1 Statistical analysis

The Shannon-Weaver index (H') data of the diet of each forage fish (univariate) were used to construct a Euclidean distance resemblance matrix (Anderson *et al.* 2008). Relative abundance data of zooplankton were transformed into log(x+1) to down-weigh undue influence of highly abundant zooplankton and a dummy species was added in the samples without any specimen (Anderson *et al.* 2008). The log(x+1) transformed data were used to construct a Bray-Curtis resemblance matrices (Anderson 2001). Environmental variables were normalised and employed to construct Euclidean distance resemblance matrices. Permutational analysis of variance (PERMANOVA; pseudo-$P > 0.05$) was run using Euclidean distance resemblance matrices of Shannon-Weaver index from the diet of each forage fish to test the diet difference among the months and regions in the Murray Estuary and Coorong (Clarke and Warwick 2001). For small-mouthed hardyhead, the analysis consisted of two factors including five sampling months as random five levels and three sampling regions as fixed three levels. In the case of sandy sprat and Tamar goby, only two sampling regions were used as fixed two levels in the analysis. PERMANOVA was also conducted using Bray-Curtis resemblance matrices to detect significant differences of zooplankton abundance, and the factors included months as random five levels and regions as fixed three levels. Pairwise post-hoc comparisons using the multivariate analog of the t-test (pseudo-t) were performed at each level to identify significant difference. Unrestricted permutation was accomplished for each factor and interaction with 999 permutations to detect differences at $\alpha = 0.05$ (Anderson 2001). A distance-based linear model (DistLM) was performed to identify the effect of environmental variables on zooplankton abundance. Normalised environmental data and log(x+1) transformed zooplankton abundance data were used in DistLM analysis (Anderson *et al.* 2008). A distance based redundancy analysis (dbRDA) was then plotted during DistLM analysis to give a visual representation of the influence of environmental variables on the variation of zooplankton abundance. All tests were performed using PRIMER v6 (Clarke and Gorley 2006) with the PERMANOVA+ add–on (Anderson *et al.* 2008).

4.3.4.2 Dietary analysis

The Shannon-Weaver index (*H*’) was used to assess the prey diversity of the dietary contents in each forage fish species. Shannon-Weaver index (*H*’) was calculated as

$H' = -\Sigma\, pi \ln pi$

where *p* is the relationship between the total number of prey of species *i* and total number of prey in the sample (Clarke and Warwick 2001; Clarke and Gorley 2006).

Diet data were expressed as composition in stomach of each forage fish by frequency of occurrence (% F) in the diets to determine diet composition (Hyslop 1980):

Frequency of occurrence (% Fi) = (Ni / N) × 100

where *Fi* = percent frequency of prey type *i*, *Ni* = number of prey *i* in the gut, and *N* is total number of prey in the gut contents.

Ivlev’s (1961) electivity index was used to identify the level of prey selection of forage fish (Chesson 1978). Electivity index was calculated as

Electivity index Ei = $\frac{ri-pi}{ri+pi}$

where ri is the proportion of prey in diet and pi is proportion of prey in ambient environment. The values (Ei) range from −1 to +1, where −1 indicates the absence of prey in guts and therefore suggests prey avoidance. Conversely, positive values suggest active selection of a prey type. Zero indicates no or little selection (i.e., random selection).

Dietary overlap of forage fish was calculated using Schoener's overlap index (Fjosne and Gjosaeter 1996).

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

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

Feeding strategy of forage fish was determined using Levins's index of niche breadth to determine whether the fish is a specialist or a generalist. Levins's index was calculated as

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

where P_i is the proportion of the $_i$th prey in the diet of a fish species and the n is the number of prey groups. The average percentage of prey numbers (N) was used as proportion. The B value ranges from 0 to 1. The index value of zero indicates low dietary breadth with high feeding specialization while the value of one indicates the widest possible trophic niche with general prey selection (Fjosne and Gjosaeter 1996; Arceo-Carranza and Chiappa-Carrara 2015).

A standardised measure of this niche breadth B_A is calculated as:

$\mathbf{B}_A = \dfrac{\mathbf{B} - 1}{\mathbf{n} - 1}$; This places the measure on a scale of 0 to 1 (Krebs 1999).

4.4 Results

4.4.1 Forage fish diets and niche breadth

A total of 574 fish (266 small-mouthed hardyhead, 190 sandy sprat, and 118 Tamar goby) were dissected to identify the gut contents. Sizes of the forage fishes in catch ranged 16-85 mm for small-mouthed hardyhead, 15-89 mm for Tamar goby and 18-70 mm for sandy sprat. Overall, 16 prey types, including seven crustaceans, four insects, two rotifers, one nematodes, one polychaetes and one acanthocephalan were identified in the gut contents of the three forage fishes during the study period from November 2013 to March 2014 (Table 4.1). The gut content of all three forage fishes was dominated by crustaceans. Gut content analysis showed different occurrences of harpacticoids (65%), ostracods (58%), amphipods (53%) and mysids (6%) in small-mouthed hardyhead. Dietary analysis indicated a high occurrence of amphipod (90%), followed by harpacticoida (31%), ostracods (28%) and mysids (18%) in Tamar goby. Similarly, harpacticoida (73%) dominated in the sandy sprat gut followed by amphipod (59%), ostracods (37%) and mysids (5%). Occurrences of insects were greater in small-mouthed hardyhead (Chironomidae 32%; Diptera 21% and Corixidae 2%) than in Tamar goby (Diptera 2%), whereas these prey items were completely absent in sandy sprat over the study period. In addition, rotifers were identified in the gut of Tamar goby but were absent from the guts of small-mouthed hardyhead and sandy sprat. Finally, nematodes and acanthocephalan commonly occurred in the diets of all three forage fishes while polycheate worms (Nereidae) were identified in small-mouthed hardyhead and Tamar goby. The occurrence of Nereidae was high (26%) in Tamar goby and low (4%) in small-mouthed hardyhead, but completely absent in sandy sprat. All 16 prey types were used to calculate dietary niche breadth of each forage fish. The niche breadth was quite low for all forage fish with the highest value observed in sandy sprat (B_A = 0.38, Fig. 4.2).

Table 4.1 Frequency of occurrence (F %) of prey types in the gut of three forage fish species.

Phyla/Class	Taxon	small-mouthed hardyhead	Tamar goby	sandy sprat
Crustacea	Amphipoda	53	90	59
	Ostracoda	58	28	37
	Harpacticoida	65	31	73
	Calanoida	0	1	6
	Bosmina sp.	0	1	4
	Mysidacea	6	18	5
	Nauplius	0	0	8
Insecta	Chironomidae	32	0	0
	Diptera	21	2	0
	Corixidae	2	0	0
	Staphylinidae	0	0	0
Rotifera	*Brachionus* sp.	0	4	0
	Filinia sp.	0	1	0
Nematoda	Nematoda	42	8	3
Polychaeta	Nereidae	4	26	0
Acanthocephala	Acanthocephala	10	31	9

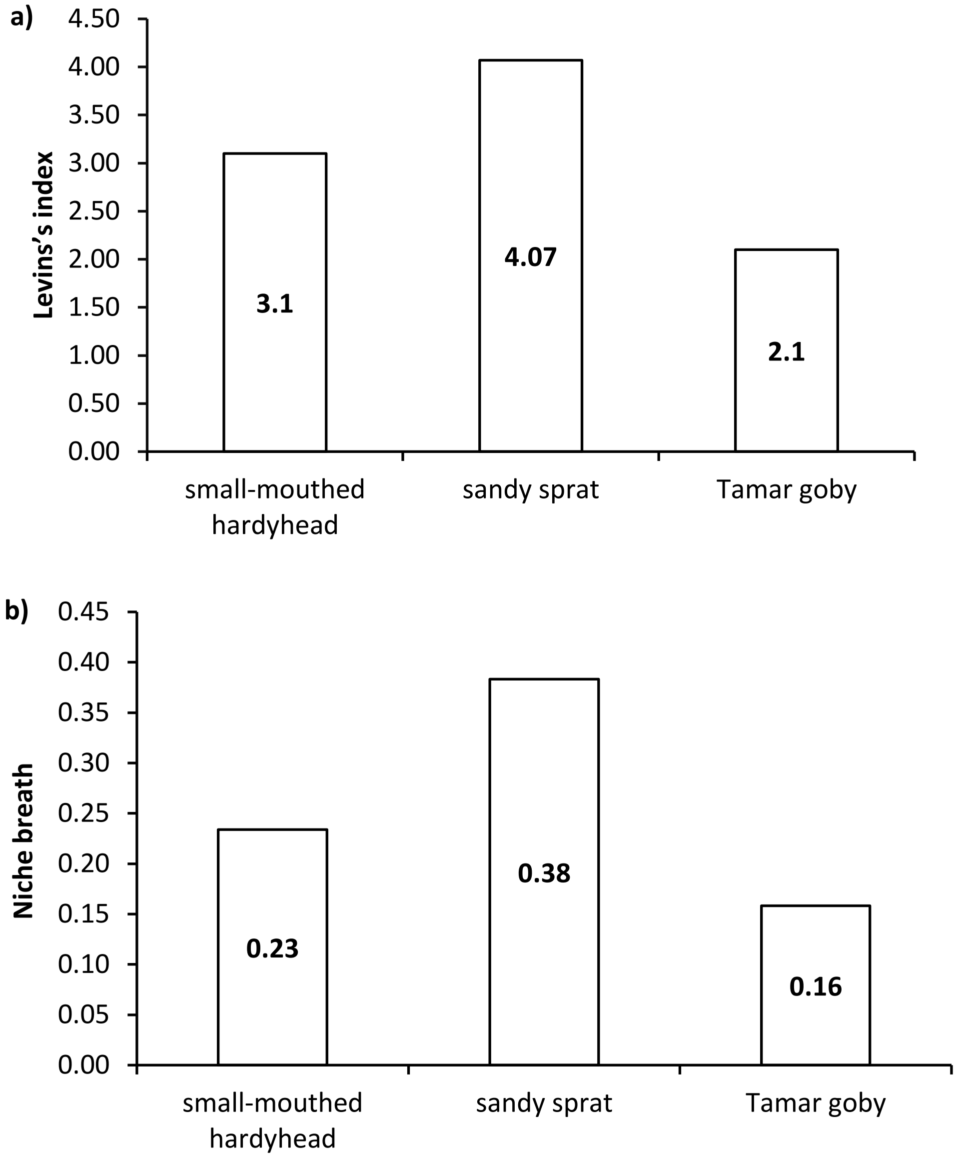

Fig. 4.2 a) Levins's index and b) Dietary niche breadth of each forage fish in the Murray Estuary and Coorong during study period. Measurement of dietary niche breadth index values of 0−1. Zero indicates the specialist while the value of one indicates generalist in feeding.

4.4.2 Dietary overlap and electivity index

The 16 prey types were also used to assess the diet overlap among the forage fish in the Murray Estuary and North lagoon (Table 4.1). Samples from the South Lagoon were excluded from diet overlap calculations due to the sole presence of small-mouthed hardyhead. The diet was highly overlapped ($\alpha = 0.8$) between sandy sprat and Tamar goby in the Murray Estuary (Fig. 4.3), where there exhibited moderate diet overlap ($\alpha = 0.53$) among the three species. In the North Lagoon, all three forage fish species showed high dietary overlap ($\alpha \geq 0.6$) (Fig. 4.3). The prey type in water samples from the field was used to calculate food selectivity for forage fishes. Seven prey types were used to calculate the electivity index for small-mouthed hardyhead, eight for sandy sprat and 10 for Tamar goby. All three forage fish species exhibited high preference and positive selection for amphipods, harpacticoids and mysids and negative selection for nematodes (Table 4.2). Sandy sprat (Ei = 0.61) and Tamar goby (Ei = 0.54) positively selected ostracods whereas small-mouthed hardyhead showed negative selection (Ei = − 0.37) for ostracods. Nereidae was negatively selected by small-mouthed hardyhead (Ei = − 0.25) and positively selected by Tamar goby (Ei = 0.54, Table 4.2).

Table 4.2 Electivity index (Ei) of each prey item of three forage fish species from three regions in the Murray Estuary and Coorong over the study period. Zooplankton collected from different sites of each region in the Murray Estuary and Coorong was used to calculate electivity index.

Taxa	Small-mouthed hardyhead	sandy sprat	Tamar goby
Amphipoda	0.75	0.83	0.88
Ostracoda	−0.37	0.61	0.54
Harpacticoida	0.54	0.86	0.30
Calanoida	0	−0.78	−0.99
Bosmina sp.	0	0.05	0
Mysidacea	0.07	0.04	1.00
Nauplius	0	−0.97	0
Chironomidae	0.54	0	0
Diptera	0	0	0
Corixidae	0	0	0
Staphylinidae	0	0	0
Brachionus sp.	0	0	0.33
Filinia sp.	0	0	−1.00
Nematoda	−0.72	−0.45	−0.55
Nereidae	−0.25	0	0.54
Acanthocephala	0	0	0

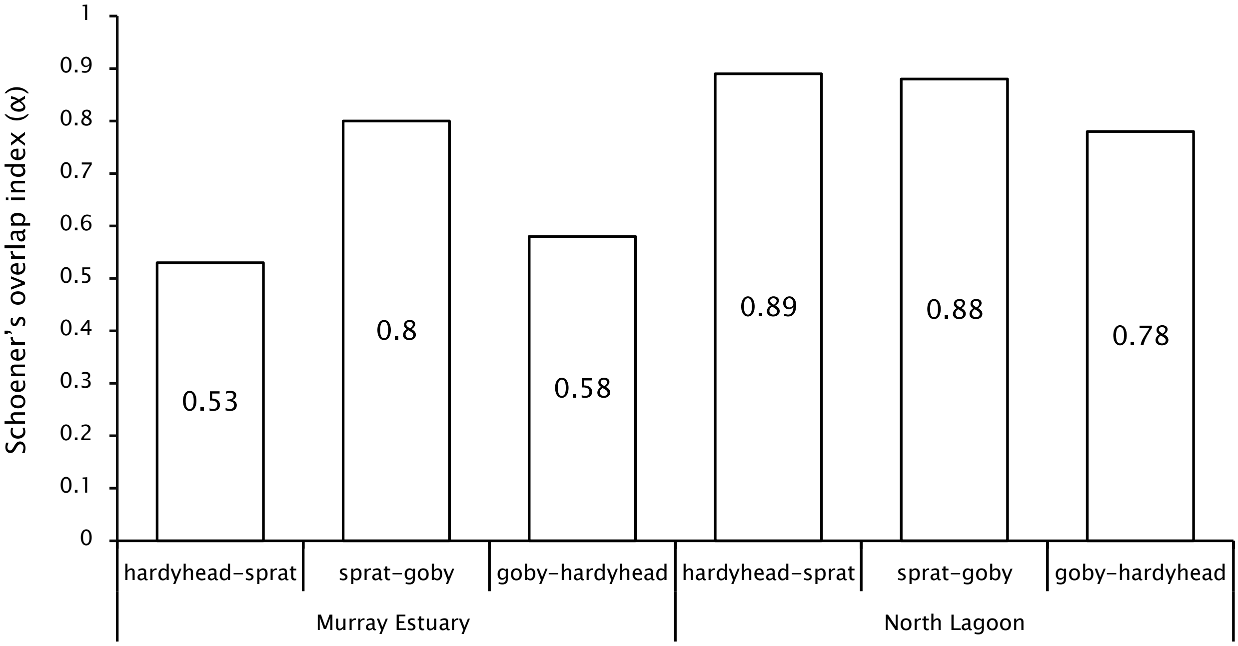

Fig. 4.3 Dietary overlap for forage fishes at the Murray Estuary and the North Lagoon. The South Lagoon was excluded from the calculation due to sole presence of small-mouthed hardyhead. Values range from 0 (no overlap) to 1.0 (complete overlap). Values 0–0.29 indicate low; 0.3–0.59 moderate; and ≥ 0.6 high dietary overlap.

4.4.3 Dietary prey diversity in forage fish

Significant spatial and temporal variation of dietary prey diversity was detected in small-mouthed hardyhead among regions (*P* = 0.028) and months (*P* = 0.032) during the study period (Table 4.3). Dietary prey diversity in sandy sprat and Tamar goby showed an inconsistent pattern in the Murray Estuary and the North Lagoon during the study period (Table 4.3). There was a significant month by region interaction of prey diversity in sandy sprat (*P* = 0.001) and Tamar goby (*P* = 0.001) but not in small-mouthed hardyhead (*P* = 0.196, Table 4.3).

Table 4.3 PERMANOVA results of Shannon-Weaver index of dietary prey of each forage fish at three regions in the Murray Estuary and Coorong during the study period. This PERMANOVA table includes fixed factors contributing to the changes of prey diversity during this study.

Forage fish	Source	df	SS	MS	Pseudo-F	*P* (perm)
small-mouthed hardyhead	Month	4	1.45	0.36	2.55	0.032
	Region	2	2.63	1.32	7.00	0.028
	Month × Region	5	1.00	0.20	1.40	0.196
	Residuals	254	36.18	0.14		
sandy sprat	Month	4	3.16	0.79	7.58	0.001
	Region	1	1.11	1.11	1.05	0.344
	Month × Region	4	4.38	1.09	10.50	0.001
	Residuals	170	17.72	0.10		
Tamar goby	Month	4	1.89	0.47	3.42	0.009
	Region	1	0.04	0.04	0.03	0.935
	Month × Region	3	3.65	1.22	8.81	0.001
	Residuals	109	15.07	0.14		

* Significant difference was set at $P < 0.05$

Pairwise tests detected significant differences in the diversity of dietary prey in sandy sprat between the Murray Estuary and North Lagoon except in February 2014 (Murray Estuary *vs* North Lagoon: pseudo-t = 1.48, P = 0.137) and March 2014 (Murray Estuary *vs* North Lagoon: pseudo-t = 1.42, P = 0.161). Similarly, pairwise comparison identified significant differences in the diversity of dietary prey in Tamar goby between Murray

Estuary and North Lagoon except in November 2013 (Murray Estuary *vs* North Lagoon: pseudo-*t* = 1.09, *P* = 0.296) and February 2014 (Murray Estuary *vs* North Lagoon: pseudo-*t* = 1.15, *P* = 0.224).

4.4.4 Temporal variation in zooplankton abundance

A significant month by region interaction (*P* = 0.001) was detected in zooplankton abundance (Table 4.4), suggesting that the pattern of spatial variation was not consistent between months.

Table 4.4 PERMANOVA results of zooplankton abundance at three regions in the Murray Estuary and Coorong during the study period. This PERMANOVA table includes fixed factors contributing to the changes of zooplankton abundance during this study.

Source	**df**	**SS**	**MS**	**Pseudo-F**	***P* (perm)**
Month	4	11016	2754	4.37	0.001
Region	2	10202	51019	1.44	0.254
Month × Region	8	28324	3540	5.62	0.001
Residuals	90	56719	630		
Total	104	105820			

*Significant difference was set at *P* < 0.05.

Pairwise test identified significant differences in zooplankton abundance among months except November 2013 *vs* December 2013 (pseudo-*t* = 1.29, *P* = 0.184); January 2014 *vs* February 2014 (pseudo-*t* = 1.57, *P* = 0.09) and February 2014 *vs* March 2014 (pseudo-*t* = 1.43, *P* = 0.14). Significant difference in zooplankton abundance was observed between the regions (Murray Estuary *vs* South Lagoon: pseudo-*t* = 4.08, *P* = 0.003 and

North Lagoon *vs* the South Lagoon: pseudo-*t* = 2.82, *P* = 0.004) in November 2013. Similarly, zooplankton abundance was significantly variable between the regions (Murray Estuary *vs* South Lagoon: pseudo-*t* = 5.67, *P* = 0.003 and North Lagoon *vs* South Lagoon: pseudo-*t* = 4.42, *P* = 0.005) in December 2013. Zooplankton abundance showed significant difference between the Murray Estuary and the South Lagoon in January 2014 (pseudo-*t* = 2.32, *P* = 0.029) and in February (pseudo-*t* = 2.56, *P* = 0.003). Similarly, zooplankton abundance was significantly variable between the Murray Estuary and the North lagoon (pseudo-*t* = 2.84, *P* = 0.022) and between the Murray Estuary and the South Lagoon (pseudo-*t* = 6.31, *P* = 0.003) in March 2014.

Salinity (DistLM, *P* = 0.001) and pH (DistLM, *P* = 0.001) were the most influential variables to predict the temporal variations in zooplankton abundance and composition in the Murray Estuary and Coorong (Table 4.5).

Table 4.5 DistLM sequential results of environmental variables on zooplankton abundance at three regions in the Murray Estuary and Coorong over the study period (SS=Sum of Square; Prop = Proportion of the variation; Cumul= Cumulative variation).

Variable	**SS**	**Pseudo-F**	**DistLM *P***	**Prop.**	**Cumul.**
Salinity	11717	12.83	0.001	0.111	0.111
pH	29583	46.77	0.001	0.280	0.390
Temperature	1229	1.96	0.104	0.012	0.402
DO	8325	15.15	0.001	0.079	0.481
Water transparency	4146	8.08	0.001	0.039	0.520
Chlorophyll-a	3198	6.58	0.001	0.030	0.550

With these two variable combined, they together contributed 39% (proportion: 0.39) to the variation in zooplankton assemblage. The DO, water transparency and chlorophyll-*a* also significantly contributed to the model (DistLM, *P* = 0.001), but these factors together explained only ~15% (proportion: 0.148) variation (Table 4.5). Similarly, in the dbRDA analysis, the first two axes (i.e. dbRDA1 and dbRDA2) explained 94% of the variability in zooplankton assemblage while pH and salinity were the key driving factors of the variability (Fig. 4.4).

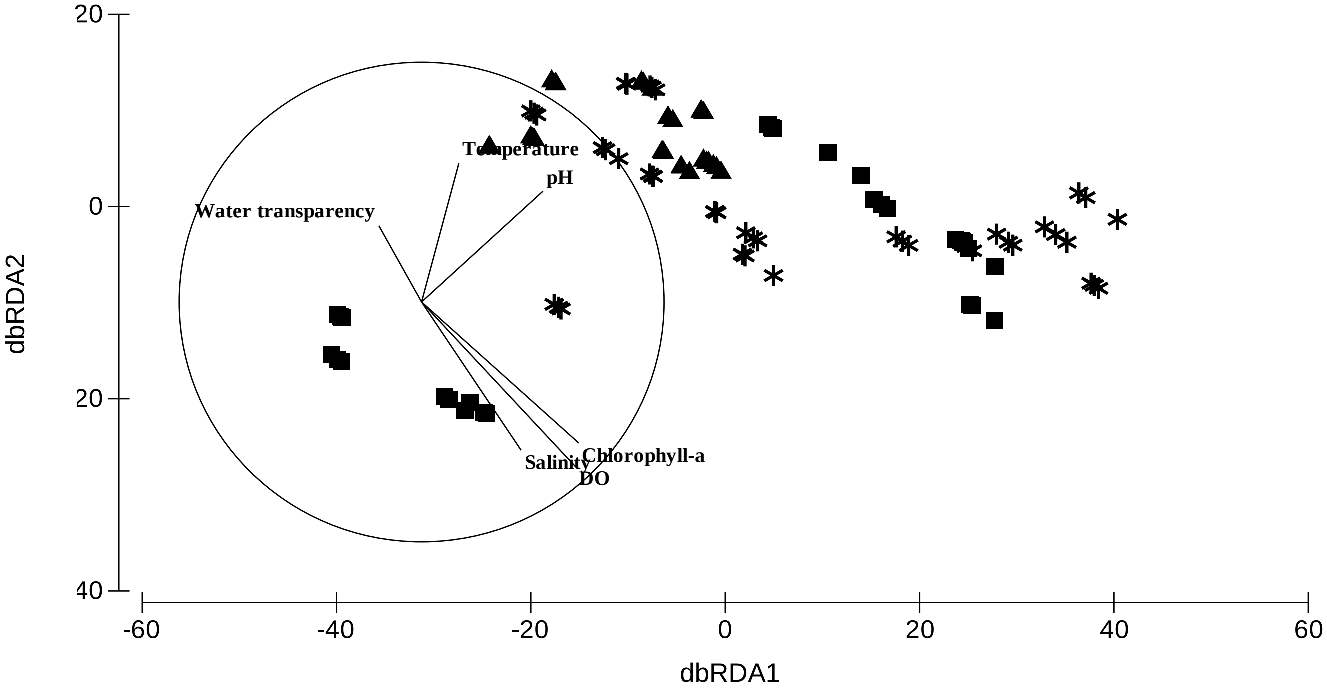

Fig. 4.4 dbRDA ordination of log(x+1) transformed zooplankton abundance data at three regions in the Murray Estuary and Coorong *versus* predictor variables: pH, salinity, water temperature, water transparency, DO and chlorophyll-a. Data points are displayed as Murray Estuary (triangle), North Lagoon (asterisk) and South Lagoon (square). Variability in Zooplankton abundance explains 79.1% of variation of the dbRDA1 model and 43.5% of the total variation, and 14.9% variation of the dbRDA2 model and 8.2 % of total variation, respectively.

4.5 Discussion

The current study addresses food selection and dietary overlap among the three forage fish species in the Murray Estuary and Coorong. This study shows that three forage fishes feed on a wide range of prey including crustaceans, insects, nematodes, polychaetes and rotifers. Here, the diets of forage fishes were dominated by crustacean followed by insects, nematodes and polychaetes. Greater occurrence of insect was detected in the diet of small-mouthed hardyhead and Tamar goby while this prey was not consumed by sandy sprat. Importantly, freshwater and estuarine rotifers also contributed to the diet of Tamar goby. In the current study, dietary analysis explores the dynamic prey selection and food sharing of all three forage fish in the Murray Estuary and Coorong. Geddes *et al.* (2016) reported that the diversity, abundance and distribution of estuarine and marine zooplankton community are significantly affected by hyper-salinity associated with low river flow in the Coorong. In the current study, the variation in occurrence and prey selection of the forage fish is more likely due to the variability in abundance and shifting of prey distribution regulated by environmental changes in the Murray Estuary and Coorong.

Previous studies in the Coorong (Geddes and Francis 2008; Deegan *et al.* 2010) reported a predominance of crustacean and polychaetes in the diet of small-mouthed hardyhead. Typically, atherinids are reported to prey on planktonic and epi-benthic preys in estuaries (Prince *et al.* 1982; Humphries and Potter 1993). The diet composition of small-bodied forage fish in the Murray Estuary and Coorong and other Australian estuaries (Humphries and Potter 1993; Becker and Laurenson 2008) suggests that this species feeds on plankton and invertebrates in the water column and at the sediment-water interface. In the present study, a high proportion of insects (chironomids, Dipterae, Corixidae and Staphylinidae) in the diet reflects the surface feeding habit of small-mouthed hardyhead. However, high occurrences of crustacean and insects in the diet of small-mouthed hardyhead are likely due to their food selection and feeding behaviour and higher abundance of these prey items in the habitats.

Likewise, dietary composition of the Tamar goby is supported by previous studies in the Murray Estuary and Coorong (Geddes and Francis 2008; Deegan *et al.* 2010) with highly abundant amphipods and low abundance of polychaetes found in previous studies. Similarly, the diets of other gobiids (*Afurcagobius suppositus*) also include copepods and polychaetes in an Australian temperate estuary (Gaughan and Potter 1997). Ordinarily, gobiids are reported to consume benthic organisms in estuaries (Humphries and Potter 1993). In the present study, the dominance of amphipods in the diet suggests that Tamar goby is an epibenthic feeder. However, the occurrences of *Brachionus* sp. and *Filinia* sp. in the diet of Tamar goby are likely due to common distribution of rotifers regulated by freshwater barrage and river inflow to the Murray Estuary and the North Lagoon in the Coorong.

In contrast, small-bodied marine clupeoids are commonly distributed near shore and migrate to estuaries and wetlands for growth and development during early life history (Gaughan *et al.* 1996; Rogers and Ward 2007). Of these clupeoids, sandy sprat is abundant around inshore of the gulf and inside the Murray Estuary and Coorong of South Australia (Rogers and Ward 2007). Typically, sandy sprat is a pelagic opportunist feeder and fed on planktons as reported in other clupeoids (Gaughan *et al.* 1996). Bice *et al.* (2016) reported the dominance of crustaceans including the harpacticoid copepods (~73% of all prey items) in sandy sprat diet from the Murray Estuary. In the current study, crustacean contributed to the major diet of sandy sprat. The high proportion of crustacean and low proportion of polychaetes in the stomach of sandy sprat are possibly due to the opportunistic feeding habit that allows this species to prey on relatively abundant planktons and benthos in the Murray Estuary and Coorong.

Food competition and dietary overlap between fish species are often observed in estuarine systems because of low and variable abundance of invertebrate prey species (Moyle and Cech 2004). The ecological degradation due to protracted drought and low freshwater flows has severely impacted prey diversity in the Murray Estuary and Coorong (Geddes *et al.* 2016). Consequently, dietary overlap and food competition can be intensified due to low diversity of food resources in the Murray Estuary and Coorong. Dietary overlap of forage fish in the present study differed between regions in the Murray Estuary and Coorong. The low overlap between the diets of small-mouthed hardyhead and other two species is likely due to the absence of preferred prey items for this species in the Murray Estuary except amphipods. In contrast, high diet overlap among forage fish species in the North Lagoon suggests high abundance of preferred prey and food sharing among forage fish species. Dietary overlap among forage fish species is patchy in the Murray Estuary and comparatively intensive in the North Lagoon. The abundance and distribution of forage fish and prey individuals are strongly affected by salinity gradients regulated by freshwater inflow to the Murray Estuary and Coorong (Hossain *et al.* 2016; Geddes *et al.* 2016). This may reflect diet sharing among the forage fish in the North Lagoon where both forage fish and their prey are exposed to the optimal salinity environment.

The current study focused on zooplankton and other invertebrates in the gut analysis as most of the forage fish were reported to be planktivorous (Geddes *et al.* 2016). The narrow niche breadth of each forage fish is likely due to the abundance reduction of prey items in the environment. Benthic prey species were dominated by harpacticoid copepods, amphipods, nematodes and polychaetes with only few insects in the diet. The lack of diverse prey is reflected by the niche breadth index, suggesting that these forage fish are specialist feeders. Despite this, the dietary overlap between benthic Tamar goby and other pelagic forage fish (sandy sprat and small-mouthed hardyhead) was found in the current study, likely reflecting prey selection and food sharing at the same dietary niche in the Murray Estuary and Coorong. The variability and abundance of prey items influence the conspecific or interspecific competition in the environment (Ward *et al.* 2006). However, other studies indicate that forage fish may feed on whatever is readily available in a large quantity rather than selecting specific prey items (Becker and Laurenson 2008). This suggests that the variability and abundance of prey items also determine the prey selection and preference of forage fish in the Murray Estuary and Coorong.

In estuaries the diversity and abundance of prey organisms such as plankton, benthos and other invertebrates are regulated by environmental variability and seasonal succession (Jendyk *et al.* 2014; Leterme *et al.* 2015). The diversity of prey organisms in this study is comparable to a recent report (Dittmann *et al.* 2015) and the status over three decades ago in the Coorong (De and Geddes 1980). Generally, the variability of prey diversity of forage fish is directly impacted by the temporal variation of zooplankton abundance and seasonal succession of phytoplankton in the Coorong (Dittmann *et al.* 2012). On the other hand, movement of euryhaline fish that have strong salinity tolerance, such as small-mouthed hardyhead, allows its access to diverse prey items in the Coorong, therefore showing an indirect effect on prey diversity (Lui 1969; Molsher *et al.* 1994). The spatial variation of dietary prey diversity in sandy sprat and Tamar goby is probably due to the limited salinity tolerance of some prey species in the Murray Estuary and the North Lagoon.

The variation of zooplankton abundance and distribution can impact the function of predators at higher trophic levels in the food web. In the current study, temporal variation of zooplankton abundance was mainly influenced by pH and salinity, and, to a lesser extent by other environmental variables. The pH in estuaries is regulated by dissolved ions such as carbonate and bicarbonate through freshwater inputs (Gillanders *et al.* 2011). Their variation is related to salinity, photosynthesis and DO levels (Ringwood and Keppler 2002). The change of pH can impact zooplankton growth, development and reproduction (Keller *et al.* 2002; Vehmaa *et al.* 2012). In the current study, the water pH was ~8 in the Murray Estuary, 7–8 in the North Lagoon and 6–8 in the South Lagoon, which is in the similar range with other Australian salt lakes (pH 8–9) (Williams 1981; Khan 2003). Although we identified pH as a significant factor affecting zooplankton, the mechanism was unclear as pH in the estuarine system usually co-varies with salinity and other factors (Williams 1998; Gillanders *et al.* 2011).

Salinity is the key variable attributing to a physiological barrier limiting zooplankton distribution, species richness (Paturej *et al.* 2015) and diversity in estuarine systems (Williams 1998; Boeuf and Payan 2001). Salinity in the Murray Estuary and Coorong controls the abundance and distribution of vertebrates and invertebrates (Geddes 2005; Noell *et al.* 2009; Webster 2010). In the current study, salinity ranged 2–30 in the Murray Estuary, 11–75 in the North Lagoon and 40–85 in the South Lagoon. Salinity can influence the variability of phytoplankton dynamics (Jendyk *et al.* 2014), picophytoplankton communities (Schapira *et al.* 2010) and microbenthic communities (Dittmann *et al.* 2015) in the Coorong. The abundance and distribution of zooplankton are impacted by the variability of salinity in the Coorong (Geddes *et al.* 2016) and in other estuaries (Marques *et al.* 2007). The seasonal variation of zooplankton in the current study is probably prevailed due to the variability of salinity in the Coorong.

Water transparency is related to the abundance of suspended materials and affect light penetration and primary productivity through photosynthesis (Herman and Heip 1999). Water transparency in this study ranged 25−200 cm in the Murray Estuary, 12−50 cm in the North Lagoon and 20− 80 cm in the South Lagoon. The depth of water transparency corresponds well with the chlorophyll *a* concentrations in the Murray Estuary (1.22 ± 0.53 μgL^{-1}), North Lagoon (2.81 ± 0.90 μgL^{-1}) and South Lagoon (2.96 ± 0.60 μgL^{-1}) and reflects the variation of phytoplankton abundance. The high chlorophyll *a* in the North Lagoon and South Lagoon might be due to high abundance of diatoms and picophytoplankton (Leterme *et al.* 2013). The variability of phytoplankton production regulated by salinity and nutrients is likely to influence the temporal variation in zooplankton abundance and distribution in the Murray Estuary and Coorong (Leterme *et al.* 2015).

In conclusion, environmental factors affected the trophic interaction through food competition and partitioning among forage fish in the Coorong. Crustaceans were actively selected by all three forage fish but small-mouthed hardyhead also selected insects as prey in the Coorong. The nematodes and acanthocephalans were commonly observed in the gut of forage fish. The diet overlap among forage fishes in the North Lagoon was more than in the Murray Estuary. The temporal variation of zooplankton abundance is predominantly driven by the variability of pH, salinity, DO, water transparency and chlorophyll *a*. The variation in prey abundance significantly affected the predator-prey interaction through trophic dynamics along the salinity gradient in the Coorong. This study enhances our understanding of diet selection and dietary overlap among the forage fish species in an estuarine-hypersaline lagoonal system. The variation of environmental factors may mediate the outcome of diet selection and food sharing among fish in this inverse Australian estuary. This study provides basal information for further study of food web and trophic ecology in this and other similar estuarine systems.

4.6 Acknowledgement

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Chapter 5

Salinity stress response in estuarine fishes from the Murray Estuary and Coorong, South Australia

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5.1 Abstract

Estuaries are unstable ecosystems and can be changed by the environmental and anthropogenic impact. The Murray Estuary and Coorong were degraded by drought and low freshwater input in the last decade and thereby transformed into the largest hyper-saline lagoon in Australia. This study evaluates the physiological stress of two estuarine fish species (small-mouthed hardyhead *Atherinosoma microstoma* and Tamar goby *Afurcagobius tamarensis*) to the induced salinity change in captivity. The test fishes were collected from the Coorong and transported to the laboratory in the water from the Coorong. Small-mouthed hardyhead was exposed to descending salinity change and an ascending salinity variation was used for Tamar goby. A number of enzymes were assessed to measure the stress response of fish to salinity change. The activity of reactive oxygen species was significantly increased with the salinity change in both fish species compared with the fish in the control. Significant salinity effect on superoxide dismutase activity was observed on Tamar goby but not on small-mouthed hardyhead. Conversely, the impact of salinity on catalase activity was detected on small-mouthed hardyhead but not on Tamar goby. The study reveals that the induction of physical stress by salinity changes occurred in both Tamar goby small-mouthed hardyhead despite the varying response of antioxidant enzymes between fish species. The study provides an insight into the understanding of physiological adaptation in estuarine fish to salinity change. The results could improve our knowledge on stress response and resilience of estuarine fish to hypo- and hyper-salinity stress.

Additional keywords: Stress, antioxidants, salinity, estuarine fish, Coorong, catalase

5.2 Introduction

Estuaries are a transitional zone linking land, freshwater and marine environments and offer productive and diverse habitats to numerous organisms for feeding, breeding and nursery (Beck *et al.* 2001; Gillanders *et al.* 2011). Despite ecological, biological and commercial significance, estuaries are often subject to anthropogenic degradation and pollution (Blaber 2002; Edgar *et al.* 2000; Madeira *et al.* 2013). Organisms in estuaries are likely to be stressed by alterations in salinity, temperature and pollution due to climate change and anthropogenic activities (Matoo *et al.* 2013). Salinity fluctuation appears to be a key factor affecting species distribution and life history in estuarine ecosystems (Telesh and Khlebovich 2010; Webster 2010; Williams *et al.* 1990). Although estuarine animals have adapted to a long-term salinity variation and developed physiological and biochemical mechanisms to cope with salinity change (Williams *et al.* 1990), it is not clear on physiological responses and biochemical variation when a stress occurs due to salinity fluctuation (Lavado *et al.* 2012; Madeira *et al.* 2013).

Environmental stressors can cause formation of reactive oxygen species (ROS) in the cell (Halliwell and Aruoma 1991; Liepelt *et al.* 1995), but the antioxidant enzymes are evolved to reduce or eradicate these free radicals from the cell. For instance, the epidemic of oxidative stress occurs when the antioxidant system loses balance between generation and removal of ROS (Lackner 1998). The increase of ROS usually causes cellular injury and inhibition of antioxidant enzymes (Valko *et al.* 2006). The excessive ROS can damage DNA (Liepelt *et al.* 1995; Lackner 1998; Abele 2002; Barber *et al.* 2006), cell membrane structure (Parihar and Dubey 1995) and protein function in aquatic animals (Sambrook *et al.* 1989; Heink *et al.* 2013). In teleost, the effect of a stressor can be minimised by physiological antibody defence mechanisms that are regulated by enzymes such as superoxide dismutase (SOD), catalase (CAT) or glutathione peroxidases.

The most important anti-oxidant enzyme SOD can catalyse the production of free superoxide anion radicals to hydrogen peroxide during dismutation (Heink *et al.* 2013). Likewise, the catalase is an enzyme that catalyse the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules (Barber *et al.* 2006).

The Murray Estuary is an inverse estuary located at the terminus of the Australia's largest river system, i.e., the Murray–Darling Basin. In the last decade, the Murray Estuary and Coorong have been ecologically degraded due to protracted drought and lack of fresh water inflow from the upper stream (Kingsford *et al.* 2011; Ferguson *et al.* 2013). As a result, water salinity has changed from estuarine to marine concentrations at Murray Mouth in the Murray Estuary, from marine to hyper-saline at North lagoon and to extremely saline (>100) at South Lagoon in the Coorong. The Murray Estuary and Coorong are an important habitat for commercial, recreational and small-bodied forage fish species (Brookes *et al.* 2015). The hyper-saline condition has negatively affected the ecology and physiology of vertebrates and invertebrates (Webster 2010). Consequently, fish abundance and diversity are significantly reduced and fish distribution is limited to a particular region at salinities of 36-52 in the Coorong (Zampatti *et al.* 2010; Wedderburn *et al.* 2016).

Small fishes, such as small-mouthed hardyhead (*Atherinosoma microstoma*) and Tamar goby (*Afurcagobius tamarensis*), are common prey for piscivorous fish and birds in the Murray Estuary and Coorong (Paton 2010). Small-mouthed hardyhead commonly live in shallow water in estuaries, coastal bays and coastal lakes and lagoons throughout south-eastern Australia (Lintermans 2007; Wedderburn *et al.* 2016). This species is highly abundant at high salinity (35-100) in the South Lagoon (Eckert and Robinson 1990) and also common in the North Lagoon and the Murray Estuary in the Coorong (Noell *et al.* 2009; Hossain *et al.* 2016). On the other hand, the Tamar goby is commonly found in the estuaries and coasts of Victoria, New South Wales, eastern South Australia and northern Tasmania in Australia (Lintermans 2007). Tamar goby is commonly distributed at the regions with low salinity (2-58) in the Coorong (Hossain *et al.* 2016). This species is highly abundant in the Murray Estuary, but there are some in the North Lagoon and none in the South Lagoon (Hossain *et al.* 2016; Wedderburn *et al.* 2016). Salinity creates a physiological barrier to limit the distribution and abundance of Tamar goby in the Coorong (Zampatti *et al.* 2010). Despite the empirical observation on the salinity dependent occurrence and distribution of estuary fishes, our knowledge on the physiological response and resilience of estuary fish to salinity variation in Coorong is poor. Most studies on the impact of salinity on fish in the Murray Estuary and Coorong have focused on fish species composition, assemblage structure and seasonal distribution (Zampatti *et al.* 2010; Bucater *et al.* 2013; Brookes *et al.* 2015; Hossain *et al.* 2016). Elevation of salinity due to low freshwater flow has reduced fish species diversity in the Coorong (Zampatti *et al.* 2010). Salinity variation could cause temporal numerical variation of prey fish and large-bodied marine and diadromous fish at the Murray mouth (Bucater *et al.* 2013). In particular, salinity fluctuation is the major determinant for the abundance and distribution of estuary dependent fish species. Salinity variation can induce the stress and in turn can impact the physiology of estuarine fish species. Lui (1969) reported a wide salinity tolerance of small-mouthed hardyhead (lethal concentration at 50% mortality, LC_{50}: 3.3-108) in captivity. The LC_{50} of salinity tolerance in Tamar goby was 73.2 at 14 ºC and 71.4 at 23 ºC in a laboratory condition (McNeil *et al.* 2013). However, other than the laboratory test on the salinity tolerance of small-mouthed hardyhead (Lui 1969) and Tamar goby (McNeil *et al.* 2013) in captivity, there is no rigorous study to evaluate stress responses of these common estuarine fishes to salinity variation in the Coorong.

The aim of the study was to investigate the stress response to salinity variation of two key estuarine fish species in the Murray Estuary and Coorong. We hypothesise that the variability in salinity would increase stress and alter the antioxidant response in estuarine fish. The elevated stress would potentially increase prey fish mortality and in turn affect the abundance and distribution of other predatory fish species. The results of this study will improve our understanding on the physiological response of estuarine fish under salinity stress.

5.3 Materials and methods

5.3.1 Fish collection and management

Adult small-mouthed hardyhead (40–70 mm total length, TL) were collected from Salt Creek (salinity 75) in the South Lagoon and Tamar goby (40–80 mm TL) were sampled at the Beacon 19 site (salinity 15) near the Murray mouth in the Murray Estuary. At both sites, these fish were sampled using a seine net in the Murray Estuary and Coorong lagoon. Fish were transported in aerated holding tanks to the laboratory at Flinders University.

Each species was acclimatized in a holding tank (200 L) at 2-3 individuals/L for two weeks. Field water was used during acclimation to adjust the salinity to the same level at the fish collection location. The photoperiod for the holding tanks was maintained 12L: 12D at a light intensity of 500 Lux. Water quality parameters (salinity, DO, pH and temperature) were monitored daily using the AquaRead multi-parameter probe (Aquaprobe AP-800). The nitrate, nitrite and ammonia levels were measured on alternative days using the Aquaspex test kit (Aquaspex Water Testing Products, Australia) during the experimental period. Fish were fed 2−3 times daily with bloodworm and brine shrimp *Artemia* sp. during the period of acclimation and salinity trials. Fish swimming behaviour, food intake, physical injury and mortality were monitored during the acclimation and trial periods. The salinity trials were conducted in 20 L aquaria with aeration, and water was replaced every other day with respective salinities in each treatment. Salinity was increased by adding ocean sea salt (Aquasonic, Australia) and reduced by adding demineralised water (Wedderburn *et al.* 2008).

5.3.2 Salinity trials

5.3.2.1 Ascending salinity trial on Tamar goby

The ascending salinity trial was conducted on Tamar goby by increasing salinity over time as the fish were collected from a region with a low salinity of 15 in the field. The experiment was designed with three treatments: (i) the Tc treatment was the control with a constant salinity of 15, which was the same as the field salinity; (ii) the T_5 treatment was with increasing salinity from the control at a 5% increment daily, and (iii) the T_{10} treatment was with increasing salinity from the control at a 10% increment daily. The range of salinity change was designed according to the tolerance level of Tamar goby (McNeil *et al.* 2013). Ten individuals of Tamar goby were placed in each aquarium in three replicates. Fish of each treatment was euthanized in AQUI-S^{TM} at 40 mgL^{-1} after the 10-day test at the ending salinities of 15, 22 and 30 in three treatments. The liver of each individual in the above treatments was collected and preserved at -80 ºC for stress determination and enzyme analysis.

5.3.2.2 Descending salinity trial on small-mouthed hardyhead

Descending salinity trial was performed on small-mouthed hardyhead as the fish are abundant at hyper-saline (> 80) region in the South Lagoon. This trial included salinity reduction from the high salinity control (T_{CT}: 75) to a low salinity at a daily reduction rate of either 1% (T_1) or 2% (T_2) by adding demineralised water. The control salinity (T_{CT}: 75) was set based on the salinity in the field from where the samples were collected. The trial was terminated after 20 days at the salinities of 75 (T_{CT}), 60 (T_1) and 45(T_2) based on the estimate of salinity tolerance (LC_{50}: 3.3–108) of small-mouthed hardyhead (Lui 1969). Five individuals were placed in each aquarium of all treatments. Fish in each aquarium of all treatments were euthanized using AQUI-S^{TM} at 40 mgL^{-1} at the end. The liver was collected from each fish in the above treatments and preserved at -80 ºC for stress determination and enzyme analysis.

5.3.3 Stress determination

This study was designed to test the oxidative stress due to salinity change on fish in captivity. Stress in both species was determined by measuring the generation of reactive oxygen species (ROS) due to salinity variation during the trial period. ROS was determined using liver of each fish species following the instruction of the assay kits.

5.3.3.1 Reactive oxygen species (ROS) activity assay

The ROS values were determined using an OxiSelect^{TM} in vitro ROS/RNS assay kit (Green Fluorescence, STA-347; Cell Biolabs, Inc. San Diego, USA). The ROS free radical content in an unknown sample was measured fluorometrically by comparing with the hydrogen peroxide standard curve. The liver tissue was homogenised on ice at 20 mg tissue per ml phosphate-buffered saline and centrifuged at 10000× *g* for 5 min. The supernatant was collected for further analysis. A 50 µL sample supernatant was transferred to a 96-well plate for fluorescence measurement. Then 50 µL of catalyst was added to all wells and incubated for 5 min at room temperature. Subsequently, 100 µL of 2, 7-dichlorodihydrofluorescein solution was added to each of 96 wells and incubated at room temperature for further 15-45 min in dark. The plate reaction was then read at 480 nm excitation / 530 nm emission on CLARIOstar (BMG Labtech).

5.3.4 Enzyme assays

5.3.4.1 Superoxide dismutase (SOD) activity assay

Superoxide dismutase (SOD) activity was determined with an OxiSelect^{TM} superoxide dismutase activity kit (STA-340; Cell Biolabs, Inc. San Diego, USA) to evaluate the ability of the xanthine/xanthine oxidase system for generating superoxide anions. The chromagen produced a water-soluble formazan dye upon reduction by superoxide anions. Liver tissues were homogenised on ice in 7 ml lysis buffer per gram tissue and centrifuged at 12000× *g* for 10 min. The supernatant of tissue lysate was collected and preserved at -80 ºC for further analysis. In brief, 20 µL tissue lysate supernatant, 5 µL xanthine solution, 5 µL chromagen solution, 10 µL 10× SOD assay buffer and 50 µL water were mixed and added on a 96-well microtiter plate. Then, 10 µL pre-diluted xanthine oxidase solution (1×) was added into each well and incubated for 1 h at 37 °C. The absorbance was read at 490 nm on a FLUOstar Omega microplate reader (BMG Labtech). The activity of SOD was measured as the inhibition for chromagen reduction.

5.3.4.2 Catalase (CAT) activity assay

Catalase activity was determined using an OxiSelect™ catalase activity assay kit (STA-341; Cell Biolabs, Inc. San Diego, USA). The kit worked on a two-step reaction. The first reaction was to dismutase hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2). The disintegration rate of hydrogen peroxide into water and oxygen was proportionate to the concentration of catalase. The second reaction was the production of quinoneimine dye by the oxidative coupling reaction of 4-

aminophenazone (4-aminoantipyrene, AAP) and 3, 5-dichloro-2-hydroxybenzenesulfonic acid in the presence of H_2O_2 catalysed by horseradish peroxidase. The quinoneimine dye coupling product was measured at 520 nm, which correlated to the amount of hydrogen peroxide remaining in the reaction mixture. In the current study, the liver tissue was homogenised on ice in 7 ml cold phosphate-buffered saline with 1 mM EDTA per gram of tissue and centrifuged at 10000× *g* for 15 min at 4 °C. The supernatant was collected and stored for further analysis. A 20 µL sample and 50 µL hydrogen peroxide working solution (12 mM) was mixed thoroughly on a 96-well microtiter plate and incubated for 1 min. Then 50 µL of the catalase quencher was added into each well and mixed thoroughly to stop the reaction. On a fresh 96-well microtiter plate, 5 µL of each reaction well was transferred and 250 µL of the chromogenic working solution was included to each well for incubation for 40-60 min with mixing. Then, the plate absorbance was read at 520 nm on the FLUOstar Omega plate reader (BMG Labtech). The catalase activity was measured using the catalase activity assay standard curve.

5.3.5 Statistical analysis

All data of fish survival, reactive oxygen species (ROS), superoxide dismutase (SOD), and catalase (CAT) activity are presented as mean ± standard error (S.E.). Data were tested for normality and homogeneity using Shapiro-Wilk and Levene’s test. The normally distributed data were analysed using one-way analysis of variance (ANOVA) to compare the differences among treatments. If significant differences were detected then data were further analysed using Tukey post hoc tests. Where assumption of normality was not met even after data transformation, then a Kruskal–Wallis non-parametric test was performed. When Kruskal-Wallis test showed significant difference among different levels, Mann-Whitney U test was performed to compare between two levels. The level of significant difference was set at $P < 0.05$. All data were analysed using the statistical package IBM SPSS Statistics 22.

5.4 Results

There was significant difference ($P = 0.002$) of fish survival among treatments in the ascending salinity trial. Overall, the survival of Tamar goby was 83.33 ± 0.64% at the end. The survival of Tamar goby in the T_5 and T_{10} treatments was 90 ± 0.57% and 60 ± 0.57%, respectively. Abnormal swimming and low feeding of Tamar goby were observed in T_5 and T_{10} from day 5 of the trial. Conversely, normal swimming and feeding were observed on the fish in the control (T_C) during trial period. However, there was no mortality in the control (T_C) during the ascending salinity trial. Post hoc tests showed significant difference between the control (T_C, $P = 0.002$) and 10% ascending salinity (T_{10}), and between the 5% ascending salinity (T_5, $P = 0.01$) and 10% ascending salinity (T_{10}) on Tamar goby during the trial period. In the descending salinity trial, the average survival of small-mouthed hardyhead was 97.77 ± 0.11%, and there was no significant variations ($P > 0.05$) among the treatments. Small-mouthed hardyhead in all treatments showed normal swimming and feeding behavior throughout the experiment.

5.4.1 Reactive oxygen species (ROS) activity

ROS activity was significantly different (Kruskal-Wallis one-way ANOVA, $\chi^2 = 7.2$, $P = 0.027$) among treatments in the descending salinity trial on small-mouthed hardyhead (Fig. 5.1a). Mann-Whitney U test revealed significant variation of the ROS activity between the treatments (T_{CT} *versus* T_1, $P = 0.050$), (T_1 *versus* T_2, $P = 0.050$) and (T_{CT} *versus* T_2, $P = 0.050$) in small-mouthed hardyhead (Fig. 5.1a).

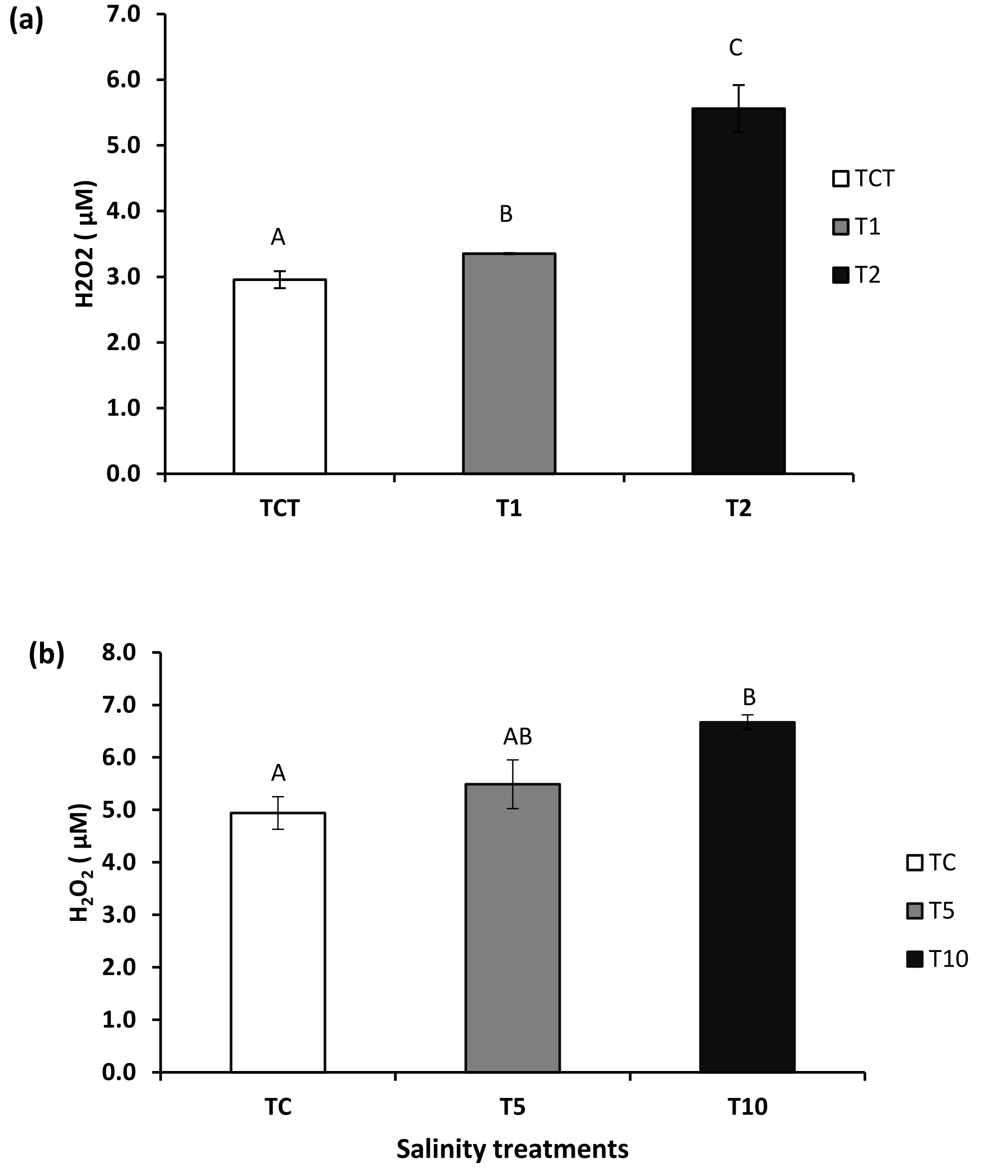

Fig. 5.1 Reactive oxygen species (ROS) activity in the liver of (a) small-mouthed hardyhead and (b) Tamar goby among different treatments in the salinity trials. The treatments are shown as T_{CT}: Control salinity; T_1: 1% daily decreasing salinity and T_2: 2% daily decreasing salinity for small-mouthed hardyhead and T_C: Control salinity; T_5: 5% daily increasing salinity and T_{10}:10% daily increasing salinity for Tamar goby. ROS activity was determined as production of H_2O_2 (µM) in the tissue. Values were expressed as mean ± S.E. The significant difference was set at $P < 0.05$.

Similarly, one-way ANOVA indicated significantly different activity (*P* = 0.026) of ROS among the treatments in the ascending salinity trial on Tamar goby. Post hoc tests showed that ROS activity was significantly changed between the control salinity (T_C, *P* = 0.024) and the 10% ascending salinity (T_{10}) but not between the control (T_C, *P* = 0.512) and 5% ascending salinity (T_5) on Tamar goby during the trial period (Fig. 5.1b). Conversely, the ROS activity did not significantly change (*P* = 0.101) between the 5% (T_5) and 10% ascending salinity (T_{10}) treatments on Tamar goby (Fig. 5.1b).

5.4.2 Superoxide dismutase (SOD) activity

The SOD activity significantly varied in Tamar goby by increasing salinity. In contrast, the SOD activity did not show significant variation in small-mouthed hardyhead by salinity reduction. One-way ANOVA indicated that SOD activity did not show any significant difference (*F* = 3.68, *P* = 0.091) among the treatments in the descending salinity trial on small-mouthed hardyhead (T_{CT}, T_1 and T_2; Fig. 5.2a). However, significant differences of SOD activity (*F* = 20.03, *P* = 0.002) were observed among the treatments in the ascending salinity trial (T_C, T5 and T_{10}; Fig. 5.2b) on Tamar goby. Post hoc comparison detected that SOD activity at the 5% salinity elevation (T_5; *P* = 0.010) or at the 10% salinity elevation (T_{10}; *P* = 0.002) was significantly different from the control (T_C) on Tamar goby in the ascending salinity trial (Fig. 5.2b). However, no significant difference of SOD activity (*P* = 0.284) was observed between 5% salinity elevation (T_5) and 10% salinity elevation (T_{10}) in the ascending salinity trial on Tamar goby (Fig. 5.2b).

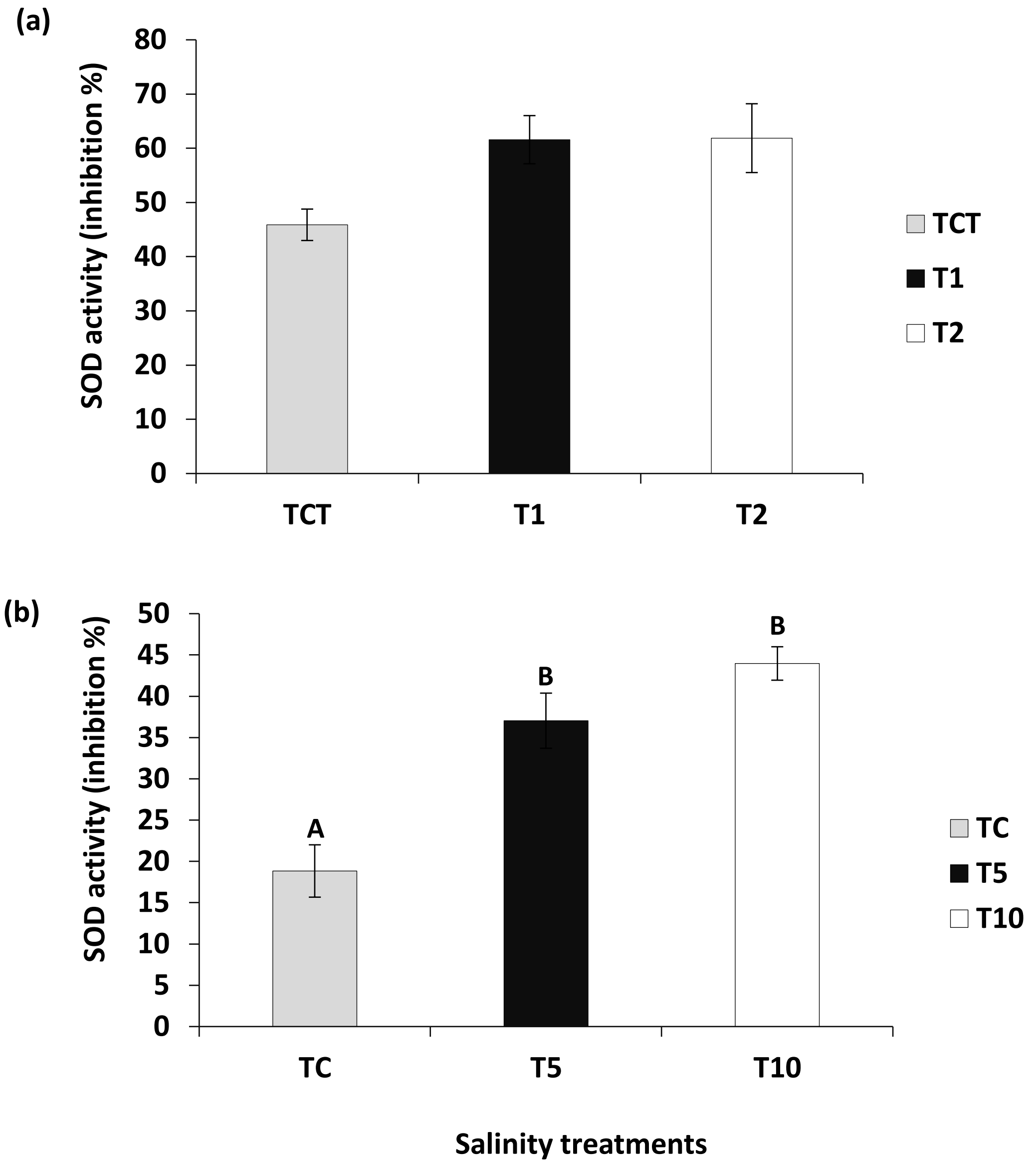

Fig. 5.2 Superoxide dismutase (SOD) activity (percent of inhibition) in the liver of (a) small-mouthed hardyhead and (b) Tamar goby among the different treatments in the salinity trials. The treatments are shown as T_{CT}: Control salinity; T_1: 1% daily decreasing salinity and T_2: 2% daily decreasing salinity for small-mouthed hardyhead and T_C: Control salinity; T_5: 5% daily increasing salinity and T_{10}:10% daily increasing salinity for Tamar goby. Values were expressed as mean ± S.E. The significant difference was set at $P < 0.05$.

5.4.3 Catalase (CAT) activity

Significant variation of CAT activity was observed on small-mouthed hardyhead but not on Tamar goby during the salinity trials. One-way ANOVA indicated that catalase activity was significantly different among the treatments in the descending salinity trial (F = 6.38, P = 0.033) on small-mouthed hardyhead (Fig. 5.3a). Post hoc comparison revealed that catalase activity at the 1% salinity elevation (T_1; P = 0.029) was significantly changed, compared with the control salinity (T_{CT}) on small-mouthed hardyhead (Fig. 5.3a). Catalase activity did not significantly change between the 1% salinity decline ($T_{1,}$ P = 0.458) and the 2% salinity decline (T_2) on small-mouthed hardyhead. However, no significant variation of the CAT activity (Kruskal-Wallis one way ANOAVA, χ^2 = 0.356, P = 0.837) was observed among the treatments in the ascending salinity trial on Tamar goby (Fig. 5.3b).

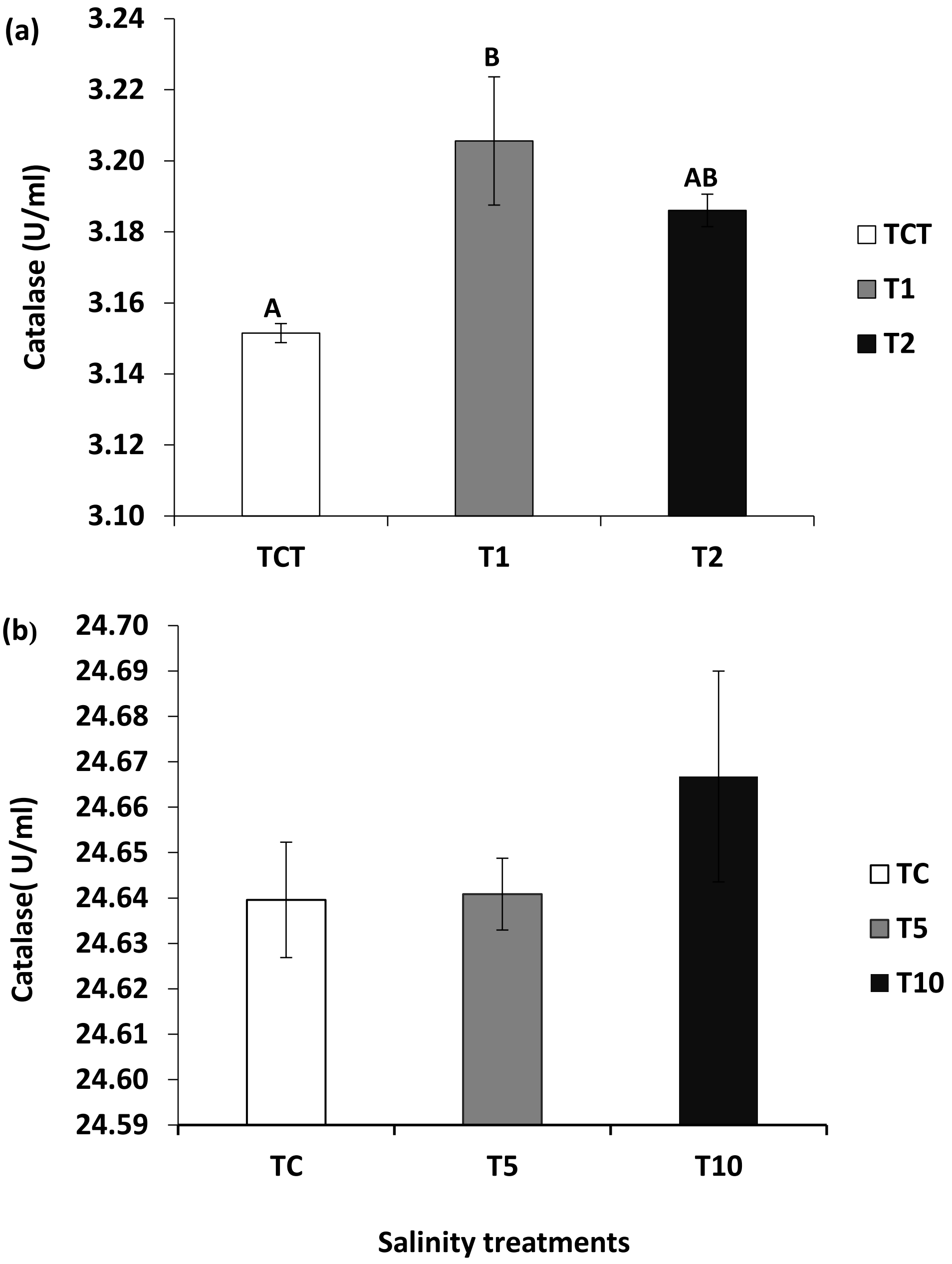

Fig. 5.3 Catalase (CAT) activity (U/ml) in liver of (a) small-mouthed hardyhead and (b) Tamar goby among the different treatments in the salinity trials. The treatments are shown as T_{CT}: Control salinity; T_1: 1% daily decreasing salinity and T_2: 2% daily decreasing salinity for small-mouthed hardyhead and T_C: Control salinity; T_5: 5% daily increasing salinity and T_{10}:10% daily increasing salinity for Tamar goby. Values were expressed as mean ± S.E. The significant difference was set at $P < 0.05$.

5.6 Discussion

5.6.1 Stress induced by salinity change

Stress usually induces the formation of reactive oxygen species such as superoxide radical (O_2^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O_1) in aquatic organisms (Ross *et al.* 2001; Abele and Puntarulo 2004). The activity of ROS in an organism is predominantly stimulated by the stress intensity associated with intrinsic and extrinsic factors such as salinity, temperature and dissolved oxygen (Ross *et al.* 2001). Hyper- and hypo-osmotic conditions can impact the tolerance of the species and often cause oxidative stress in estuarine organisms (Ferreira *et al.* 2005; Freire *et al.* 2011). Organisms usually need to spend extra energy for osmoregulation either in a hyper- or in a hypo-osmotic condition, which in turn induces ROS production in the cell (Gonzalez 2012). In the present study, the increased ROS level and observed symptoms in both trials indicates that the Tamar goby were significantly stressed by the ascending salinity while small-mouthed hardyhead were stressed by the descending salinity in terms of ROS induction, but they could cope with the salinity stress and display normal swimming behaviour.

In the present study, abnormal swimming and feeding behaviours were observed in Tamar goby at the 5% (T_5) and 10% (T_{10}) of increasing salinity trials, indicating a stress response of Tamar goby to elevated salinities. The normal swimming and feeding behaviours of Tamar goby in the control suggest the fish were not stressed during the salinity trial and the ROS level in the control salinity could serve as a reference point for fish exposed to varying salinities. In this study, the ROS activity in Tamar goby at the 5% (T_5) or at 10% (T_{10}) of increasing salinity was higher than in the control. This result is supported by another study where the ROS level in sturgeon *Acipenser naccarii* was increased due to exposure to increasing salinity (0–35) (Martinez-Alvarez *et al.* 2002). In the current study, the induced stress on Tamar goby was more intensified in the T_{10} treatment than in the T_5 treatment. The high production of ROS as H_2O_2 in the T_5 and T_{10} treatments in Tamar goby was mainly stimulated by salinity elevation that caused the stress in Tamar goby.

Similarly, the ROS activity in small-mouthed hardyhead at the T_1 or T_2 salinity treatment was also increased in comparison to the control salinity (T_{CT}) although the fish showed normal swimming and feeding behaviour at all treatments throughout the salinity trial. This result is supported by another study where the ROS activity in European sea bass (*Dicentrarchus labrax*) was intensified due to salinity reduction from 32 to 2.5 in captivity (Sinha *et al.* 2015). The induction of ROS and the increase of antioxidant activity were reported in estuarine fish (Ross *et al.* 2001; Doherty *et al.* 2010; Freire *et al.* 2011), mollusc (Rudneva 1999), crustaceans (Liu *et al.* 2007) and other aquatic organisms (Lushchak 2011) in response to environmental stress. For instance, Liu *et al.* (2007) reported that the high ROS in shrimp (*Litopenaeus vannamei*) was produced due to the exposure of the organism from 30 to other salinities at 5, 15 and 50 for 24 h. The small-mouthed hardyhead is a euryhaline species and able to tolerate a wide range of salinity variation (LC_{50}: 3.3 – 108; Lui 1969). Small-mouthed hardyhead is abundant and widely distributed from a region of hyper salinity (>100) in the South Lagoon to a region with low salinity (>2) near the mouth of Murray River (Noell *et al.* 2009; Hossain *et al.* 2016). Despite the wide salinity tolerance of small-mouthed hardyhead in the field, the present study detected stress signs on this species under a decreasing salinity regime in captivity. In the laboratory, the reduction of salinity from 75 to 60 and 45 in 20 days accelerated the production of ROS in small-mouthed hardyhead due to salinity reduction. The ROS generation in small-mouthed hardyhead indicates its stress response to salinity reduction. The impact of elevated ROS on small-mouthed hardyhead is possibly minimised by antioxidant activities (e.g., CAT activity) during the trial period in captivity. Nevertheless, the normal swimming and feeding behaviours of small-mouthed hardyhead during the trial suggest that this fish could cope with salinity variation despite the increase of ROS in small-mouthed hardyhead at descending salinities.

5.6.2 Antioxidants response to stress

Superoxide dismutase (SOD) and catalase (CAT) enzymes can directly scavenge reactive oxygen species (ROS) in the cell (An *et al.* 2010). In the current study, the SOD activity in small-mouthed hardyhead at 1% (T_1: 61.58% inhibition) and at 2% (T_2: 61.86% inhibition) daily decreasing salinity was higher than in the control (T_{CT}: 45.88% inhibition). This result is similar to the results of another study where SOD and CAT activities in euryhaline black porgy *Acanthopagrus schlegeli* increased after exposing the animals to a hypo-osmotic environment at a salinity of 10 (An *et al.* 2010). Similarly, SOD and CAT activities were enhanced in mud crab (Paital and Chainy 2010) and in ark shell (An and Choi 2010) after the animals were transferred from the full strength seawater (salinity 35) to a hypo-osmotic environment (salinity 25). In the current study, the production of SOD at the 1% and 2% decreasing salinity in small-mouthed hardyhead was slightly higher than in the control, but the differences between treatments and the control were not significant. The similar SOD activity in the regimes of 1% and 2% decreasing salinity (T_2) in small-mouthed hardyhead is probably due to its euryhaline nature. Therefore, it is possible that the SOD activity could instantaneously counteract with the generation of ROS in the plasma of small-mouthed hardyhead and result in no significant variation despite the change of salinity change in this study.

In Tamar goby, the increase of SOD activities at 5% or 10% increasing salinity corresponded to the high levels of ROS, suggesting a clear response of the antioxidant to salinity stress in Tamar goby. This result is similar to the physiological response of sturgeon *Acipenser naccarii* in which the SOD and CAT activities in the blood were raised due to the increase of salinity from 0.5 to 35 over 20 days (Martinez-Alvarez *et al.* 2002). The increased SOD activity indicates that the production of antioxidants is associated with salinity stress. This study demonstrates that the antioxidant defence mechanism in the estuarine fish is a strategy to adapt to salinity elevation through reduction of free radicals.

In contrast, the activity of CAT showed low variation compared to the SOD activity in both fish species. Typically, the catalase enzyme converts the hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2), thereby counteracting the toxicity of H_2O_2 in the cell (Barber *et al.* 2006). In the present study, however, the change of catalase activity is not concomitant with the SOD activity in both fish species as the responses of SOD and CAT to ROS can vary among organisms (Wdzięczak *et al.* 1982; Ferreira *et al.* 2005). The CAT activity is more sensitive to the existing free radicals and the SOD activity is usually more responding to superoxide radicals (Smith 1976; Alam *et al.* 2013; Carocho *et al.* 2013). The CAT activity in small-mouthed hardyhead at the 1% descending salinity was significantly higher than in the control, but there was no significant change at the 2% descending salinity. This result corresponds with the elevated CAT activities in other estuarine fish such as sea bass *Dicentrarchus labrax* (Roche and Bogé 1996) and black porgy *Acanthopagrus schlegeli* (An *et al.* 2010). For instance, Roche and Bogé (1996) reported that osmotic shock by decreasing salinity from 37 to 5 in 2 h could increase the CAT activity in sea bass (*Dicentrarchus labrax*). In the current study, the high CAT activity of the hardyhead in the T_1 salinity treatment suggests the ROS stress is probably owing to peroxide radicals.

On the other hand, the CAT activity in Tamar goby was not significantly varied among salinity treatments although CAT at the 10% increasing salinity was numerically greater than at the 5% increasing salinity and in the control. The CAT activity in Tamar goby is expected to be stimulated by induced ROS in response to hyper-osmotic stress. The proportion of CAT activity in different tissues of teleost often varies among freshwater, marine and estuarine species (Smith 1976; Wilhelm Filho *et al.* 1993). For instance, the CAT activity was reduced in sturgeon (*Acipenser naccarii*) due to the elevated contents of hepatic protein during the increase of environmental salinity (Martínez-Álvarez *et al.* 2002). In crustaceans such as penaeid shrimp *Litopenaeus vannamei*, salinity variation increases both SOD and CAT activities (Liu *et al.* 2007; Li *et al.* 2008). In golden pompano *Trachinotus ovatus*, the increase of salinity from 10 to 26 reduced CAT but did not change SOD (Ma *et al.* 2016). In Tamar goby, salinity variation only stimulated the response of SOD but not significant response in CAT. It seems that Tamar goby is probably stressed by superoxide radicals rather than by hydrogen peroxide stress.

This study shows an induction of oxidative stress on Tamar goby in hyper-osmotic environments and on small-mouthed hardyhead in a hypo-osmotic condition. The high production of ROS in the liver indicates greater stress in Tamar goby while small-mouthed hardyhead could well cope with the stress and display normal swimming and feeding behaviours during the salinity trial. The oxidative stress is neutralised by the immuno-response mechanism regulated by the SOD and CAT activities in estuarine fish, suggesting that the functional antioxidant system is important for fish adaptation to salinity change. The change of SOD and CAT activities is an indication that estuarine fish can adapt the stress due to salinity change. This study improves our knowledge on immuno-response and adaptation of estuarine fish to stress associated with salinity fluctuation and provides an insight to understand resilience of small-bodied fish in a hyper-saline estuarine system.

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Chapter 6

General Discussion and Conclusion

Forage fish have a role in linking energy transfer between trophic levels, are used commercially as fishmeal to feed other animals and also contribute to human food, thus making them an important component of food webs (Alder *et al.* 2008). However, forage fish populations are ecologically vulnerable due to environmental variations in the marine and estuarine system and overexploitation (Peck *et al.* 2014). In the Murray Estuary and Coorong, forage fish are consumed by piscivorous fish and water birds and thus play a significant trophic role in the estuarine system (Paton 2010). Recently, the abundance and composition of small-bodied forage fish populations have been heavily affected by river regulation and environmental variability in the Coorong estuary (Brookes *et al.* 2015). Therefore, understanding the response of forage fish to environmental variation is essential for sustainable management and conservation of the ecosystem and fishery resources in the Coorong. The present research project explores the effects of environmental changes and associated regulating factors on the life history, ecology and physiology of three important forage fish species (small-mouthed hardyhead, *Atherinosoma microstoma*; sandy sprat, *Hyperlophus vittatus* and Tamar goby, *Afurcagobius tamarensis*) in the Coorong, South Australia. In particular, this research focuses on physiological stress in forage fish induced by environmental changes and the subsequent variation in population dynamics. The present thesis contributes to new knowledge on the assemblage structure (Chapter 2), growth response (Chapter 3), feeding ecology (Chapter 4) and physiological adaptation (Chapter 5) of forage fish in the Coorong. This chapter (Chapter 6) summarises the key findings in the above studies and discusses their collective implications to understanding of fish biology and ecosystem management in the Coorong.

6.1 Summary of major findings

1. There was spatial variation in forage fish assemblage across the salinity gradients in the Murray Estuary and Coorong. Forage fish assemblage was characterized by (i) a greater abundance and dominance of small-mouthed hardyhead in the South Lagoon, (ii) a low abundance of sandy sprat and Tamar goby in the North Lagoon, and (iii) a complete absence of sandy sprat and Tamar goby in the South Lagoon. The differences observed in forage fish assemblages are mainly driven by the salinity fluctuation in the Coorong region.
2. Forage fish showed a trend of fast growth rates in the Coorong. Fish condition factors varied among fish sampled across different salinity gradients over time. The growth performance of forage fish was mainly regulated by chlorophyll-*a*, water transparency, salinity and to a less extent by temperature and oxygen in the Coorong.
3. Forage fish diets were characterized by the dominance of crustaceans such as amphipods, ostracods and harpacticoids. However, nematodes and acanthocephalans were also commonly found in the gut of all forage fishes. Sandy sprat and Tamar goby showed high dietary overlap in the Murray Estuary while the diets of all three forage fishes were potentially overlapped in the North Lagoon. The prey abundance was temporally variable and predominantly regulated by salinity, pH, dissolved oxygen, water transparency and chlorophyll *a* in the Coorong.
4. Salinity changes in a laboratory condition were found to result in measurable physiological stress in forage fish. The antioxidant superoxide dismutase

responded to salinity variation in Tamar goby but not in small-mouthed hardyhead. Conversely, catalase responded significantly to salinity change in small-mouthed hardyhead but not in Tamar goby. This study indicates that salinity fluctuation can differentially induce physiological stress among species in the Coorong fish assemblages.

6.2 Knowledge advance and research significance of this study

6.2.1 Environmental impacts on forage fish assemblages

Estuaries include dynamic habitats and are influenced by environmental variability (Gillanders *et al.* 2011). As a result, estuarine fishes are often affected by continuous changes in environmental factors and habitat alteration via river flow regulation (Martino *et al.* 2003), which can be reflected in the variation in abundance and distribution of fish assemblages in the estuarine ecosystem. This study identified the variation in relative abundance and distribution of forage fish species in the Coorong. Previous studies reported that low freshwater flow and subsequent hyper-salinity in the Coorong have a strong influence on abundance, diversity and distribution of estuarine fishes (Noell *et al.* 2009; Zampatti *et al.* 2010). The current study further discovered the spatial and temporal distribution patterns of forage fish assemblages across different salinity gradients in the Coorong and key environmental drivers. Although the small-mouthed hardyhead mainly occurs in the South Lagoon and the North Lagoon in 2009 (Noell *et al.* 2009), this species was also found in the Murray Estuary, indicating its euryhaline nature in the Coorong region. In contrast, the sandy sprat and Tamar goby were not found in the South Lagoon. The spatial variation of fish assemblage in this study contributes to 94% of the abundance variation of forage fish in the Coorong.

The abundance and distribution of fish populations have previously been shown to be influenced by environmental variables in the estuarine system (James *et al.* 2013). In the present study, spatial variation in forage fish assemblages was predominantly regulated by changes in salinity and water transparency. Low freshwater flow during the drought period has made the environment hypersaline, and causing deterioration of the ecological condition in the Coorong (Brookes *et al.* 2009). In particular, salinity is the major environmental determinant and varies spatially and temporally in the Coorong (Webster 2010). Hyper-salinity of the Coorong has caused the change in assemblages of estuarine fishes (Zampatti *et al.* 2010; Ye *et al.* 2012), reduced zooplankton diversity (Geddes *et al.* 2016) and phytoplankton dynamics (Jendyk *et al.* 2014). In this study, salinity, pH and water transparency were the best environmental predictors of the spatial variation in forage fish assemblages. In the present study, salinity ranged from 2–30 in the Murray Estuary, from 11–75 in the North Lagoon and from 40–85 in the South Lagoon. It appears that 96% of the variability observed in forage fish assemblages is predominantly driven by the salinity fluctuation in the Coorong. In particular, salinity in the Coorong is driven by drought and low freshwater flow from upstream. Thus, this study suggests that the assemblages of forage fish population are significantly impacted by drought and low freshwater flow input from the Murray River into the Coorong. The results of this study have added new understanding of environmental impacts on small-bodied fish populations, which provides new knowledge for ecosystem management to improve their populations and food web resilience in the Coorong.

6.2.2 Relationship between environmental variability and growth characteristics of forage fish

Growth and development of estuarine fishes are influenced by environmental factors (Gillanders and Munro 2012; Madeira *et al.* 2013). Chapter 3 reveals the rapid growth of forage fish species in the Coorong. The estimated growth rates were 0.019 mm day^{-1} for small-mouthed hardyhead, 0.016 mm day^{-1} for sandy sprat and 0. 038 mm day^{-1} for Tamar goby. The fast growth rate of small-mouthed hardyhead corresponds to a similar growth trend in this species over 20 years ago in the Coorong (Molsher *et al.* 1994) and in other estuaries in Australia (Prince and Potter 1983; Potter *et al.* 1986). In the present study, the rapid growth of small-mouthed hardyhead is likely related to its tolerance of a wide range in salinity (LD_{50}: 3.3 – 108 g L^{-1}; Lui 1969) that allows this species to explore multiple food resources. Although the growth rate of sandy spat was lower than small-mouthed hardyhead and Tamar goby in this study, it is faster than the sandy spat in a previous study (0.012 mm day^{-1}) in the Coorong (Rogers and Ward 2007). Indeed, the sandy sprat prefers to spawn in marine conditions even if this species uses estuaries as a feeding and nursery ground (Gaughan *et al.* 1996). In the present study, the marine salinity at the Murray Estuary and the North Lagoon might stimulate early spawning of sandy sprat and result in rapid growth, possibly due to less food competition with other species in the early season. However, Tamar goby are usually abundant in the Murray Estuary and the North Lagoon (Noell *et al.* 2009) and spawn exclusively during spring (October to December) but the spawning persists over five months (Cheshire *et al.* 2013). In this study, the fast growth rate of Tamar goby is likely related to the new recruits during the extended spawning season (October to February) and mediated by improved food availability with more freshwater input to the Murray Estuary (Cheshire *et al.* 2013).

Food abundance has especially been shown to affect fish growth performance in estuaries (Ricker 1975). In this study, the growth performance of forage fishes is mainly explained by variable levels of chlorophyll-*a*, water transparency and salinity in the Coorong. The condition factors of the forage fish are mainly explained by variations in chlorophyll-*a* level, and transparency (~30% variability) and in salinity (6% variability). A recent study revealed that primary production is regulated by the abundance of phytoplankton in the Murray Estuary and by benthic diatoms and picophytoplankton in the North and South Lagoons (Leterme *et al.* 2015). Changes in primary production have been shown to affect the diversity and abundance of grazers (zooplankton) and particulate feeders (benthic invertebrates), whose abundance impacts fish growth via trophic interactions in the ecosystem (Geddes *et al.* 2016). The findings of this study that the growth rates of forage fish are predominantly influenced by transparency and chlorophyll-*a* level may thus be explained by a similar effect on all trophic levels in the estuarine ecosystem.

6.2.3 Prey selection, dietary overlap and prey variability of forage fish

Prey selection in the estuarine system is often determined by prey abundance and distribution (Cantanhêde *et al.* 2009). Chapter 4 addresses prey selection and dietary overlap among forage fishes in the Coorong. In the current study, a wide variety of prey types including crustaceans, insects, nematodes, polychaetes and rotifers were identified in the fish gut, which is supportive of previous studies that found the diets of forage fish in the Coorong were dominated by crustacean and polychaete species (Geddes and Francis 2008; Deegan *et al.* 2010). The high proportion of insects (chironomids, Dipterae, Corixidae and Staphylinidae) in the diet of small-mouthed hardyhead reflects its planktonic and epi-benthic feeding behaviour (Humphries and Potter 1993). Likewise, the diet composition of the Tamar goby is in accordance with the result of Geddes and Francis (2008) and Deegan *et al.* (2010), who reported the high abundance of amphipods and low abundance of polychaetes in the diet of this species. On the other hand, the sandy sprat is a pelagic opportunist feeder and mainly feeds on plankton like other clupeoids (Gaughan *et al.* 1996). In this study, the diet of the sandy sprat includes a majority of crustaceans with a low proportion of polychaetes, which is supported by a recent study (Bice *et al.* 2016) where crustaceans were found to comprise 73% of the prey observed in the sandy sprat gut in the Murray Estuary. This indicates that the opportunistic feeding strategy of sandy sprat allows this species to prey on planktonic and benthic prey that are relatively abundant in the Coorong.

Chapter 4 explored food competition and dietary overlap among forage fish species in the field. Food competition between fish species occur in the Coorong because of their dietary overlap and abundance fluctuation in prey species (Moyle and Cech 2004). In the Coorong, protracted drought and low freshwater flow to the system has resulted in a significant reduction in prey diversity (Geddes *et al.* 2016). Consequently, low diversity of food resources can intensify dietary overlap and food competition. In the present study, dietary overlap among the forage fish varied between regions in the Coorong, being relatively low in the Murray Estuary, intensive in the North Lagoon and apparently non-existent in the South Lagoon. The low dietary overlap among forage fish in the Murray Estuary is likely due to the absence of preferred prey in the environment. In contrast, the high dietary overlap among forage fish in the North Lagoon suggests high availability of preferred prey species and food partitioning among forage fish. The abundance and distribution of forage fish and prey species are predominantly impacted by salinity gradients in the Coorong (Geddes et al. 2016). Thus, this study suggests that the dynamic prey selection and food sharing are associated the fish distribution mediated by salinity in the Coorong.

Chapter 4 also explored the temporal variations of zooplankton abundance that is predominantly influenced by pH and salinity, and, to a lesser extent by other environmental variables. Among the environmental variables, pH is regulated by dissolved ions such as carbonate and bicarbonate through freshwater inputs into the estuary (Gillanders *et al.* 2011). Change in pH is related to salinity, photosynthesis and dissolved oxygen (Ringwood and Keppler 2002). The results of the current study are supported by other studies (Keller *et al.* 2002; Vehmaa *et al.* 2012) where zooplankton growth and reproduction are influenced by pH change in estuaries. Although pH was identified as affecting zooplankton in this study, the mechanism is unclear as pH in the estuarine system usually co-varies with salinity and other environmental factors (Williams 1998; Gillanders *et al.* 2011).

Salinity has a strong impact on abundance, distribution, species richness (Paturej *et al.* 2015) and diversity of zooplankton in estuaries (Boeuf and Payan 2001). In this study, salinity has significant influence on zooplankton abundance in the Coorong. The findings of the current study correspond to previous studies where salinity was found to influence phytoplankton dynamics (Jendyk *et al.* 2014), picophytoplankton communities (Schapira *et al.* 2010) and microbenthic communities (Ross *et al.* 2001; Gonzalez 2012; Matoo *et al.* 2013; Dittmann *et al.* 2015). The abundance and distribution of zooplankton have been shown to be impacted by seasonal occurrence of hyper-salinity in the Coorong (Geddes *et al.* 2016) and in other estuaries (Marques *et al.* 2007). This study suggests that seasonal variation in zooplankton abundance is likely a driving force underlying food competition among forage fishes.

6.2.4 Physiological response of forage fish to salinity stress

Salinity can cause oxidative stress in estuarine organisms, especially where the input of freshwater flow is low in dry seasons (Ferreira *et al.* 2005; Freire *et al.* 2011), and this could affect the life history of forage fish populations (Webster 2010). Chapter 5 examined the physiological responses of estuarine forage fish species to stress induced by salinity fluctuation under laboratory conditions. In the current study, increases in salinity induced stress in Tamar goby, but not in small-mouthed hardyhead. The ROS activity was higher than in the control in Tamar goby in the 5% salinity and 10% salinity treatments. This result is supported by another study where ROS level in sturgeon *Acipenser naccarii* was elevated due to the exposure of the animal to increasing salinity (0−35) (Martinez-Alvarez *et al.* 2002). This indicates that the high production of ROS as H_2O_2 in Tamar goby is mainly due to salinity elevation.

In contrast, the ROS activity in small-mouthed hardyhead in the treatments of decreasing salinity to 1% or 2% was augmented in comparison to the control salinity, which is consistent with a study where the ROS level in European sea bass (*Dicentrarchus labrax*) was intensified due to salinity reduction from 32 to 2.5 in captivity (Sinha *et al.* 2015). Although small-mouthed hardyhead are tolerant of a wide variation in salinity level, evidence of stress in this species appeared under a decreasing salinity treatment in captivity. In this study, the reduction of salinity from 75 to 60 and 45 in 20 days enhanced the production of ROS in small-mouthed hardyhead. This study indicates that the level of ROS is more closely related to salinity change in Tamar goby than in small-mouthed hardyhead.

Stress is usually minimised by the physiological antibody defence mechanism in organisms (Ross *et al.* 2001). In the present study, antioxidants responded to the salinity-induced stress in both forage fish. In small-mouthed hardyhead, SOD activity was higher than in the control at 1% and 2% daily decreasing salinity, consistent with a previous study of estuarine fish species where SOD and CAT activities in euryhaline black porgy *Acanthopagrus schlegeli* increased after exposing the animals to a hypo-osmotic environment at a salinity of 10 (An *et al.* 2010). Likewise, SOD was significantly increased at 5% or 10% increasing salinity, suggesting a clear response of the antioxidant to salinity stress as evidenced by high ROS in Tamar goby. This result agrees with the previous report on the antioxidant response of sturgeon *Acipenser naccarii* in which the SOD and CAT activities in the blood were elevated due to the increase of salinity from 0.5 to 35 over 20 days (Martinez-Alvarez *et al.* 2002). The response of SOD in the hardyhead and Tamar goby indicates that the production of antioxidants is associated with salinity stress. This study suggests that the antioxidant defence mechanism in estuarine fish may be a strategy to adapt to salinity fluctuation through reduction of free radicals.

In the current study, the variation in catalase (CAT) activity was lower than that of the SOD activity in hardyhead or Tamar goby. CAT counteracts the toxicity of hydrogen peroxide (H_2O_2) in the cell by breaking down H_2O_2 to water (H_2O) and oxygen (O_2) (Barber *et al.* 2006). In this study, CAT levels were higher in small-mouthed hardyhead at the 1% descending salinity than at the 2% descending salinity and the control. Likewise, CAT levels in Tamar goby were stimulated more at the 10% increasing salinity than at the 5% increasing salinity and the control. The results of this study are in line with the elevated SOD and CAT activities in other estuarine fish (Roche and Bogé 1996) where CAT activity in sea bass (*Dicentrarchus labrax*) increased due to osmotic shock. The catalase activity in this study was not synchronised with the SOD activity in hardyhead or Tamar goby as the responses of SOD and CAT are species-specific (Wdzięczak *et al.* 1982; Ferreira *et al.* 2005). In addition, the CAT response to the immediate free radicals and the SOD activity usually differs between *in vivo* and *in vitro* determinations (Smith 1976; Alam *et al.* 2013). The results of this study indicated that the variation in CAT response in hardyhead and Tamar goby may be related to the changes in hydrogen peroxide concentration in fish under varying salinity stress.

6.3 Conclusions and recommendations for estuarine and fishery management

This thesis investigated the life history strategy of forage fishes in a variable environment in the Coorong. The following conclusions on the dynamics of forage fish population in the Coorong are based on the field and laboratory studies.

1. Distribution of forage fish assemblage is mainly associated with salinity variation across regions in the Coorong. Such a response of forage fish

reflects the physiological adaptation of fish to change in environmental conditions. The change in fish distribution allows forage fish to explore a wider range of habitats and broader food resources in a reverse estuary system. The new understanding on the dynamics of small-bodied forage fish species and key environmental drivers can form a baseline for further research to understand food web dynamics and trophic interactions in the Coorong and other similar temperate estuaries.

2. Fish length-weight analysis and the von Bertalanffy model reveal the trend of fast growth in the early life history of all three forage fish, which may be related to the high primary production level of the Coorong during this study. The growth performance of forage fish varied between the regions in the Coorong across a range of salinity gradients. Chlorophyll *a* and water transparency appeared to have the greatest influence on growth of forage fish.
3. The diet of forage fish was dominated by crustacean species with a higher dietary overlap in the North Lagoon than in the Murray Estuary. Prey availability to forage fish is mainly driven by changes in pH, salinity, dissolved oxygen, water transparency and chlorophyll *a* in the Coorong. Variation in prey abundance was found to affect the predator-prey interaction along the salinity gradient in the Coorong, suggesting that salinity variation can mediate the diet selection and food partitioning among forage fish in this Australian inverse estuary.
4. Significant oxidative stress was induced in the forage fish in the hypo- or hyper-osmotic environments. High production of ROS indicates that

salinity fluctuation can mediate the response of forge fish to environmental stress. The measurements of antioxidants such as SOD and CAT provide an effective way to detect the immuno-response of organisms to salinity stress. The immuno-response reflects the adaptation of estuarine fish to stress associated with salinity, which is essential to understand resilience of small-bodied fish in a hyper-saline estuarine system.

Sustainable management of small-bodied forage fish populations in the Coorong would depend on an ecosystem-based management approach. Specifically, the following recommendations are suggested to improve the management of estuary and ecosystem health in the Coorong:

1. Salinity is the key driver influencing the spatiotemporal distribution and critical life history processes of forage fish species in the Murray Estuary and Coorong. Freshwater releases through the barrages to maintain a ‘freshwater to hyper-marine’ salinity gradient is essential to maintain a diverse range of habitats and food resources to support resilient forage fish assemblage in the Coorong system.
2. Freshwater inflow from the upstream and reduced salinity will enhance the primary productivity in the Coorong. This will increase growth performance of forage fishes, and thus benefit higher trophic levels including commercial fishes and piscivorous birds through estuarine food web interactions.
3. Hyper-salinity in the Coorong reduces the abundance and distribution of food items such as zooplankton and benthos for forage fish. Reduction of salinity

by increasing freshwater input would improve the diversity and abundance of plankton and benthos, increase food resources for small-bodied forage fish, and thus benefit the food web in the Coorong.

6.4 Future Research

The outcomes of this research contribute new knowledge to our understanding on response of forage fish species to environmental changes in the hyper-saline Coorong Lagoon in South Australia. Nevertheless, some questions are still outstanding and future research should be focused on the following issues:

1. The current study was conducted for five months from November 2013 to March 2014, which covered only the dry period with relatively low freshwater flow to the Coorong. Therefore, longer term studies, including both dry and wet periods, will be needed to further understanding of the dynamic responses of small-bodied forage fish to freshwater flows in the Coorong.
2. This research focuses on the ecology of only three small-bodied forage fish species in the Coorong. Further study of other forage fish species will provide a more comprehensive understanding of the ecological responses of forage fish assemblages to environmental variation.
3. The findings of this study emphasise on how growth performance of adult forage fish species vary with environmental changes. Further research is needed to understand how forage fish species respond to environmental change in the Coorong during early developmental stages.
4. The present study focused on the diets and prey selection of forage fish species in the Coorong. Future research could examine the behaviour and dynamics of predator-prey interaction in the field and/or in the laboratory, to

determine whether feeding behaviour of forage fish is influenced by environmental conditions such as light, temperature and salinity.

5. In addition, further study will be required on the functional role of forage fishes (e.g. trophic pathway) and the influence of freshwater inflows on trophic dynamics in the Coorong.

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