Trophodynamics of plankton communities subjected to environmental fluctuations in an inverse estuary: A case study of the Coorong; South Australia

By

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Table of Contents

ABSTRACT

Aquatic ecosystems contribute to the majority of earth's surface and play a vital role in sustaining life on the earth. They are highly diverse in terms of physio-chemical properties, which in turn drive their biological properties. Yet, they are interconnected and influence the physical, chemical and biological properties of each other. Aquatic systems tend to be highly dynamic. In particular, estuaries are complex systems interconnecting coastal waters and riverines systems. The physio-chemical dynamics of estuaries are highly driven by the influences of freshwater flow from rivers and tidal input of marine water. As such, estuaries are dominated by gradients of environmental factors that fluctuate considerably. Organisms living in estuaries tend to be adapted to the variability of environmental factors. However, spatial and temporal changes remain a major influence on the populations, communities, physiologies and phenologies of estuarine dwelling organisms. Modification of natural spatial and temporal environmental variations often discrupts the natural fluctuations community structure and interactions. Such modifications often cause serious impairments to ecosystem health and functioning. The Coorong, South Australia, is a Ramsar listed inverse estuary that is highly affected by anthropogenic disruption of water flow. It is a system that has suffered extreme ecosystem function and health degradation associated to reduced freshwater input due to anthropogenic and drought influences. This research thesis uses planktonic organisms to study the health and functioning of the Coorong. Planktonic organisms are very closely linked to water quality parameters. As such, this study identified planktonic bioindicators of environmental health of the Coorong. Moreover, it examined the changes in zooplankton population structure and recruitment in relation to variations in water quality and, finally, it examined the changes in ecosystem functioning in relation to water flow in the system. Overall, this study uses innovative approaches for studying changes in plankton communities in relation to environmental fluctuations and provides important tools and information for managing the health of a degraded inverse estuary.

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.

Deevesh A. Hemraj

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LIST OF FIGURES

vectors represent major phytoplankton groups with spearman correlation coefficient > 0.3 with any of the first two ordination axes. (B) dbRDA of phytoplankton communities from November 2013 to October 2014 based on Bray-Curtis similarity matrix. Vectors represent environmental parameters with spearman correlation coefficient > 0.3 (strength and direction indicate the effect of the parameters on the ordination plot).

Figure 10: (A) Principal Coordinate Analysis (PCO) of zooplankton communities from November 2013 to October 2014. Ordinations are based on Bray-Curtis similarity index and vectors represent major phytoplankton groups with spearman correlation coefficient > 0.3 with any of the first two ordination axes. (B) dbRDA of zooplankton communities from November 2013 to October 2014 based on Bray-Curtis similarity matrix. Vectors represent environmental parameters with spearman correlation coefficient > 0.3 (strength and direction indicate the effect of the parameters on the ordination plot).

Figure 11: Plot of indicators against salinity and ammonia levels. (A) Polychaete and gastropod as a function of salinity of the North Lagoon. (B) *Acartia* cf. *fancetti* nauplii, copepodite and adults as a function of salinity of the South Lagoon. (C) *Cyclophora* sp. and *Scrippsiella* spp. against salinity of the South Lagoon. (D) *Cyclophora* sp. and *Scrippsiella* spp. against ammonia levels of the South Lagoon.

Figure 12: Schematic diagram showing experimental protocol developed and followed in chapter 3.

Figure 13: Bar graphs of mean pre-naupliar data representing (A) brood size, (B) embryonic development time and (C) percentage hatching success for two salinities and four pH levels. Mean for 8 replicates were calculated (N=8) and error bars represent standard deviation.

Figure 14: Percentage of individuals from salinity 2 treatments that successfully developed from (A) nauplii to copepodite, (B) copepodite to adults and (C) nauplii to adult against pH values measured during experimental procedure.

Figure 15: Percentage of individuals from salinity 10 treatments that successfully developed from (A) nauplii to copepodite, (B) copepodite to adults and (C) nauplii to adult against pH values measured during experimental procedure.

Figure 16: Overall rates of mortality (individuals per day) from nauplii to adult for each pH treatments at (A) salinity 2 and (B) salinity 10. Mean for 8 replicates were calculated (n=8) and error bars represent standard deviation.

Figure 17: Cumulative frequency plot of nauplii (N1-N6), copepodites (C1-C5) and adult at salinity 2 and pH (A) 7.77, (B) 7.51, (C) 7.29, and (D) 7.18. The plots exclude the <10 % and >90 % tails.

Figure 18: Cumulative frequency plot of nauplii (N1-N6), copepodites (C1-C5) and adult at salinity 10 and pH (A) 8.09, (B) 7.86, (C) 7.68, and (D) 7.41. The plots exclude the <10 % and >90 % tails.

Figure 19: Schematic diagram of population experiment (A) and egg production experient (B) developed and used for chapter 4.

Figure 20: Population size in relation to salinity and temperature. Black dotted trendline (R^2 = 0.6897) represents population size at 15 °C (Spearman's ρ = -0.891, n = 12, P < 0.01) and grey dotted trendline (R^2 = 0.6916) represents population size at 20 °C (Spearman's ρ = -0.867, n = 12, P < 0.01).

Figure 21: Box plot of data distribution for number of nauplii (A), copepodite (B) and adults (C) across salinity and temperature treatments. The lower edge of the box represents Quartile 1, the upper edges represents Quartile 3, while the middle line is the median. Error bars indicate the minimum and maximum numbers of individuals per treatment.

Figure 22: Percentage contribution of nauplii, copepodite and adults to the overall population structure at each salinity and temperature treatments. At 20 °C and salinity 60 no individuals were observed in the experiment.

Figure 23: Male to female ratio of adult population observed at each salinity and temperature treatment. At 20 °C and salinity 60 no individuals were observed in the experiment.

Figure 24: Daily egg production per female at each salinity and temperature treatment. Error bars represent standard deviation.

Figure 25: Average egg hatching success per treatment condition. Error bars represent standard deviation.

Figure 26: Map of the Coorong and Lower lakes showing lagoons separation and the Murray Mouth. Sampling sites are represented by asterix. Basemap was created using ArcMap (version 10.3.3; www.arcgis.com) and Coreldraw X7 (http://www.coreldraw.com/en/product/technical-suite-education-edition/).

Figure 27: Principal Component Analysis of water quality parameters of the Coorong showing differentiation between the North and South lagoons. The vectors represent the highest contributing parameters (Spearman's correlation $\rho > 0.2$).

Figure 28: Distance Based Redundancy Analysis of plankton community differentiation in relation water quality changes across the Coorong. The vectors represent the highest correlating water quality parameters (Spearman's correlation $\rho > 0.6$).

Figure 29: Distance Based Redundancy Analysis of plankton communities in the (a) North lagoon and (b) South lagoon in relation to water quality parameters and barrage flow as factor. The vectors represent the highest correlating water quality parameters (Spearman's correlation $\rho > 0.3$).

Figure 30: Network of water quality and plankton interactions for the North lagoon of the Coorong under high (a) and low (b) barrage flow. Edges are undirected and the thickness represent edge-betweenness centrality. A Bray-Curtis similarity threshold of 0.6 was applied to capture the most significant interactions.

Figure 31: Network of water quality and plankton interactions for the South lagoon of the Coorong under high (a) and low (b) barrage flow. Edges are undirected and the thickness represent edge-betweenness centrality. A Bray-Curtis similarity threshold of 0.6 was applied to capture the most significant interactions.

LIST OF TABLES

CHAPTER 1

GENERAL INTRODUCTION

Understanding aquatic systems

Aquatic ecosystems, including fresh and saline systems, contribute to the majority of earth's surface. These systems play an important role in sustaining life on the earth. They are indeed involved in regulating global climate by a two-way interaction with the atmosphere whereby physical properties of water affect the atmosphere while winds, cloudiness and precipitation affect water temperatures, currents and water cycles (Webster 1994; Vihma et al. 2015). Secondly, they encompass atmospheric-aquatic fluxes of oxygen and carbon dioxide, along with other biogeochemical cycles (Bopp et al. 2002; Ciais et al. 2014). Finally, aquatic systems are responsible for more than half of the net global primary production (Field et al. 1998; Duarte and Prairie 2005) and provide habitats for a large variety of organisms, with higher levels of taxonomic diversity than terrestrial habitats (Carr et al. 2003). For example, they are home to 28 animal phyla, out of which approximately 45% are marine endemic, compared to 11 terrestrial ones (Mittelbach et al. 2001).

From streams and lakes to estuaries and oceans, aquatic systems are highly diverse in terms of physio-chemical properties, which in turn drive their biological properties. Yet, these systems are interconnected and influence the physical, chemical and biological properties of each other. For example, river discharge contributes to a vast majority of nutrient, organic matter and/or pollutants to coastal systems (Nicolao et al. 2006) and, therefore, influence their productivity (Whitney et al. 2005). On the other hand, several marine organisms migrate up lacustrine and riverine systems to complete their life cycles (Bond et al. 2015). Marine and freshwater systems are connected by estuaries. Estuarine systems are arguably the most dynamic aquatic systems. They are influenced both by freshwater, through river discharge, and by seawater, through tidal exchange. As an important interface between freshwater and marine environments, estuaries are affected by very high spatial and temporal variations in environmental parameters.

Over the years, estuaries have been subjected to ample natural and/or anthropogenic modifications, pertaining to water flow changes, water quality changes, coastal development,

pollution, over fishing and/or introduction of invasive species (Mallin et al. 2000; Williams et al., 2008; Wang et al. 2016). These have led to changes in biodiversity and ecosystem states, and thus management measures have been developed to maintain ecosystem health and sustain biodiversity. Such measures include water flow management, waste water treatment, better fish stock assessment, assessment of ecosystem health, and analysis of ecological thresholds, to inform management. Active adaptive management is often required to sustain ecosystem states and, especially, to escalate the recovery of degraded systems (Groffman et al. 2006). Often, biological conservation is focused on the restoration of ecosystems, favouring specific species or habitats. The adequate conservation and restoration of an ecosystem involves an understanding of the overall physical, chemical, biological and functional characteristics of the ecosystem (Duarte et al. 2015). However, there remains a lack of focus on the assessment and understanding of ecosystem function when looking at the management, protection and restoration of estuarine system worldwide.

Estuaries

One of the most straightforward definition of estuaries is given by Prichard (1967) and states that “An estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurable diluted with freshwater derived from land drainage”. However, other authors have argued about physical, chemical and biological relevance of definitions, which emphasise the variety of estuaries. In particular, estuarine systems are of several different geomorphological structures. They are generally described as drowned river valley (coastal plain), tectonic estuaries, bar-built (lagoons) and fjords (glacial eroded estuaries). Although there are different types of estuaries and each estuary is arguably unique in its way, they generally share common hydrological features.

For example, estuaries are all affected by land drainage, tides, as well as precipitation and evaporation. Freshwater inflow can alter estuarine hydrodynamics by creating modifications to the circulation, stratification and water flushing (Azevedo et al. 2010). Moreover, it affects

the intrusion of salt water, whereby increased freshwater inflow is inversely related to salt water intrusion (Gong & Shen 2011). Freshwater input from the upper part of the estuary forms a horizontal density gradient, as well as vertical stratification, with saltwater from the lower end, coming in by tidal action. This usually results in increased ebb tide velocities at the surface and increased flood tide velocities at the bottom, especially related to stratification due to the forces related to flow of two different water masses (Snedden et al. 2012).

Ebb and flood tides may also be associated with the depth and morphology of an estuary. Deeper parts of estuaries, for example, are often more influenced by ebb tides while shallower regions are dominated by flood tides due to tidal velocity distortions as described by Friedrichs et al. (1992). Finally, precipitation and evaporation affect the hydrology mainly by causing increase and decrease in freshwater inflow into the estuary, which is a significant part of the water circulation. The influences of hydrodynamics and mixing are key to the chemical properties of estuaries and, therefore, highly important for biological communities.

Estuarine environmental fluctuations

Estuaries have long been regarded as naturally stressed environments due to high environmental fluctuations, especially regarding salinity, nutrients, temperature, pH and gaseous exchange dynamics (Elliot & Quintino 2007). Salinity gradient is the main environmental factor influencing the structural and functional characteristics of estuarine biota (Telesh et al. 2011). In most estuarine systems, salinity gradients are unstable because of the varying regimes of tidal action and freshwater inflow. However, in tideless estuaries, such as the Baltic Sea, more stable salinity gradients can be found (Telesh et al. 2013). To understand the distribution of organisms in estuaries, a few models and salinity range zones have been described based on observed biotic distributions. The most recognised model is the Remane diagram (Fig. 1), which describes the change in number of species (species richness) along a salinity gradient, whereby an Artenminimum (i.e. species abundance declines to a minimum, normally in salinity range of 5 to 8) is present. Also, salinity classification schemes such as the

Venice System (0-0.5, 0.5-5, 5-18, 18-30, 30-40) as mentioned by Greenwood (2007) and the statistically derived Bulger et al. (1993) scheme (0-4, 2-14, 11-18, 16-27, 24-marine) have been suggested to describe potential salinity ranges where differences in faunal and floral diversity may occur.

Moreover, waters with salinity between 5 and 8 have been identified to radically change in ionic composition and suggested to be a physio-chemical barrier between marine and freshwater fauna (Khlebovich 1969). Therefore, this range of salinity is suggested to be a diversity minimum zone, named 'Horohalinicum' by Kinne (1971). Finally, Attrill (2002) suggested another model whereby the diversity of fauna was described to decrease linearly with salinity range, where salinity range is expressed as the difference between mean low water salinity and mean high water salinity. This model therefore accounted for marine and freshwater inflow regimes. Further arguments were made implementing the presence of two ecoclines, one from freshwater towards brackish water and the other from marine water towards brackish water (Attrill & Rundle, 2002). Although they have been applied in tidally influenced estuaries, all the above models, except for the Attrill (2002) linear model and both salinity classification schemes, are based on the Baltic Sea, which is not tidally influenced and has a stable salinity gradient. This likely increases the relevance of the linear model and schemes for most estuaries. The Venice and statistical schemes are relatively similar, except for some higher levels of separation and overlaps in the brackish zones of the statistical scheme. They are relatively old but very important as they form the basis of newer schemes such as the 'two ecoclines' and 'linear models' that are now more comprehensive and applicable to different systems. The variety of different salinity models used to understand the distribution of organisms in estuaries shows the complexity of understanding the influence of salinity on physio-chemical and biological traits in these systems. Moreover, it emphasises the dissimilarity in response of different estuarine systems to salinity influences, showing that although generalised models are good indicators, the individual assessment of systems may be more beneficial.

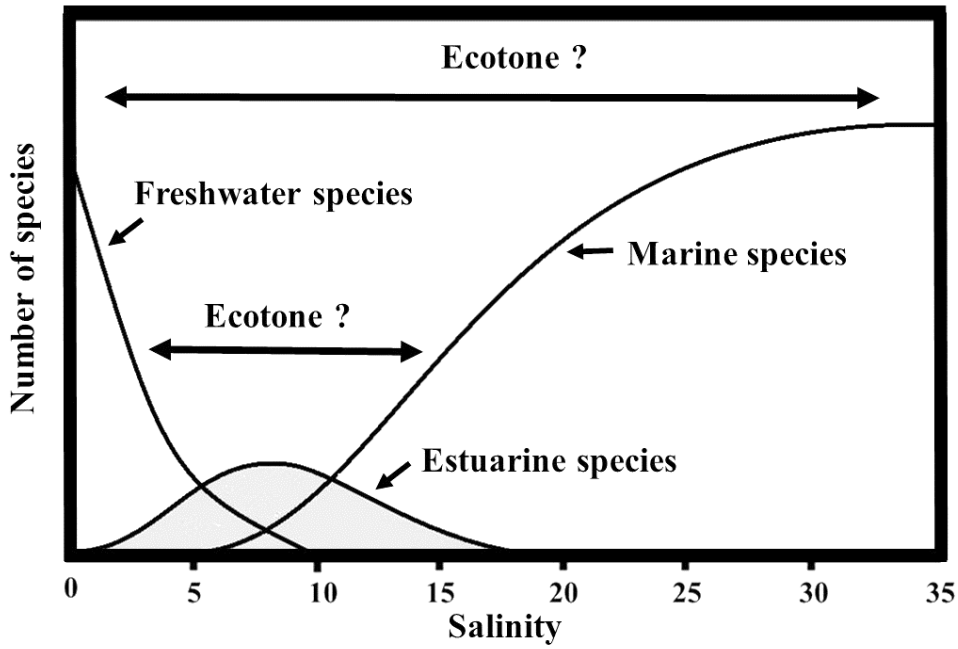

Figure 1: "Revised Remane diagram" implementing ecotones and ecoclines concept and showing freshwater, estuarine and marine species distribution in relation to salinity. Graph redrawn from Whitfield et al. (2012).

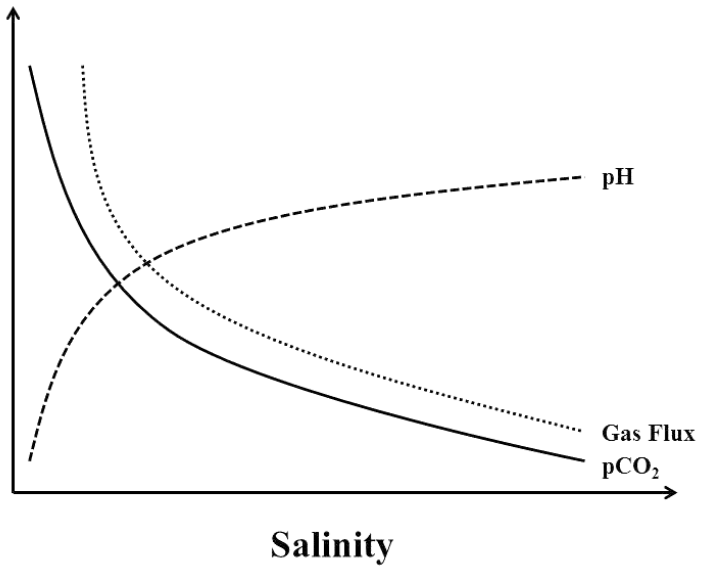

Figure 2: General relationship between salinity and pCO_2 (solid line), with resulting changes in pH (dash line), and atmospheric flux of CO_2 (dotted line) in estuarine waters. Adapted from Evans et al. (2013), Hunt et al. (2014) and Maher et al. (2015).

While rivers discharge freshwater into estuaries and cause changes in salinity, they also contribute to high amounts of nutrients, especially nitrate and phosphate, as well as organic matter. Nutrients dynamics is often dependent on variations in hydrological and flushing characteristics (Pinckney et al. 2001), and are therefore dependent on water retention.

Estuaries with long water residence times, generally have abundant nitrogen fixing cyanobacteria and high denitrification, but tend to be phosphate-limited. On the other hand, those with shorter residence time tend to be nitrogen regulated due to reduced nitrogen fixation, and generally have higher phosphate availability through desorption from the sediment (Pinckney et al. 2001; Longphuirt et al. 2016). Estuaries, therefore, tend to have relatively high nutrient concentrations compared to other aquatic systems but also remain temporally and spatially variable, thus influencing variations in productivity. The discharge of allochthonous organic matter also influences estuarine dynamics and productivity. Organic matter is an important component of biogeochemical cycles in estuarine systems, fuelling detritus-based food webs (Goñi et al. 2003). Moreover, the heterotrophic consumption of allochthonous organic matter is inversely related to estuarine primary production, thus consuming more oxygen than produced and often being saturated with carbon dioxide (CO_2) produced from heterotrophy (Soetaert et al. 2006). The fluctuation between primary production and heterotrophy is influential on the fluxes of oxygen and carbon dioxide in estuaries.

Recently, several studies have focused on the variabilities in gaseous flux, especially CO_2 levels (Cai 2011; Hu & Cai 2013; Reum et al. 2014) in estuaries. These have shown that estuaries can potentially be exposed to much higher CO_2 partial pressures (pCO_2) than coastal and open ocean environments. For example, the Pearl River estuary (China) is known to reach up to 7,000 ppm pCO_2 (Zhai et al. 2005) while the Tyne (United Kingdom) and the Scheldt (Netherlands/Belgium) can reach up to 5,500 and 9,500 ppm pCO_2, respectively (Borges et al. 2006; Ahad et al. 2008). These values reflect pCO_2 levels that have been forecasted to occur in marine environments in the next thousands of years. In estuaries, such high pCO_2 levels are mostly derived from net heterotrophy as an *in-situ* respiration by-product, or from

riverine inputs of high pCO_2 water or organic matter (Frankignoulle & Borges 2001; Maher et al. 2015). Estuaries are thus, now, recognized as environments susceptible to very high seasonal pCO_2, and resultant pH, fluctuations from low-pH riverine water discharge or heterotrophy. However, the physical, chemical, and, especially, biological effects of these variations in estuaries are relatively unexplored.

River discharge contributes considerable amounts of water to estuaries and oceans (McKee et al. 2004), having a biogeochemical role of transporting organic and inorganic carbon from terrestrial environments to the ocean (Abril et al. 2000; Yao et al. 2007; Benstead & Leigh 2012). In particular, rivers play an important role in the global carbon cycle and are typically supersaturated with CO_2 derived from allochthonous sources, autochthonous sources (Yao et al. 2007; Butman & Raymond 2011; Mayorga et al., 2005) and entrainment of soil-based CO_2 (i.e. from root respiration and terrestrial decomposition; Richey et al. 2002). Ground water input and exchange of CO_2 between riverine water and the atmosphere also account for CO_2 concentration changes in rivers (Cai & Wang 1998; Koné et al. 2009; Hunt et al. 2011). The pCO_2 measured in rivers is thus highly dependent on temporal and spatial variations in individual environments, therefore directly affecting the amount of carbon that is eventually discharged into estuaries (Gupta et al. 2009; Hunt et al. 2011; Sarma et al. 2011).

Metabolic processes such as primary production and respiration, are also key factors influencing pCO_2 and pH levels in aquatic systems (Duarte & Prairie 2005; Hu & Cai 2013). In fact, Duarte and Prairie (2005) argue that most aquatic systems, contrary to prior belief, tend to be net heterotrophic in nature because of higher respiration levels than primary production. Such systems would thus be considered secondary sources of CO_2 to the atmosphere, when aquatic CO_2 is higher than atmospheric CO_2, thus not in equilibrium (Duarte & Prairie 2005; Laruelle et al. 2010). In situ production and dissolution of CO_2 in estuaries are affected by variations in temperature and salinity (Fig. 2), whereby a decrease in solubility of CO_2 is observed with increasing temperature and salinity (Bachu & Bennion 2009). Waters of higher salinities have better pH buffering capacity than lower salinity waters and, therefore, tend to

be more stable in terms of CO_2 exchange with the atmosphere (Hu & Cai 2013). Because of the natural chemical and salinity gradients of estuaries, lower pCO_2 values and air-water exchange are observed around the river mouth area rather than in the fresher riverine, as demonstrated through pCO_2 measurements along salinity gradients in different systems (Ahad et al. 2008; Borges & Abril 2011, Cai 2011; Evans et al. 2013; Hunt et al. 2014, Maher et al. 2015). The influence of temperature is often observed with higher pCO_2 levels in warmer months compared to cold months (Koné et al. 2009; Zhai & Dai 2009; Borges & Abril 2011; Reum et al. 2014). Overall, although the solubility of CO_2 decreases with higher temperature (Bachu & Bennion 2009), the higher pCO_2 values generally observed in warmer months can be linked to the higher respiration rates and CO_2 production at higher temperatures (Jankowski et al. 2014). Being CO_2 saturated, estuaries tend to have high water to air gas flux and, therefore, are major contributors of CO_2 to the atmosphere (Jiang et al. 2008; Maher et al. 2015). Consequently, the amount of CO_2 in estuarine systems is also dependant on the variability of gas flux rates along the whole estuary. The exchange rate can be assessed using $F= k\alpha\Delta C$ (Maher et al. 2015), where k is the gas transfer velocity at specific wind stress and bottom stress, α is the solubility coefficient at in situ salinity (Fig. 2) and temperature, and ΔC is the difference between the gas partial pressure in the air and surface water (pCO_2water – pCO_2air). While it is known that pCO2 and pH levels tend to vary with other environmental parameters in estuaries, the resultant influences on biological communities in estuaries is poorly understood.

Anthropogenic impacts and health management

Estuaries have long been used by humans for various purposes as they provide a link between land and sea, and are highly dynamic and productive, therefore, recognised as prodigious systems for harbours, industries, fisheries, tourism and recreation (Attrill 1998). The use of estuaries by humans has impacted on the ecological integrity and several ecological traits associated with estuaries (Kennish 2002). For example, land claim for coastal development

has removed substantial parts of intertidal areas, creating significant habitat loss and degradation of faunal biomass, production and/or fitness (Mclusky et al. 1992; Burton et al. 2006). Moreover, activities such as shipping, industrial discharge, agriculture and urbanisation have contributed to extensive pollution in several estuarine systems by causing oxygen depletion, accumulation and bioaccumulation of toxic organic compounds and heavy metals, eutrophication and thermal pollution (Kennish 2002; Sun et al. 2012; Strokal et al. 2016). Finally, modification of freshwater flow and tidal mixing by dams or barrages have altered hydrodynamics, ecosystem function and ecological interactions by forcing changes in connectivity, habitat complexity, species composition and seasonal patterns (Grange et al. 2000; Adams et al. 2009; Olin et al. 2015).

Anthropogenic disturbances modify the natural ecosystem and recovery towards pre-existing conditions can be attained by natural restoration through secondary succession (passive process) or targeted ecological restoration programs (active process), or be unattainable, depending on the severity of degradation (Elliot et al. 2007; Borja et al. 2010; Duarte et al. 2015). However, as estuaries are naturally stressed environments and estuarine-dwelling organisms are generally well adapted to high temporal and spatial variations in environmental conditions (Elliot & Quintino 2007), detecting differences in organism response in relation to additional anthropogenically-induced stresses can be especially difficult in estuaries. This is known as the 'Estuarine Quality Paradox', as described by Dauvin (2007) and further explored by Elliot and Quintino (2007). For example, from studies on the Seine estuary, France, different sources of stress and their implications for estuarine quality were clearly discriminated (Dauvin, 2007). Similarly, a clear discrimination between natural stressors and anthropogenic-related stressors is essential to better understand and manage estuarine systems, especially those heavily impacted by anthropogenic activities (e.g. Hudson-Raritan Estuary, USA, and Pearl River Estuary, China). Nonetheless, ecosystem response often involves shifts from states in relation to stress thresholds and magnitudes (Duarte et al. 2015). Under higher stress levels, several trends may be observable, including imbalance between

primary production and respiration with respiration generally being higher, increases in nutrient loss or turnover, parasitism, and changes in community structure favouring r-strategists, low species number but higher abundance (Elliot & Quintino 2007).

Due to the complexity of the response of estuarine systems to additional anthropogenic stress, developing an understanding to underpin restoration and health management is particularly challenging. Moreover, the growing level of estuary use for various human purposes poses difficulties for managers in balancing the restoration of ecosystem health, maintaining ecosystem services and sustaining socioeconomic properties (Lonsdale et al. 2015; Boerema & Meire 2017). To this end, Lonsdale et al. (2015) have developed an 'Estuarine Planning Support System', accounting for different aspects of estuarine management (ecological, economic and legislative). Moreover, Boerema and Meire (2017) have developed an ecosystem services reference matrix for estuarine management using 39 specific management measures categorised in hydrology, water and sediment quality, morphology and habitat characteristics. However, achieving a balance in sustaining ecosystem health while promoting ecosystem services of estuaries requires comprehensive, applicable, research to monitor and model the response of a system in relation to stress. Furthermore, as indicated by Lonsdale et al. (2015) and Boerema and Meire (2017), a comprehensive understanding of system-specific responses is required to develop and apply adequate management measures for different estuarine systems.

Plankton and environmental fluctuations

In aquatic environments, various planktonic organisms, including planktonic bacteria, nano- and pico-phytoplankton, larger phytoplankton and zooplankton form the base of the food web. They are essential for supporting the whole trophic web and are impacted by both bottom-up and top-down controls. Phytoplankton and several groups of bacteria are key primary producers in aquatic systems, utilising light energy and nutrients to produce organic carbon. Zooplankton are the link between primary producers and higher consumers. They are the main

grazers of phytoplankton and bacteria and serve as prey for ichthyoplankton and higher predators (Turner 2004; Saiz et al. 2007; Morozov & Arashkevich 2010). As such, plankton support the diversity in aquatic systems. Although food web interactions are important in regulating the distribution and diversity of organisms, water properties are also of key significance in playing a similar role. For example, phytoplankton distribution, biomass and diversity in any water body are influenced by the availability of nutrients, light levels, salinity levels and temperature (Carpenter & Kitchell 1987; Cloern 1999; Juhl & Murrell 2005), while zooplankton life cycle, population structure and community structure are affected by salinity, temperature and acidity (Havens et al. 1993; Cervetto et al. 1999; Orr et al. 2005, Cripps et al. 2014). Therefore, plankton communities are subjected to geographical and seasonal variations. Planktonic communities react very rapidly to water quality changes as plankton generally reproduce and grow rapidly, depending on geographical locations. For example, Le Coz et al. (2017) have described the patterns of zooplankton distribution in relation to river-estuary continuum theories, such as the Remane's diagram, River Continuum Concept and Riverine Ecosystem Synthesis. The have revealed the importance of tidal influences and river flow velocity on zooplankton development, which are not included in these theories. Moreover, they have reiterated the major influences of salinity and other environmental factors on the spatial communities, showing sepatation of four distinct zones. As such, plankton community changes represent excellent indicators of water quality or environmental health change (Parmar et al. 2016).

Estuarine plankton communities are subjected to intense fluctuations in environmental parameters, derived from both natural and anthropogenic causes. Plankton communities are highly sensitive to changes in water quality. Therefore, temporal and spatial variations in water quality parameters drive the community structure and interactions of planktonic organisms in estuaries. Freshwater and sea-water mixing in estuaries creates a salinity gradient along which planktonic organisms are generally distributed based on their salinity tolerance and ability to reproduce. Several studies have shown clear variations in community structure of

protists and phytoplankton in estuarine systems (Gasiūnaitė et al. 2005; Jendyk et al. 2014; Balzano et al. 2015; Leterme et al. 2015; Azhikodan & Yokoyama 2016). These studies show that diatom-dominated communities are generally observed at higher salinities, while chlorophytes and cyanobacteria are more typical of lower salinity areas. Along with salinity, nutrient dynamics are highly influential on phytoplankton community structure. Because of temporal variations in freshwater inflow, and therefore nutrient loading, nutrient levels in estuaries are variable. In particular, the ratios of nitrogen to phosphorus concentrations are most commonly influential on phytoplankton community structure, but also on population blooms, especially in relation to anthropogenically-derived excess nutrients (Anderson et al. 2002; Morse et al. 2014; Wood et al. 2014; Paerl et al. 2016). Although salinity and nutrient levels are most directly influential on phytoplankton growth and communities, the interactions with several other parameters, such as turbidity, temperature, organic matter and gas fluxes, are also important (Cloern et al. 2014; Helbling et al. 2015; Kruk et al. 2015). As such, the relationships between phytoplankton communities and water quality dynamics are highly complex, even more so in estuaries.

Similarly to phytoplankton, zooplankton are directly affected by water quality fluctuations. Such fluctuations often drive the reproductive success, population dynamics and community interactions of zooplankton. For example, several copepod species can tolerate and reproduce in a range of salinity levels (e.g. *Acartia tonsa* and *Eurytemora affinis*; Devreker et al. 2009; Aguilera et al. 2013). The have higher phenotypic plasticity, enabeling them to sustain or adapt to changed environmental conditions. On the other hand, the distributions of several cladoceran and rotifer species are often negatively correlated with salinity (Park & Marshal 2000; Silva et al. 2009). In recent years, research on the influence of acidity (CO_2-derived acidity) on zooplankton has increased. Studies have shown varied effects of acidity on zooplankton, whereby the reproductive success of several species is severely impacted, while others show low or insignificant effects (Kurihara 2008; Pedersen et al. 2014; Almén et al. 2016). In estuaries, however, relatively fewer studies have examined the effects of acidity

on zooplankton (Aguilera et al. 2013). Although several estuarine zooplankton species are well known to tolerate environmental changes due to osmoregulation and/or phenotypic plasticity, the combined effects of different water quality parameters on zooplankton reproduction and population dynamics are relatively unexplored. Furthermore, there is a lack of understanding on the changes in food web structure and interactions of planktonic organisms in relation to environmental variability that are encountered, naturally or anthropogenically, in estuarine systems.

The Coorong

The Coorong is a coastal lagoon, approximately 110 Km long, located along the southern coastline of South Australia (Fig. 3). It is an important part of a RAMSAR listed wetland, the Murray Mouth, Lower Lakes and Coorong wetland, situated around the mouth of Australia's largest river basin, the Murray-Darling Basin. This wetland supports a high diversity of flora and fauna, including migrating shore birds, fish populations, planktonic organisms and benthic macroinvertebrates (Zampatti et al., 2010; Paton and Bailey. 2011; Shiel & Tan 2013; Paton & Bailey 2012; Dittmann et al. 2015; Leterme et al. 2015; Ye et al. 2015). Moreover, it is an important system for recreation and fisheries. The Coorong itself supports a wide array of marine and estuarine fauna and flora. It is relatively shallow (mostly < 3 m deep) and is separated from the sea by sand dunes. It naturally divides into two sections, known as the North lagoon and the South lagoon. These two sections are connected by a relatively narrow channel (approximately 50m to 75m) where water exchange and mixing occur. Unlike most estuaries, freshwater input from the MDB occurs close to the estuary mouth, and is primarily drawn along through the Coorong by salinity-driven gradients in response to evaporative water loss (Webster 2010). Therefore, it exhibits inverse estuarine characteristics, whereby evaporation exceeds freshwater input, and salinity is higher at the head of the system than at the mouth, forming an inverse estuarine system (Pritchard 1952; Wolanski 1986; Leterme et al. 2015; Lester & Fairweather 2009). The main source of freshwater for the Coorong is from

the Murray River, through the Lower Lakes. Freshwater discharge is controlled by barrages built in the 1940s to prevent saltwater intrusion into the Lower Lakes. Since the building of the barrages, over the years, the hydrodynamics and water quality of the Coorong have changed with reduced mixing and flushing, along with an increase in salinity, especially in the South lagoon (Leterme et al. 2015). From 2001 to 2010, Australia suffered a severe drought and there was a significant reduction in freshwater release into the Coorong. During this period, the system significantly dried out, reaching salinity levels up to 150 in the South lagoon and the ecosystem experienced a severe health degradation with loss of several species, such as birds, fish and invertebrates (Lester & Fairweather 2009; Dittmann et al. 2015). Since 2011, freshwater release has resumed, however, the system remains under recovery and in an unsteady health state, and therefore, requires further research towards better management.

Aims of the project

Over the years, several estuarine systems worldwide have been impacted by flow modifications, pollution, overuse, or coastal development that have affected or degraded their health. Estuaries are highly dynamic systems and any modification to their functioning is likely to have accumulating effects over time, even more so if affected by extreme events. A typical example of such a system is the Coorong, whereby water flow, connectivity and water quality have been significantly affected. The degraded health of the Coorong has prompted multiple research and population assessment of birds, macrobenthos and fish. Moreover, hydrodynamic changes have been investigated to generate advice on water levels and freshwater release management. However, this research tended to focus more on biological conservation, mostly in the northern part of the system, and there is still a lack of comprehensive understanding of the overall nutrient dynamics, production and changes in food web linkages along the Coorong in response to changes in environmental conditions.

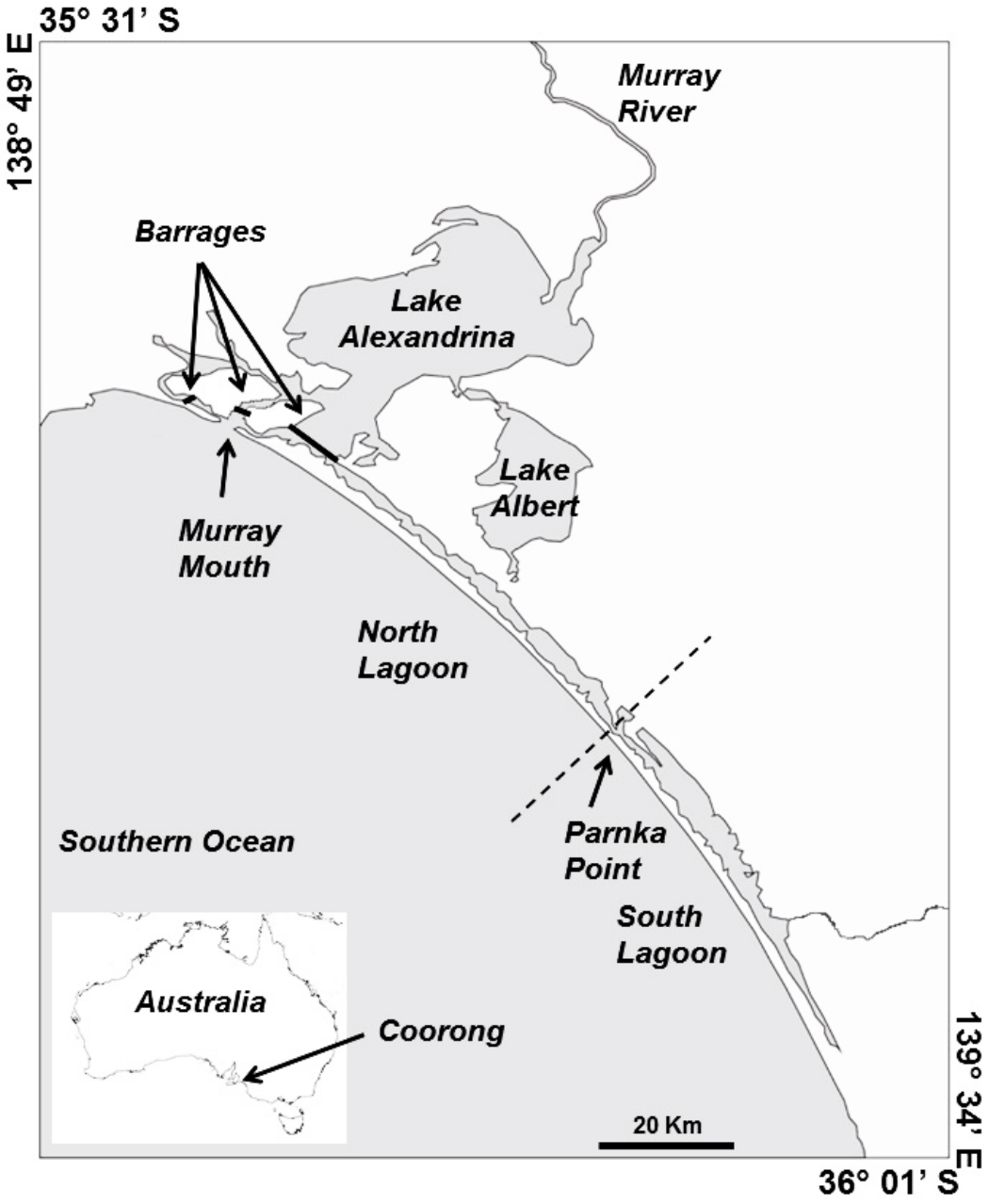

Figure 3: Map of the location of the Coorong in South Australia showing the Murray Mouth, Lower Lakes and barrages. The dotted line shows the division between the North and South lagoons at Parnka Point.

The plankton communities of the Coorong are, especially, poorly studied. The dynamics that drive changes in the plankton community structure and interactions along the system are not fully understood. A few studies have attempted to link the community structure of phytoplankton and smaller microbes to water quality fluctuations in relation to spatial, temporal and hydrodynamics changes (Jendyk et al. 2014; Balzano et al. 2015, Leterme et al. 2015). Relatively less is known about the effects of water quality and phytoplankton communities on changes in zooplankton communities along the system. Most importantly, prior to this study, no account of the zooplankton community was reported along the whole Coorong, especially in the southern parts of the system.

The overall aim of this study was to attain a detailed understanding of the changes in plankton communities in relation to fluctuating water quality. From this, applicable ecological information and methods can be deduced so as to underpin the management of the ecosystem health recovery of the Coorong by understanding changes in ecosystem functioning due to environmental variations.

The specific objectives are as follows:

1. Provide a comprehensive description of the plankton communities of the Coorong, including virus, bacteria, nano/picoplankton, phytoplankton and, especially, zooplankton.
2. Identify bioindicator species that will accurately define the state of the water quality, different habitat structures and ecosystem health.
3. Examine the influences of multi-stressors, likely encountered in the Coorong and other estuaries, on zooplankton lifecycle in relation to recruitment and population.
4. Understand the shifts in overall plankton community interaction and ecosystem functioning in relation to fluctuations in environmental conditions.

Overall, this study is presented in four main data chapters, each addressing specific parts of the overall aim and objectives. Information and methods presented can be applied in estuaries

and coastal lagoons worldwide to facilitate the understanding of the functioning of such ecosystems.

Thesis structure

Chapter 2: Plankton bioindicators of environmental conditions in coastal lagoons

In this study, the identification of adequate planktonic indicator species suitable for monitoring and managing environmental variability, habitat structure and health of the Coorong is undertaken. To achieve this, the spatial and temporal changes in phytplankton and zooplankton communities along the Coorong and their relation to environmental variability was studied by providing the first account of the zooplankton populations of the hypersaline part of the system.

Chapter 3: A combination of salinity and pH affects the recruitment of Gladioferens pectinatus (Brady) (Copepoda; Calanoida).

This chapter focuses on reproductive response and recruitment of copepod *Gladioferens pectinatus*, collected from the Coorong, in relation to changes in pH levels, that can be attained from low-pH freshwater input or acidsulphate soil washout, and salinity changes, from freshwater flow, that are encountered in the Coorong. More specifically, reproduction is studied in terms of pre-naupliar data, including brood sizes, embryonic development and hatching success, as well as post-naupliar data, including percentage of nauplii that grow into copepodites and adults, mortality rate, development in relation to time, and sex ratio of copepods under different levels of salinity and pH.

Chapter 4: Population structure of Acartia fancetti in relation to hyperhaline and thermal stresses; a neritic species in a hypersaline lagoon.

In this chapter, the aim is to identify the reproductive response of a population of *Acartia fancetti* to hypersalinity and temperature variations. More specifically, the egg production, hatching success and population dynamics of this species under a variety of hypersaline and temperature treatments are investigated to have a better understanding of the effects of

seasonal and anthropogenically derived changes in salinity and temperature on the population structure.

Chapter 5: Anthropogenic shift of plankton food web structure in a coastal lagoon by freshwater flow regulation.

In this study, the effects of freshwater water release on the planktonic food web of the Coorong is investigated, to understand the effects of fluctuations in flow regimes on the overall food web interactions and ecosystem functioning. The overall plankton communities (virus, bacteria, nano/picoplankton, phytoplankton and zooplankton) present along water quality gradients is examined and changes in community interactions are examined to provide an insight into the understanding of the microbial and zooplankton food web, based on flow regime. Due to the high difference in periodic water release into the system, the shifts in trophic interactions are studied in relation to environmental and habitat variability from freshwater release. This study uses a food web network approach rather than single trophic levels to gain better understanding of ecosystem functioning.

All four data chapters have been either publilshed or submitted to peer-reviewed journals. Although this study was primarily conducted by the author, supervisors and co-workers are included in published papers as co-authors due to their contribution in terms of study concept development, experimental design, sample analysis and data analysis.

CHAPTER 2

PLANKTON BIOINDICATORS OF ENVIRONMENTAL CONDITIONS IN COASTAL LAGOONS

This chapter has been published in the journal Estuarine, Coastal and Shelf Science.

Reference:

Hemraj, D.A., Hossain, M.A., Ye, Q., Qin, J.G. and Leterme, S.C., 2017. Plankton bioindicators of environmental conditions in coastal lagoons. *Estuarine, Coastal and Shelf Science*, *184*, pp.102-114.

2.1. Abstract

Coastal lagoons are characterised by strong spatial gradients of environmental parameters, especially hypersalinity, and are prone to anthropogenic disturbance. The Coorong (South Australia) is an inverse estuarine coastal lagoon separated from the sea by sand dunes. It is exposed to extreme water quality changes that affect its aquatic communities. Here, we used plankton as indicators of extreme environmental fluctuations to monitor and manage the environmental health of such complex systems. We defined the relationship of different plankton communities with water quality fluctuations and determined plankton species suitable for monitoring the ecosystem health. Two distinct communities of phytoplankton and zooplankton were identified, with salinity and nutrients being the principal factors impacting species distribution. Thus, two sets of indicator species were selected based on the different communities observed. Polychaete and gastropod larvae were positive indicators, showing salinity range restriction of brackish to marine. The distribution *Acartia* cf. *fancetti* represented healthy hypersaline conditions (salinity 40 – 60), while *Cyclophora* sp. and *Scrippsiella* sp. were negative indicators, correlating with extreme salinity and ammonia levels. The implementation of planktonic organisms as environmental indicators provided a constructive tool for the management of ecosystem health of the Coorong and will be applicable to similar coastal lagoons.

2.2. Introduction

A number of comprehensive and multidisciplinary studies (Barnes 1980; Lasserre and Marzollo 2000; Roselli et al. 2013; Giménez et al. 2014; Coelho et al.,, 2015) have shown that the ecology of coastal lagoons is highly variable, depending on the prevalent physical and chemical environment. In particular, tidal to wind-driven water movements and strong spatial gradients of environmental parameters can be observed (Lamptey and Armah 2008; Leterme et al. 2015). Coastal lagoons are prone to anthropogenic interference and disturbance by agricultural drainage, discharge of sewage or change in hydrology, thus modifying their structure and function (Brehmer et al. 2013; Dhib et al. 2013; Leterme et al. 2015). Such modifications, coupled with climatic variations, affect the overall ecosystem health and environmental gradients of specific coastal lagoons (Lester and Fairweather 2009; Paul et al. 2016), therefore causing stress and changes in communities of resident species (Paul et al. 2016). The active interaction of climatic and anthropogenic stressors presents a serious challenge in terms of managing and predicting the water quality and ecosystem health of estuaries and coastal lagoons.

Plankton communities are often used as bioindicators to monitor ecological change in aquatic systems (Paul et al. 2016; Lemley et al. 2016) and are considered good natural bioindicators due to their rapid response to fluctuating environmental conditions (Livingston 2002; Albaina et al. 2009; Amengual-Morro et al. 2012). In particular, phytoplankton populations reflect climate variability and changes that occur in aquatic ecosystems (Edwards and Richardson 2004; Richardson and Schoeman 2004; Leterme et al. 2005). Similarly, zooplankton communities are considered ideal bioindicators for estuarine conditions as they have the potential to remain in the water body of appropriate water quality (Wilson., 1994; Albaina et al. 2009). Assessing plankton dynamics is, therefore, of particular importance when considering the impact of environmental variability on ecological changes of coastal lagoons, which are amongst the most productive and dynamic habitats on earth (Alongi 1998; Gönenç and Wolflin 2005). However, fundamental information on how climatic variability affects population

dynamics, abundance and taxonomy in those environments is still relatively limited (Anthony et al. 2009; Hall et al. 2013).

The Coorong lagoon (South Australia) is one of Australia's most important wetland areas and a RAMSAR listed wetland. It is known to have a high biological diversity, supporting a wide variety of local and migrating shore birds, fish populations, planktonic organisms, benthic macroinvertebrate populations and flora (Zampatti et al. 2010; Patton and Bailey. 2011; Shiel and Tan 2013; Patton and Bailey 2012; Dittmann et al. 2015; Leterme et al. 2015; Ye et al. 2015). The Coorong is a long, narrow coastal lagoon at the mouth of Australia's largest river system, the Murray-Darling Basin (MDB). This shallow water system is connected to the sea at the mouth of the River Murray (Murray Mouth), which is subject to infilling and scouring on a seasonal basis. Unlike most estuaries, freshwater input from the MDB occurs close to the estuary mouth, and is primarily drawn along through the Coorong by salinity-driven gradients in response to evaporative water loss (Webster 2010). The Coorong lagoon is a complex system as it also exhibits inverse estuarine characteristics, whereby evaporation exceeds the freshwater input (Pritchard 1952; Wolanski 1986; Lester and Fairweather 2009). Moreover, salinity is higher at the head of the system than at the mouth (Leterme et al. 2015; Lester and Fairweather 2009). The salinity levels of the Coorong have increased dramatically between 2004-2010 (> 90) because of the lack of water inflow due to the increased retainment of water further up the Murray river (Nayar and Loo 2009), but also due to the drought (2004-2010; Zampatti et al. 2010). Since 2010, however, there has been a substantial increase in freshwater release into the Coorong and the connectivity to the sea has improved. This resulted in a change of fauna and flora communities as clearly shown by Patton and Bailey (2014), Dittmann et al. (2015), Leterme et al. (2015) and Ye et al. (2015).

Few studies have looked at the spatial distribution of planktonic organisms in hypersaline inverse estuaries such as the Coorong (Silva et al. 2009) and, therefore, relatively less is known compared to that in positive estuaries. Moreover, few studies have explored the use of planktonic organisms as ecosystem health and environmental variability indicator in such

systems. Understanding and modelling the dynamics of an aquatic system without knowledge of its base of productivity is often not possible (Jaanus et al. 2009). In this study, we identify potential planktonic indicator species suitable for monitoring and managing environmental variability and health of ecosystems such as inverse estuaries. To achieve this, we study the spatial and temporal changes in plankton communities along the Coorong (phytoplankton and zooplankton) and look at their relation to environmental variability, also providing the first account of the zooplankton populations of the hypersaline part of the system. We hypothesise a significant decrease in the number of plankton species and the presence of a different plankton community in the hypersaline region. We identify the main water quality parameters that influence the plankton dynamics of the system and then categorise suitable species to serve as indicators of water quality variability and ecosystem health following the criteria given by Hilty and Merenlender (2000), Carignan and Villard (2002) and Siddig et al. (2016).

2.3. Materials and methods

2.3.1. Study area

The Coorong is a shallow (< 3 m) and narrow (< 4 km) coastal lagoon over 110 km in length (Fig. 4). It is divided into two main lagoons, the North lagoon (NL) and the South lagoon (SL), and presents an estuarine area around the mouth of the Murray River. The North and South lagoons are separated by a narrow and shallow channel at Parnka Point. The Coorong is separated from the Southern Ocean by peninsular dunes, except at the Murray Mouth where marine water enters the system by tidal action. Freshwater, from the Murray River and Lower Lakes (Lake Alexandrina and Lake Albert), is released into the system, in the North lagoon, through a series of barrages which prevent salt water intrusion into the lakes and regulate freshwater inflow into the Coorong. Freshwater flow is also limited by seasonal availability, while water exchange between the North and South lagoons is restricted at Parnka Point. Due to its hydrological and physical properties, the Coorong is predisposed to an increasing salinity gradient; higher in the South lagoon (Webster 2005; Nayar and Loo. 2009). In this study, seven

sites were sampled monthly from November 2013 to October 2014 along the length of the Coorong (NL and SL) and in the Goolwa Channel (GC) (Fig. 4).

2.3.2. Environmental parameters

At each site, salinity, pH, temperature (°C) and turbidity (NTU) were measured using an AquaRead multi-parameter probe (Aquaprobe AP-800). Dissolved oxygen (DO) was measured using a Thermo Scientific portable meter (Orion Star A323 RDO/DO). Hourly wind data were obtained from the South Australian Research and Development Institute (SARDI). Wind stress (ε_W, $m^2.s^{-3}$) was calculated for individual sites using the formula $\varepsilon_W = (5.82 \times 10^{-9}) \times (W^3/Z)$ where W is the wind speed ($m.s^{-1}$) and Z is the water depth (Seuront et al. 2002). Tide data were downloaded from the Bureau of Meteorology website (www.bom.gov.au) and the amplitude was measured as the difference between high and low tide levels. Freshwater flow data for the barrages and Salt Creek were sourced from the WaterConnect website (www.waterconnect.sa.gov.au) and from the Department of Environment, Water and Natural Resources (DEWNR).

2.3.3. Nutrient concentrations

Triplicate water samples (10 mL) were collected at each site for the determination of dissolved inorganic nutrients concentrations (silica (SiO_2), ammonia (NH_3), orthophosphate (PO_4^{3-}) and nitrate/nitrite (NO_x^-)). Samples were filtered through SARSTEDT syringe polyethersulfone membrane filters (Filtropur S 0.45 µm pore size) into Greiner centrifuge tubes (15 mL, SARSTEDT) and stored at -20 °C until analysis. Prior to analysis, samples were thawed, mixed and placed into the auto-sampler (Latchat XYZ Autosampler ASX-520) of the Latchat QuikChem Flow Injection Analyser (FIA QuikChem 8500 Series 2). Samples were analysed following published methods (Hansen and Koroleff 2007).

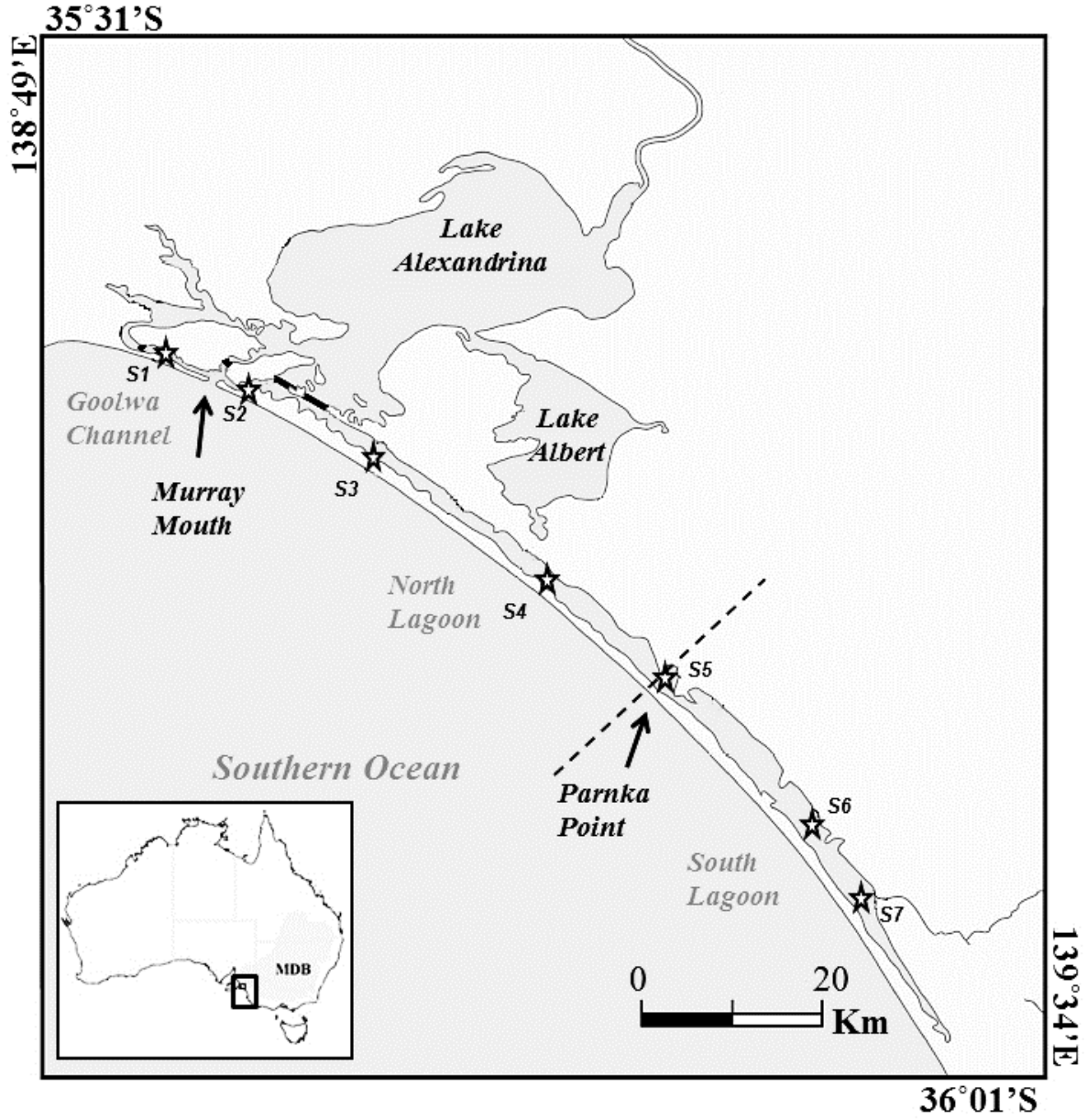

Figure 4: Map of the Coorong and Lower Lakes indicating the seven locations sampled monthly from November 2013 to October 2014. MDB: The Murray Darling Basin.

The FIA calibration standards were prepared with 0.6 M sodium chloride (salinity 35) to minimise refraction errors as several samples were of salinities higher than 40. This did not affect the detection limits of the FIA. The detection limit was 0.2 µM for SiO_2, 40 µM for NH_3, 0.02 µM for PO_4^{3-} and 0.009 µM for NO_x^-.

2.3.4. Phytoplankton

Chlorophyll *a* (Chl *a*) concentrations were measured as a representation of phytoplankton biomass (Bowes et al. 2012), but not solely relied upon to understand the phytoplankton dynamics (Kruskopf and Flynn 2006). Triplicates of 250 mL water samples were filtered on 47 mm glass microfiber filters (Filtech Grade 393, pore size 1 µm) using a vacuum pump (MILLIPORE Millivac Maxi SD1P014M04) and a filtration rack (Marine Laboratories, Engineering and Technical Services, CSIRO). These were subsequently stored at -20 °C, until analysis. Chl *a* was extracted in 5 mL methanol at 4 °C in the dark for 24 hours and the fluorescence was measured with a Turner 450 Fluorometer. The total Chl *a* concentration ($\mu g.L^{-1}$) was calculated as Chl *a* = *k*fluo(v/V)*, where *k* is the gain value of the fluorometer, *fluo* is the fluorometer reading, *v* is the extraction volume (mL) and *V* is the sample volume (mL).

For phytoplankton identification and enumeration, 1 L water samples were fixed with 5 mL Lugol's iodine (5% final concentration) in order to preserve the structure of the organisms' chloroplast and subsequently identified and enumerated by Microalgal services (Ormond, Victoria) as described in Leterme et al. (2015).

2.3.5. Zooplankton

Zooplankton samples were taken using a modified Schindler-Patalas (cylindrical and open top instead of a cubic and closed top; Fig. 5) plankton trap (35 L), filtered through a 50 µm mesh into 250 mL plastic jars and preserved in formalin (2% – 5%). Organisms were allowed to

settle at the bottom of the sampling jar and the excess formalin solution was carefully removed with a 60 mL syringe to avoid resuspending the organisms. The sample was then poured onto a gridded Greiner square petri dish (120 x 120 mm; Sigma-Aldrich) and zooplanktonic organisms were identified to the lowest taxonomic level possible and enumerated under a Nikon inverted microscope (Eclipse TS100) based on their morphological taxonomic features (Bayly 1992; Hamond 1971; Hamond 1973; Hebert 1977; Shiel 1995; Smirnov and Timms 1983; www.imas.utas.edu.au).

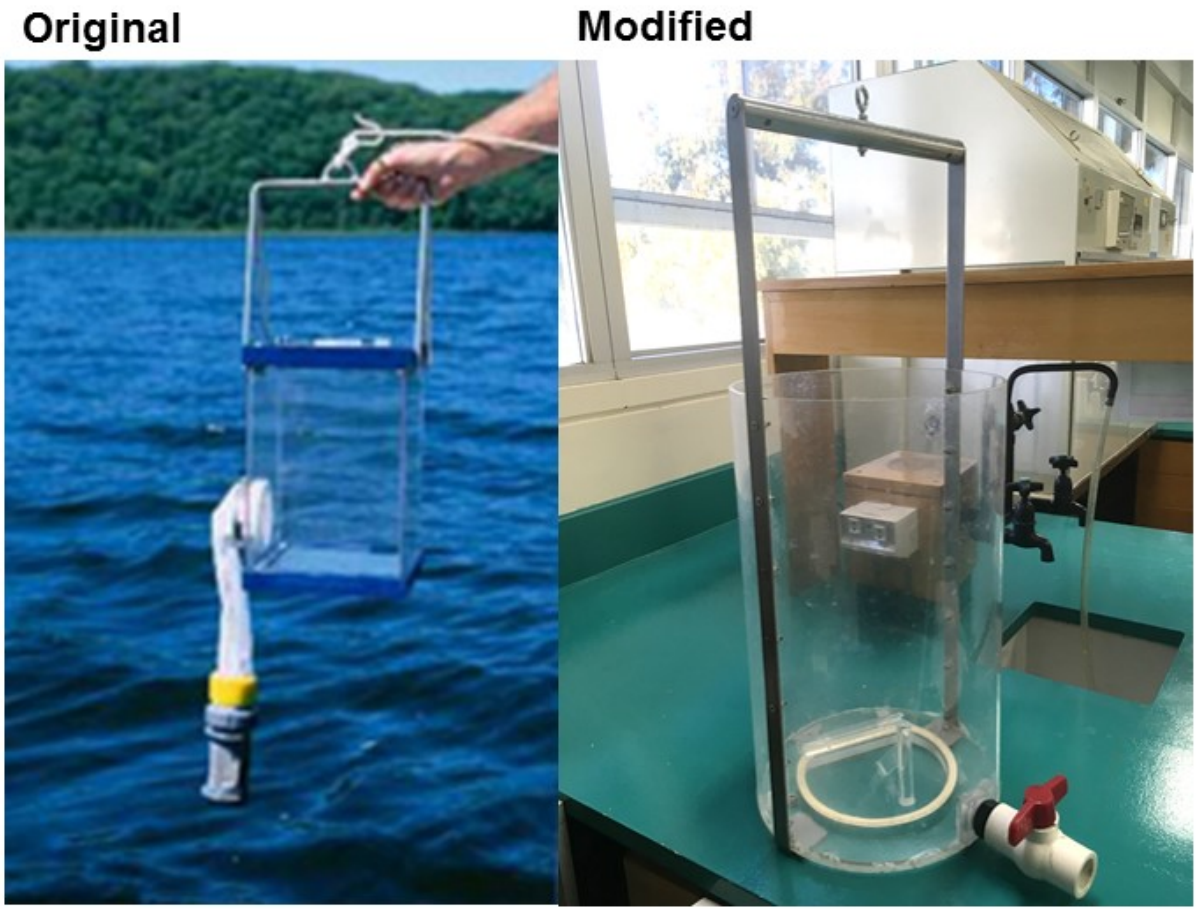

Figure 5: Comparison between original and modified Schindler-Patalas trap used for this project. The modified trap (on the right) has open top and is cylindrical.

2.3.6. Statistical analysis

Univariate statistical analyses were performed using IBM SPSS Statistics 20. Environmental data were tested for normality through a Kolmogorov-Smirnov test (Zar 1996). As the data did not follow a normal distribution, non-parametric analyses were used to test for correlations (Spearman's ρ correlation coefficient) or for differences in individual parameters over space and time (Kruskal-Wallis 1-way ANOVA).

Multivariate statistics were performed using PRIMER v6 with the PERMANOVA+ add–on (Clarke and Gorley, 2006; Anderson, 2008). Environmental data were examined in draftsman scatter plots to ascertain whether some variables were highly correlated with one another, and if the assumptions made about the data were valid. Draftman plots were examined before and after log X or X+1 transformation and normalisation, prior to building a Euclidean distance resemblance matrix. This ensured that the data were approximately multivariate-normally distributed before performing a Permutational analysis of variance (PERMANOVA) to test for significant differences in overall water quality between the Goolwa Channel (GC) and the two lagoons (NL and SL), using a 3-factor, nested design, (month - *fixed*, location - *fixed* and site nested in location – *random*) on the Euclidean distance resemblance matrix (Anderson 2008).

We then used PERMANOVA to identify whether populations and communities distribution of phytoplankton and zooplankton were significantly different between locations and seasons. As specimens were identified to different taxonomic levels, they were treated as operational taxonomic units (OTUs) for analysis (Sokal and Sneath 1963; Dittmann et al. 2015). Data were first tested for normality using a Kolmogorov-Smirnov test (Zar 1996), as they did not follow a normal distribution; phytoplankton and zooplankton data were transformed to meet the assumptions for further statistical analyses using log X+1 and square-root, respectively, prior to analysis. Spearman's *ρ* correlation coefficient was used to test for correlations between the taxonomic richness (total OTUs), and salinity. Each dataset was used to build a Bray-Curtis resemblance matrix (which measures the similarity in species composition) and PERMANOVA was applied to the resemblance matrix to test for significant differences in overall community

in space and time, using a 3-factor (month - *fixed*, location - *fixed* and site nested in location – *random*), nested design, as for environmental parameters (Anderson 2008).

A two-way crossed SIMPER (Similarity Percentages - species contributions) analysis was carried out to investigate which genera are responsible for the multivariate community patterns within and between sites and location (Clarke and Gorley 2006). Finally, to explore the taxonomic groups correlated to the different community, a Principal Co-ordinate analysis (PCO; Clarke and Gorley 2006), with vector overlay (major phytoplankton groups for phytoplankton PCO and OTUs for zooplankton PCO), was performed for both phytoplankton and zooplankton to investigate differences in communities between locations (GC, NL, SL).

In order to determine the importance of each environmental parameter in influencing the distribution of phytoplankton and zooplankton communities, a BEST (BIO-ENV Stepwise; Clarke and Gorley 2006) procedure was performed to identify the set of environmental parameters most correlated to the multivariate phytoplankton and zooplankton communities, separately (Clarke and Gorley 2006). A Distance-Based Redundancy Analysis (dbRDA) was then performed based on the Bray-Curtis resemblance matrix and normalised environmental parameters which best describe the variations observed in community composition (different for phytoplankton and zooplankton) to represent the community distributions in relation to environmental parameters.

Finally, to identify suitable environmental health indicator OTUs, an Indicator Value (IndVal) for each OTU was calculated as per Dufrêne and Legendre (1997):

$IndVal_{ij} = A_{ij} \times B_{ij} \times 100$

with $A_{ij} = Nindividual_{ij} / Nindividual_i$ and $B_{ij} = Nsites_{ij} / Nsites_j$

where, $Nindividual_{ij}$ is the mean number of individuals of the OTU in the groups of sites j (in our case NL or SL), $Nindividual_i$ is the sum of mean number of individuals of the OTU i across all site groups, $Nsites_{ij}$ is the number of sites in group j where the OTU i is present, and $Nsites_j$ is the total number of sites in group j. A script for the calculations was written and executed

with MATLAB R2015b. The significance was tested with 999 random bootstrap permutations and a z test, assuming approximate normality of distribution (Dufrêne and Legendre 1997). Only OTUs with IndVal > 30% and $p < 0.05$ were considered as potential indicators (Dufrêne and Legendre 1997; Fortunato et al. 2013). The specific correlations of OTUs of good indicator potential to environmental parameters were then calculated to explore the predictability (positive or negative) of the OTUs.

2.4. Results

2.4.1. Environmental parameters

Environmental parameters showed strong spatial gradients along the length of the Coorong and temporal changes across seasons.

Overall, all locations (GC, NL and SL) were significantly different in terms of environmental parameter characteristics throughout the year (PERMANOVA, $p < 0.01$). Water temperature showed significant temporal variation (Kruskal-Walis, $p < 0.05$), while pH was only significantly lower in November and December 2013 in SL (Kruskal-Walis, $p < 0.05$). Salinity varied from 0.49 (at S1 in August 2014), to 98.42 (at S7 in April 2014). Salinity levels in GC, NL and SL were significantly different (Kruskal-Walis, $p < 0.05$; Fig. 6), throughout the period of study. In addition, salinity was negatively correlated to the freshwater flow through the barrages and through Salt Creek (Spearman $\rho = -0.152$ and $\rho = -0.164$, respectively, $p < 0.05$; Fig. 6).

GC, NL and SL were also characterised by different nutrient concentrations (Fig. 7). GC and NL showed significantly higher concentrations of NO_x^- and PO_4^{3-}, compared to SL (Kruskal-Walis, $p < 0.05$), which had significantly higher concentrations of NH_3 (Kruskal-Walis, $p < 0.05$). The concentrations of SiO_2 were higher in SL in colder months (Kruskal-Walis, $p < 0.05$) and higher in NL in warmer months (Kruskal-Walis, $p < 0.05$). Barrages flow was significantly correlated to NH_3 and NO_x^- concentrations (Spearman $\rho = -0.289$ and $\rho = 0.330$, respectively, $p < 0.05$). Overall, nutrients were found to be unlimiting based on nutrient ratio data (N:P

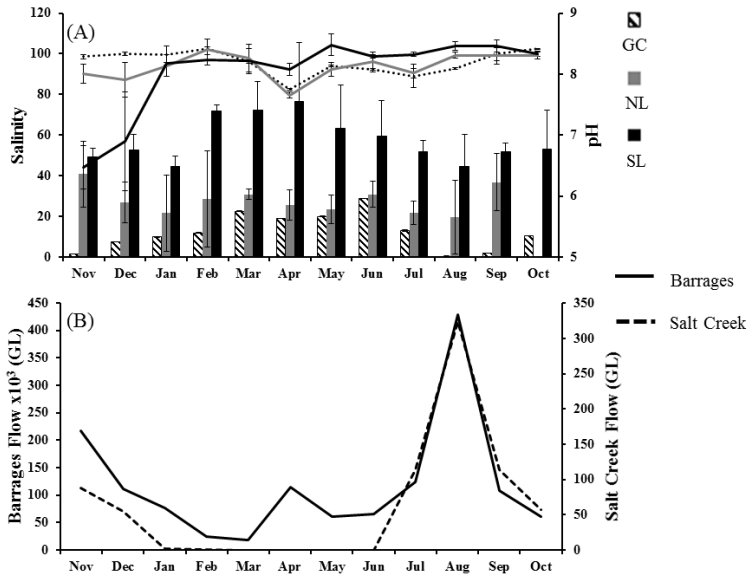

Figure 6: (A) Salinity (bars) and pH (lines) fluctuations from November 2013 to October 2014 in the Goolwa Channel and Coorong representing differences in levels across the system as well as temporal variations. (B) Monthly freshwater flow from barrages and Salt Creek.

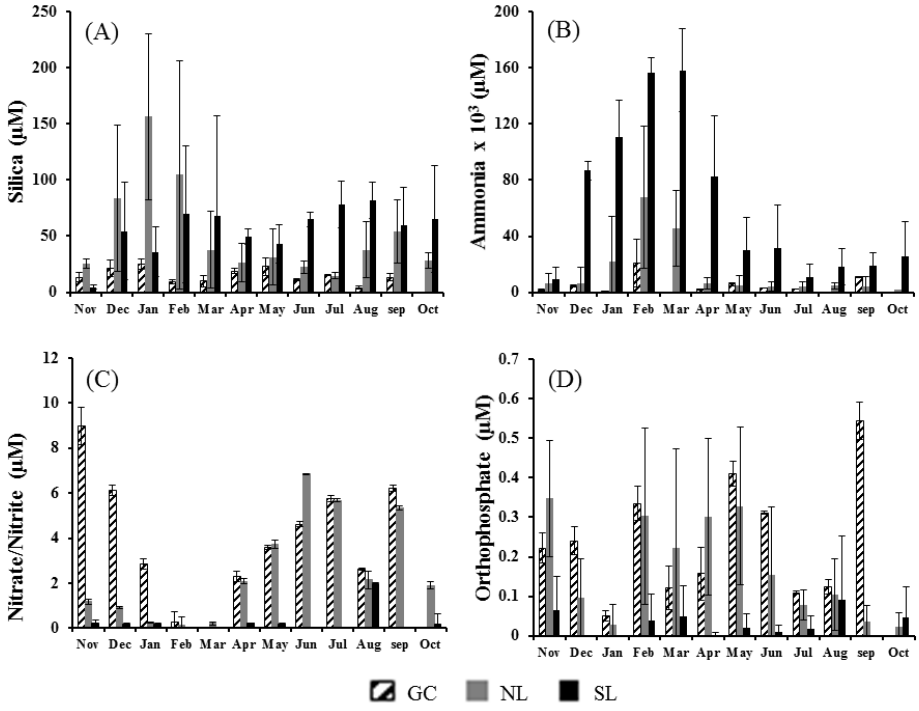

Figure 7: Nutrient fluctuations in the Goolwa Channel, North Lagoon and South Lagoon from November 2013 to October 2014. (A) Silica, (B) Ammonia, (C) Nitrate/Nitrite and (D) Orthophosphate.

>16:1, Si:P > 15:1, N:Si > 1:1; Redfield et al., 1963). Finally, SL was significantly more turbid than GC and NL (Kruskal-Walis, $p < 0.05$). Although GC and NL were characterised by different environmental parameters than SL, seasonal variations were observed, especially around S4 in the NL. Moreover, a clear distinction can be seen between colder (July – September) and warmer months (December – May).

2.4.2. Phytoplankton Communities

Phytoplankton biomass, in terms of chlorophyll *a* concentrations, was positively correlated to salinity (Spearman $\rho = 0.682$, $p < 0.05$; Fig. 8A). However, OTUs richness showed a negative correlation with salinity (Spearman $\rho = -0.267$, $p < 0.05$; Fig. 8B). A total of 152 phytoplankton OTUs were identified in the Coorong throughout the period of study (Supplement Table 1). Overall, GC, NL and SL were significantly different in terms of community structure (PERMANOVA, $p < 0.05$). GC was dominated by chlorophytes and cyanoprokaryotes, and SL was dominated by diatoms and chrysophytes, while NL had a high number of different taxonomic groups (108 OTUs; Appendix Table 1) contributing to its phytoplankton community (Fig. 9A). SIMPER analysis showed that GC and NL had an average dissimilarity of 58.06%, while GC and SL had an average dissimilarity of 68.28%, and NL and SL had an average dissimilarity of 54.28%. The variability observed in the phytoplankton community was analysed by PCO (Fig. 9A), which presented an unconstrained ordination plot based on the Bray-Curtis resemblance matrix. The vectors, on the plot, show which phytoplankton taxonomic groups are the major contributors to the communities. The lengths of the vectors represent the level of correlation (Threshold of Spearman $\rho > 0.3$, based on Critical values for Spearman Rank Correlation from Zar 1996). A clear differentiation in phytoplankton community can be seen between SL and the other regions. The BEST test showed that salinity and NO_x^- concentrations were the variables explaining most of the variations in phytoplankton community, with Spearman $\rho = 0.526$ ($p < 0.01$). Other significant variables included 24 hour wind stress, N:Si ratio and N:P ratio (Spearman $\rho = 0.506$ ($p < 0.01$). The dbRDA test represents the distribition of phytoplankton communities in relation to the environmental

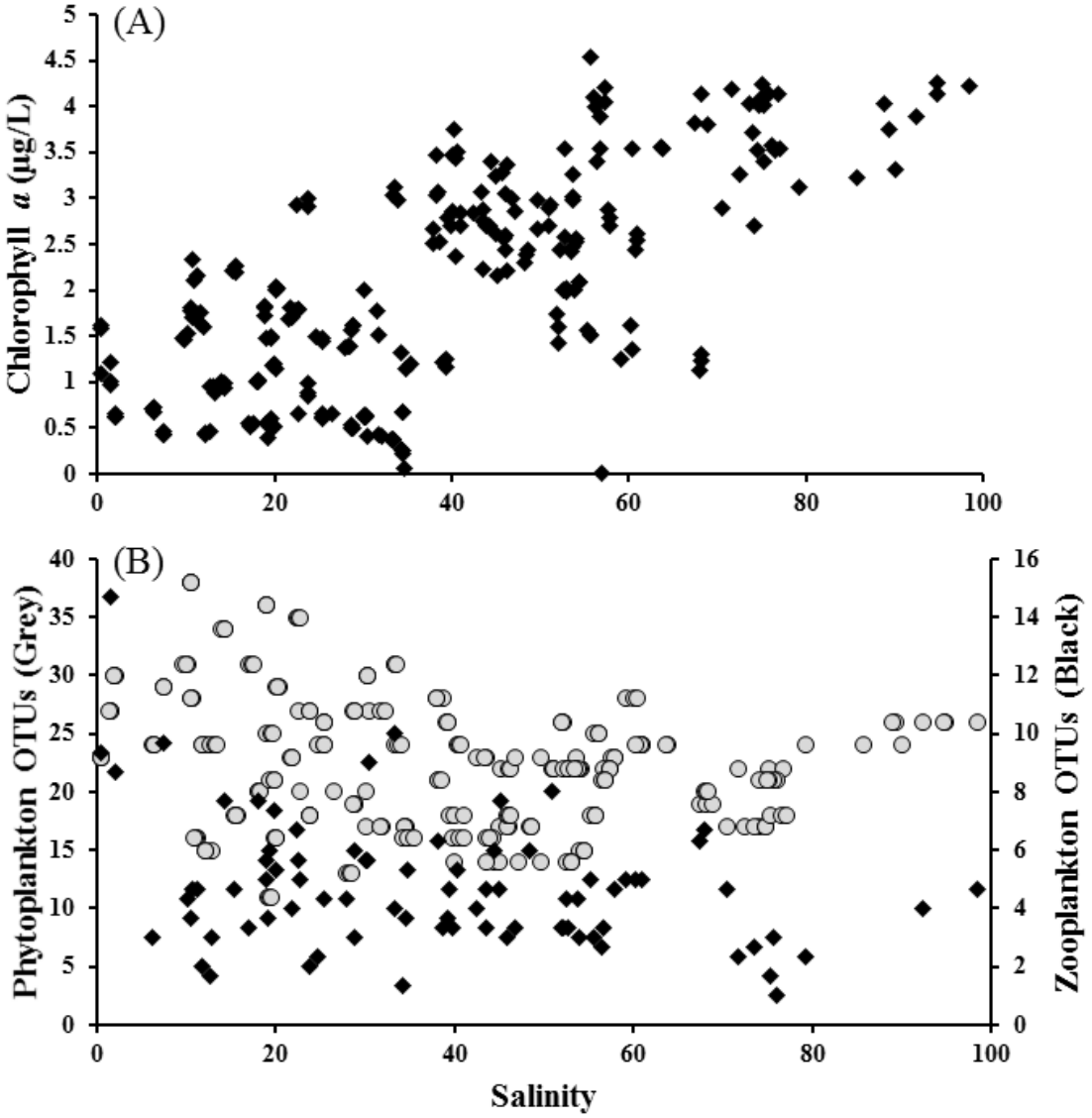

Figure 8: (A) Chlorophyll *a* concentration and (B) Phytoplankton and zooplankton OTUs, as a function of salinity.

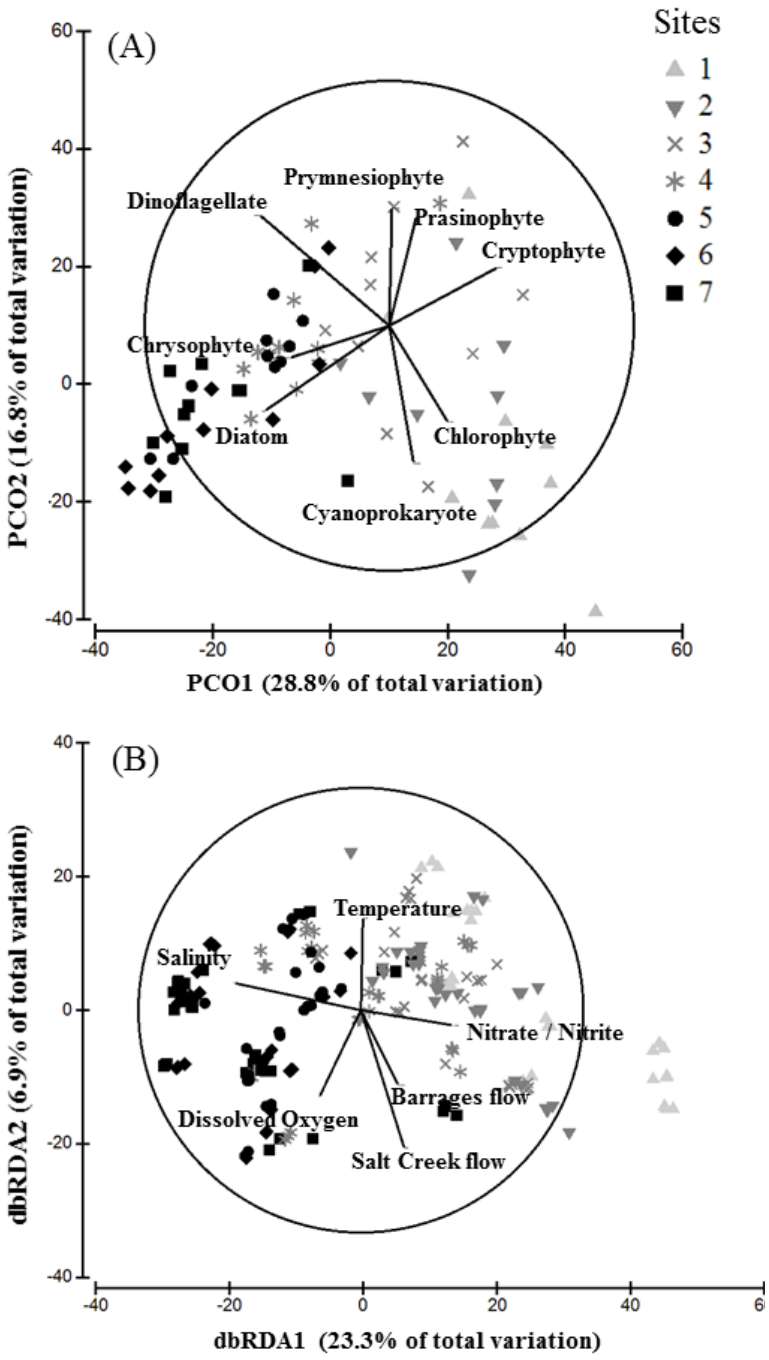

Figure 9: (A) Principal Coordinate Analysis (PCO) of phytoplankton communities from November 2013 to October 2014. Ordinations are based on Bray-Curtis similarity index and vectors represent major phytoplankton groups with spearman correlation coefficient > 0.3 with any of the first two ordination axes. (B) dbRDA of phytoplankton communities from November 2013 to October 2014 based on Bray-Curtis similarity matrix. Vectors represent environmental parameters with spearman correlation coefficient > 0.3 (strength and direction indicate the effect of the parameters on the ordination plot).

parameters (Fig. 9B). Salinity and NO_x^- were correlated to the first dbRDA axis, while barrages flow, Salt Creek flow and temperature were correlated to the second dbRDA axis. The phytoplankton community at SL was noticeably distinct to that of the NL and GC, however, some variations were seen around S4 (asterisk) and S5 (circles).

2.4.3. Zooplankton Communities

Zooplankton OTUs richness and abundance were negatively correlated with salinity (Spearman ρ = -0.243 and ρ = -0.309, respectively, $p < 0.05$; Fig. 9B) but positively correlated to phytoplankton OTUs richness (Spearman ρ = 0.188, $p < 0.05$). Forty zooplankton OTUs were identified in the Coorong during the study period (Appendix Table 2).

While some differentiation in zooplankton community was seen between SL compared to GC and NL, a high variability in community structure was observed (Fig. 10A). The PCO showed an unconstrained ordination plot of the zooplankton community based on the Bray-Curtis similarity matrix (Fig. 10A). The vectors on the PCO represent the zooplankton OTUs contributing to the communities and the length of the vectors represents the levels of correlation. The zooplankton communities in GC, NL and SL were significantly different (PERMANOVA, P = 0.012). The SIMPER analysis showed that GC and NL had an average dissimilarity of 65.81%, GC and SL had an average dissimilarity of 75.59% and NL and SL had an average dissimilarity 72.59%. Five of the zooplankton OTUs found in SL (*Filinia pejleri, Keratella australis, Bosmina meridionalis, Daphnia lumholtzi, Boeckella triarticulata*) were only present once at Salt Creek (S7) in August when the freshwater flow from the Salt Creek was at the maximum (Fig. 6B).

The BEST test showed that salinity, chlorophyll *a* concentration and cryptophytes were the variables explaining most of the variations in zooplankton community, with spearman ρ = 0.301 ($p < 0.01$). The dbRDA represents the distribution of zooplankton in relation to the most correlated environmental parameters and phytoplankton groups. Salinity and cryptophytes were correlated to the first dbRDA axis while total chlorophyll *a* and tidal amplitude were correlated to the second dbRDA axis (Fig. 9B).

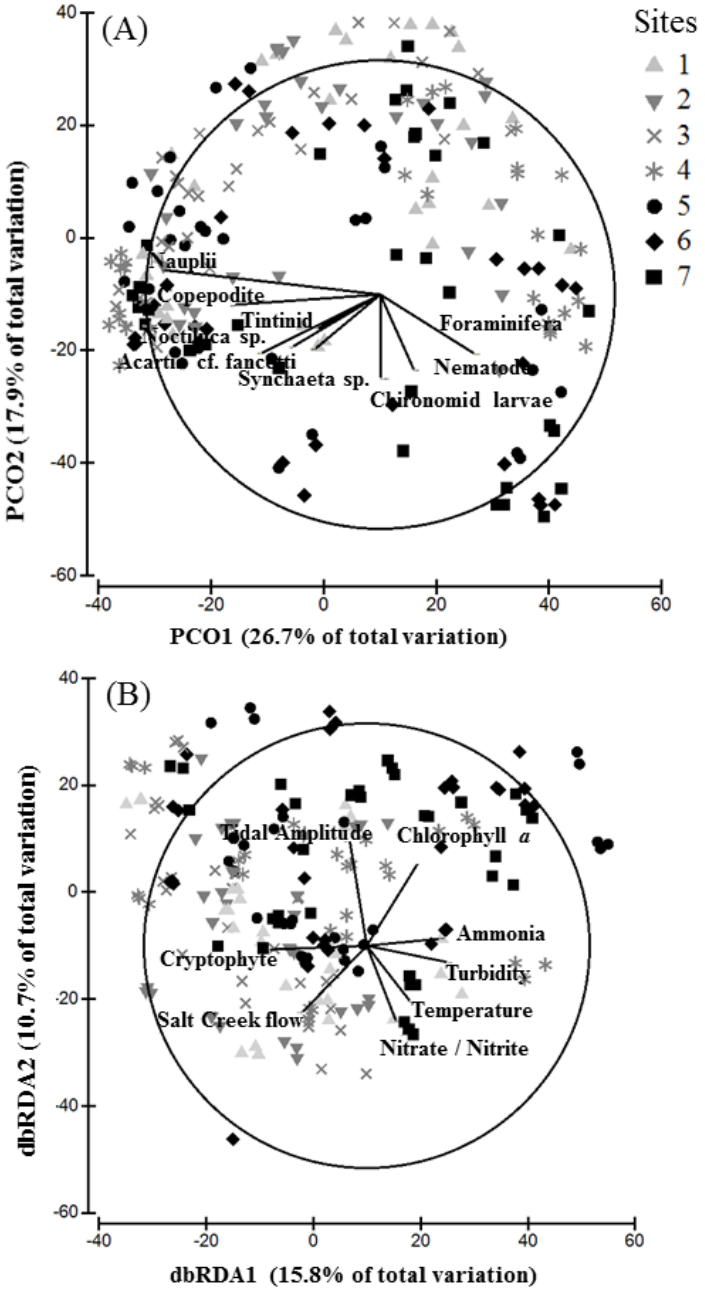

Figure 10: (A) Principal Coordinate Analysis (PCO) of zooplankton communities from November 2013 to October 2014. Ordinations are based on Bray-Curtis similarity index and vectors represent major phytoplankton groups with spearman correlation coefficient > 0.3 with any of the first two ordination axes. (B) dbRDA of zooplankton communities from November 2013 to October 2014 based on Bray-Curtis similarity matrix. Vectors represent environmental parameters with spearman correlation coefficient > 0.3 (strength and direction indicate the effect of the parameters on the ordination plot).

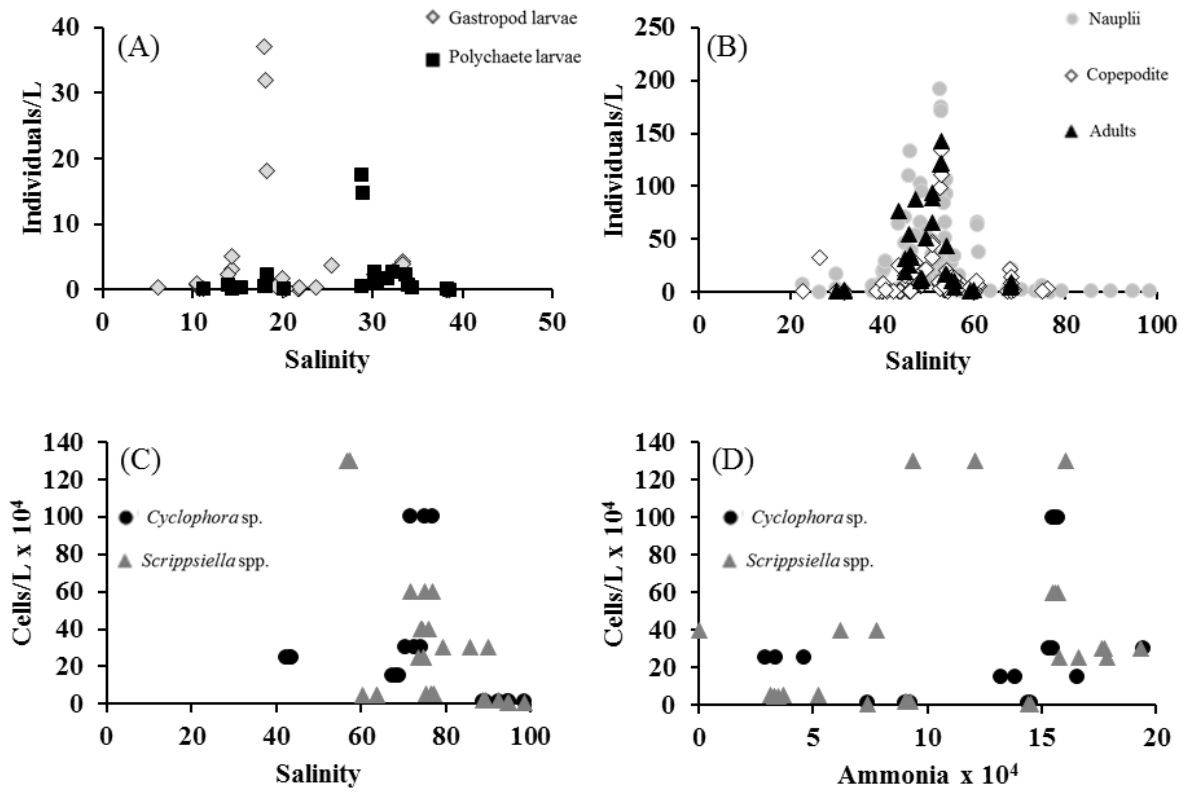

Figure 11: Plot of indicators against salinity and ammonia levels. (A) Polychaete and Gastropod as a function of salinity of the North Lagoon. (B) *Acartia* cf. *fancetti* nauplii, copepodite and adults as a function of salinity of the South Lagoon. (C) *Cyclophora* sp. and *Scrippsiella* spp. against salinity of the South Lagoon. (D) *Cyclophora* sp. and *Scrippsiella* spp. against ammonia levels of the South Lagoon.

2.4.4. Water quality indicator

Indicator values attributed to each OTU differed depending on the locations in the system and therefore, different OTUs were selected for each part of the system. Indicator OTUs were only selected for locations inside the Coorong lagoon (NL and SL). The life cycle (especially for zooplankton) and correlation coefficients of phytoplankton and zooplankton OTUs of higher IndVals to salinity and nutrient levels were also considered for the selection of indicators. In the NL, the diatom *Rhizosolenia* spp. (IndVal 96.6 %, $p < 0.05$) represented a potential good indicator. *Rhizosolenia* spp. correlated negatively to NO_x but positively to NH_3 (Spearman ρ = -0.347 and 0.444, respectively, $p < 0.05$). The distribution of polychaete and gastropod larvae (IndVal 91.9 %and 70.9 %, $p < 0.05$, respectively) spanned from salinity 10 to 40 (Fig. 11A). Gastropod larvae were negatively correlated to salinity (Spearman ρ = -0.303, $p < 0.05$). Three strong indicators were identified for the SL. *Acartia* cf. *fancetti* adults (IndVal 100%, $p < 0.05$) were only present in salinity 30 to 70 (Fig. 11B) and was negatively correlated to both salinity and NH_3 levels (Spearman ρ = -273 and -0.315, respectively, $p < 0.05$). Diatom *Cyclophora* sp. (IndVal 100%, $p < 0.05$) and dinoflagellate *Scrippsiella* sp. (IndVal 99.3 %, $p < 0.05$) correlated positively to salinity (Spearman ρ = 0.354 and 0.639, respectively, $p < 0.05$) and NH3 (Spearman ρ = 0.430 and 0.460, respectively, $p < 0.05$; Fig. 11 C and D).

2.5. Discussion

2.5.1. Environmental parameters

This study showed strong spatial and temporal differences in water quality in the Coorong and Goolwa Channel. Salinity gradually increased in NL with sharp increases around S4 towards SL. Those changes in salinity are related to freshwater input from the barrages, freshwater from Salt Creek, tidal action and evaporation (Webster 2005; Webster 2010). The volume of freshwater input varies significantly on a seasonal basis (Fig. 6B), especially in the more saline sites of SL, where considerably lower volumes are discharged compared to the NL. In addition, evaporation rates are high in the Coorong, especially during the summer months (Webster.

2010), which is typical of an arid area (Nunes-Vaz et al. 2012). Another highly important feature affecting salinity is the physical restriction of water exchange between NL and SL at Parnka point (Webster 2005), limiting the amount of hypersaline water that is flushed out of SL, even under favourable wind forcing (Webster 2005). While GC and NL are more dynamic in terms of tidal action and freshwater input, SL is isolated and receives limited freshwater input, therefore being constantly more saline than GC and NL. An increase in microbial populations and activity may also have caused the decreased pH in November and December, likely by increased respiration and bacterial decomposition.

A significant difference was also observed in the nutrient composition between GC, NL and SL. These nutrient concentrations are in accordance with Ford (2007), Haese et al. (2009) and Leterme et al. (2015), whereby very high ammonia and silica concentrations were observed in SL. These higher concentrations are typical of environments where high evaporation concentrates nutrients in the water column (Haese et al. 2009). Even so, as argued by Ford (2007) and Haese et al. (2009), such concentrations generally exceed those usually expected from high evaporation. Seepage of underground water has been speculated to be a contributor of nutrients (Haese et al. 2009), but needs further investigation. Although NL showed clearly distinct water quality to that of SL, S4 showed a high variability in water quality, reflecting either NL or SL conditions. This suggests that the area between S4 and S5 is a highly dynamic region where most of the interaction between NL and SL water happens.

Finally, even though the drought period has passed and the Coorong is still in a state of recovery, based on persistent hypersalinity levels and hydrological features, SL tends to behave as a separate, non-drying, salt lake. Indeed, it has typical restricted water outflow and exchange at Parnka Point, higher evaporation than water inflow from Salt Creek (Eugster and Hardie 1978; Webster, 2010) and significant seasonal water level changes (Dummont 1998; Enzel et al. 2003) as shown by data from www.waterconnect.sa.gov.au. The Coorong is, therefore, unique in physiochemical characteristics compared to other inverse estuarine

systems or coastal lagoons (Panfili et al. 2006; Yáñez-Arancibia et al. 2014), where such clear differentiation and sudden sharp change in chemistry are much less prevalent.

2.5.2. Phytoplankton distribution

During the period of study, phytoplankton biomass was observed to be constantly higher at sites with elevated salinity. This is in accordance with Yin et al. (2000), Jendyk et al. (2014), Leterme et al. (2015) and Nche-Fambo et al. (2015), but in conflict with other studies (Putland et al. 2014; Haraguchi et al. 2015), showing the variability between individual systems. Concentrations of different nutrients are an important driver of phytoplankton biomass in estuarine systems, especially if some nutrients are limiting (Haraguchi et al. 2015).

The increase in phytoplankton biomass with salinity is associated with the spatial variation in phytoplankton community structure along the system. Indeed, our results show that OTUs richness correlated negatively with salinity and that the community was mostly dominated by diatoms in the more saline areas. These results corroborate previous descriptions of the community changes along the Coorong, as well as the adaptability of diatoms to hypersaline waters (Leterme et al. 2010; Leterme et al. 2013; Jendyk et al. 2014; Leterme et al. 2015).

While salinity is the main driver of the phytoplankton community in the Coorong, the nutrient composition of the different locations (GC, NL and SL) also affected the community structure along the system. A higher concentration of Si was observed in the SL, which would be associated with higher diatom numbers. Previous studies have shown that the N:Si ratio is important for the growth of diatoms (at least 2 µM Si, N:Si ≥ 1:1) and reported that diatoms have lower half-saturation values for nitrate and ammonium uptake. Moreover, diatoms have better capabilities of utilising ammonium than flagellates (Egge and Aksnes 1992; Clark et al. 2002; Esparza et al. 2014), which would be highly beneficial in environments dominated by high silica and ammonia concentrations. Finally, the multivariate analysis showed that the phytoplankton communities of SL and NL varied mostly around S4, similarly to water quality. This indicates the close relationship between phytoplankton and water quality in the Coorong. Similarly, phytoplankton community composition has been shown to change with salinity and

nutrient changes in other systems (Varona-Cordero et al. 2014; Domingues et al. 2015), especially with increase of diatoms with higher nitrogen levels. Moreover, Badylac and Philips (2004) have shown that diatom dominated communities are prevalent in systems of intermediate water turnover, which is especially the case for SL in the Coorong.

2.5.3. Zooplankton distribution

The OTUs richness and abundance of zooplankton were negatively correlated to salinity. However, although the SIMPER test showed reasonable dissimilarities, the distinction between zooplankton communities of GC, NL and SL were less obvious in the multivariate analyses. This is likely related to distinct differences in total abundances but not overall community composition.

Our results showed that the community in SL was characterised by nematodes, chironomid larvae and foraminifera, although harpacticoids, ostracods and the calanoid *Acartia* cf. *fancetti* also significantly contributed to the community. Several of these organisms have commonly been observed in hypersaline environments (Brock and Shiel 1983; Ólafsson et al. 2000) including holohaline groups such as ostracods and harpacticoids (Brock and Shiel. 1983). Here, *Acartia* cf. *fancetti* occurred in salinities ranging from > 30 to 70 in SL, during November and December 2013, as well as September and October 2014. *Acartia* is a genus of calanoid copepod, generally observed in coastal marine areas and inshore waters and *Acartia fancetti* has been reported to occur in marine waters of Port Phillips Bay, Victoria, Australia (McKinnon et al. 1992). Several species of this genus can survive salinities ranging from 1 to 35 (Cervetto et al. 1999; Gaudy et al. 2000; Svetlichny and Hubareva 2014). However, Cervetto et al. (1999) have also shown that *Acartia tonsa* was able to survive at salinities up to 70. Moreover, Ohs et al. (2009) have shown that *Acartia* eggs can remain viable in hypersaline water. Such characteristics suggest that the population encountered in SL could be highly halotolerant. As it was not observed in NL, GC or around the Murray Mouth, the ocean was less likely the

source of the species in the Coorong. We, thus, suggest that the species reappeared from dormant eggs.

GC and NL, on the other hand, were mostly characterised by mesohaline, polyhaline and euhaline zooplanktonic organisms. However, several species predominantly present in SL, were also observed in significant numbers in GC and NL, especially holohaline species such as harpacticoids and ostracods (Brock and Shiel 1983). Moreover, seasonal peaks in freshwater species, such as some cladoceran, rotifer and copepod species were observed. These occurrences were predominantly related to high barrage flows. Those species thus likely originate from the Lower Lakes and are observed in the GC and NL after being carried through the barrages under high flow conditions. The occurrence of these species in GC and NL can be expected to persist when the salinity is < 2 (Shiel and Tan 2013).

In addition, copepod nauplii were present throughout the system during the whole year, while adults only appeared seasonally. Several factors, including water quality parameters, food availability and food type can affect the growth and development of copepods (Ismar et al. 2008; Devreker et al. 2009; Kimmerer et al. 2014). In fact, chlorophyll *a* concentration and cryptophyte numbers were some of the main factors governing the zooplankton communities of the Coorong. Several studies have also shown that temperature and salinity affect copepod development (Milione and Zeng 2008; Pan et al. 2016), including differences in life-stage composition at different salinities such as very high nauplii numbers with relatively low adult numbers (Pan et al. 2016), therefore requiring appropriate environmental conditions for full reproductive cycle.

Phytoplankton densities are important determinants of copepod development (Durbin et al. 1983; Ban 1994; Lin et al. 2013), although the phenology and timing of hatching, in relation to food availability and predator numbers, are also of significance (Seebens et al. 2008). Food type and selectivity have been argued to influence development. For example, Ismar et al. (2008) has shown that cryptophytes such as *Rhodomonas* and diatoms such as *Thalassiosira* favoured *Acartia* survival, while the dinoflagellate *Prorocentrum* caused significant negative

effects, even if *Rhodomonas* and *Thalassiosira* were present. In the Coorong, phytoplankton abundances are relatively high compared to some other estuaries and, therefore, changes in water quality along with phytoplankton species composition are most likely related to the development of copepod nauplii and, thus, occurrences of adults of different species.

2.5.4. Water quality Indicators

Based on Hilty and Merenlender (2000), Carignan and Villard (2002) and Siddig et al. (2016), some of the main criteria for selection of potential indicator species include the ability of the species to indicate changes and the cause of change, provide continuous assessment, have an established correlation with water quality, be dispersal-limited and be cost-effective to monitor. As shown by the multivariate analyses, water quality parameters and plankton communities were distinct in the two Coorong lagoons i.e. NL and SL. Accordingly, different OTUs were selected as indicators for NL and SL due to the different habitats and possible differences in ecological niche of the organisms.

In NL, the distribution of both polychaete and gastropod larvae spanned in salinity approximately 10 to 40 (Fig. 11A), indicating habitat and recruitment restriction, for benthic dwelling adults, based on salinity ranges in the Coorong. These distributions are also in accordance with those of adults in the system across salinity ranges, as shown by Dittmann et al. (2015). The presence of larval stages of these organisms is a positive indicator for salinity levels across NL, representing healthy brackish to marine environment. Indeed, Marsden and Baharuddin (2015) have experimentally shown that the reproductive cycle of gastropod can serve as good indicators of water quality. On the other hand, the correlation of *Rhizosolenia* spp. to NH_3 indicates an increase in abundance with increased NH_3 levels in NL. As shown by Figures 6 and 7, high NH_3 is not typical of NL. Excess NH_3 levels in aquatic systems is considered a toxicant and widely associated with high stress in a variety of aquatic organisms that are not tolerant, especially teleost species (Marsden and Baharuddin 2015; Sinha et al.

2015). Therefore, the association of *Rhizosolenia* spp. with higher NH_3 illustrates that high abundances of *Rhizosolenia* spp. in NL is a negative indicator of the water quality.

In the hypersaline SL, adults of the copepod *Acartia* cf. *fancetti* were only observed from salinity 30 to 70 and were most abundant between salinity 40 and 60 (Fig. 11B). Copepods have commonly been shown to be reproductively impaired by changes in water quality, especially with input of organic pollutants and change in salinity (Forget-Leray et al. 2005; Seuront et al. 2011; Pan et al. 2016). Although nauplii and copepodite stages of *Acartia* cf. *fancetti* were present in a wider salinity range, their abundances were significantly lower (Fig. 11 B). The distribution of adults of this species and the negative correlation show that the lifecycle is restricted by salinity levels. Therefore, it is a highly suitable indicator for healthy hypersaline conditions (< 60) in SL. On the contrary, diatom *Cyclophora* sp. and dinoflagellate *Scrippsiella* sp. were only present at high salinity and NH_3 levels (Fig. 11 C and D), and showed positive correlation to both salinity and NH_3. This shows that both *Cyclophora* sp. and *Scrippsiella* sp. are good indicators of water quality degradation in SL and therefore negative indicators.

2.5.5. Applications for other inverse estuaries

The use of indicator species in aquatic systems is relatively common. However, most studies have used a variety of species as indicators to monitor pollution levels and environmental health, especially in terms of excess nutrient loading and heavy metals in estuaries and marine environments (Böcük et al. 2013; Ponnusamy et al. 2014; Ogden et al. 2014). In the Coorong, natural water quality gradients and major differentiation in water quality across the system are prevalent. The health of the system has degraded over the past years, due to a reduction in freshwater input and high freshwater loss, changing the aquatic communities. Although freshwater input into the system has increased, there is still the occurrence of very high salinities and fluctuating water level and quality. Lester and Fairweather (2009) have shown

that such fluctuations affect the system by creating different ecosystem health states, including healthy, unhealthy and degraded marine and hypersaline environments.

The use of selected plankton OTUs for monitoring such health states of the system is highly beneficial in terms of promptly indicating changes as well as thresholds in the system and, therefore, can be a constructive tool for managing the ecosystem health of the Coorong. Similarly, the use of planktonic organisms as indicators is potentially of high relevance to monitor the occurrence of extreme conditions or changes in water quality resulting from hydrological changes in other inverse estuaries and coastal lagoons, such as the Sine Saloum, Senegal, Mossoró River estuary, Brazil or Gulf of Cádiz (Panfili et al. 2006; Echevarria et al. 2009; Medeiros et al. 2010). As shown in this study, defining the habitats and plankton-environment interrelation in a system is important for the selection of appropriate indicators of each habitat and managing ecosystem health.

2.6. Conclusion

In this study, a comprehensive description of the plankton communities in relation to environmental parameters of the Coorong, South Australia was provided. The water quality of the system was found to be highly influential on the community structure, creating two distinct communities. Phytoplankton community was mostly dominated by diatoms in SL. On the other hand, zooplankton communities between the GC, NL and SL were less distinct. The distribution of zooplankton was also influenced by phytoplankton biomass and distribution. *Acartia* cf. *fancetti* populations were observed in SL, which could be associated with the hatching of resting eggs. Based on the different communities and habitats, two sets of indicator OTUs were selected for NL and SL. These include positive and negative indicators that correlate with salinity and nutrient levels and indicate thresholds of change in both NL and SL. Based on the different habitats observed in the Coorong, the indicator species likely occupy different ecological niches. The niches occupied by these species in different ecosystems may also influence their utility as indicators. The implementation of plankton indicators in monitoring

of ecosystem health of coastal lagoons can be highly beneficial for studying the effects of extreme hydrological and water quality changes on organisms and foodweb structure of such complex systems. Moreover, the use of planktonic organisms relates highly to the criteria of indicator species selection for aquatic systems.

CHAPTER 3

A COMBINATION OF SALINITY AND pH AFFECTS THE RECRUITMENT OF GLADIOFERENS PECTINATUS (BRADY) (COPEPODA; CALANOIDA)

This chapter has been published in the journal Limnology and Oceanography.

Reference:

Hemraj, D.A., Allais, L. and Leterme, S.C., 2017. A combination of salinity and pH affects the recruitment of *Gladioferens pectinatus* (Brady) (Copepoda; Calanoida). *Limnology and Oceanography*.

3.1. Abstract

Carbon dioxide levels in many estuaries fluctuate and, in several cases, reach extremes much higher than those predicted for oceans by the end of the century. Moreover, estuaries are characterised by natural fluctuations in salinity, and reduced pH, from increased pCO_2, exposes estuarine organisms to multiple stresses. Although the effects of low pH on the reproduction of several marine copepod species have been assessed, studies examining effects of pH in estuarine copepod species are extremely scarce. Here, we aim at understanding the reproductive response of *Gladioferens pectinatus* to the stress posed by both salinity and pH. *G. pectinatus* was exposed to salinities 2 and 10, at four different pH levels each. Our results show no impairment in the brood size, embryonic development time and hatching success under low pH levels at either salinities. However, at salinity 2, the percentage of nauplii growing into adults significantly decreased at low pH, whereas at salinity 10, no major effect was observed. We argue that the combination of osmoregulation and acidity induced stress response can affect the development of nauplii and copepodites, as well as adult recruitment, likely due to energy reallocation and molting impairment. We also argue that resilience and phenotypic plasticity highly influence the ability of different copepod species and populations to reproduce and grow under stressful combinations of environmental parameters. This study points out the importance of understanding the effects of multiple stresses or parameters on the adaptability of organisms to water acidification.

3.2. Introduction

Estuaries impose a variety of environmental stressors on organisms. Fluctuations in water quality, especially salinity, temperature, pH and dissolved oxygen, are common features of estuaries and influence the distribution of biotic communities (Elliot and McLusky 2002; Telesh and Khlebovich 2010). Dissolved greenhouse gasses, especially carbon dioxide (CO_2), have been reported to reach extremely high concentrations (5000-10000 µatm) in several estuaries around the world, causing fluctuations in water parameters, especially pH (Cai 2011; Hu and Cai 2013; Maher et al. 2015). The foremost cause of variations in carbon dioxide partial pressures (pCO_2) in estuaries is the seasonal fluctuation in the difference between net autotrophy and net heterotrophy (Borges et al. 2008). Many coastal and inland aquatic systems, including estuaries, tend to be net heterotrophic in nature, thus decomposing more carbon than what is produced by autotrophy (Duarte and Prairie 2005). In estuaries, in situ respiration and riverine input water with high concentrations of organic carbon and CO_2 contribute to the variations in pCO_2 levels and pH (Cai 2011). These are derived from the accumulation of dissolved terrestrial and aquatic respiratory CO_2 and in some estuaries, pCO_2 reach higher values (5000-10000 µatm; Cai 2011) than those that have been predicted for oceans by the end of the century (~ 700 µatm; IPCC, 2007). Such systems can also be sources of CO_2 to the atmosphere, resulting from air-water fluxes (Duarte and Prairie 2005; Jiang et al. 2008; Maher et al. 2015). Air-water flux rates can influence retainment of CO_2 in heterotrophic systems and likely depend on the response of air-water fluxes to increased atmospheric CO_2 (Roy et al. 2011). Moreover, air-water CO_2 fluxes are dependent on water salinity levels due to the buffering capacities that are associated to different salinities (Hu and Cai 2011), therefore also producing differences of pH levels at different salinities. This is especially important in estuarine systems as, in addition to a salinity gradient which causes osmoregulatory stress (Towle 1997), lowered pH can cause additional stress for estuarine organisms (Biswas et al. 2011; Waldbusser et al. 2011; Dickinson et al. 2012; Aguilera et al. 2013).

The stress caused onto aquatic organisms by fluctuations in environmental conditions, such as changes in salinity and pH, often affect their reproductive capabilities (i.e. egg production, development, sex ratio, dispersal and survival) and growth rates, which may in turn have repercussions at population, community or food web levels (Devreker et al. 2009; Engels et al. 2011; Pan et al. 2016). Zooplankters are among the organisms that are affected by changes in environmental conditions as they often only tolerate specific ranges of water quality parameters (Devreker et al. 2012; Sperfeld and Mangor – Jensen 2014; Roy et al. 2015). They are an important component of the aquatic foodweb, serving as a major pathway of energy from primary producers to higher trophic levels (Morozov and Arashkevich 2010; Muylaert et al. 2010; Winder and Jassby 2011). Changes in zooplankton communities will likely be reflected in the communities of other organisms for which they serve as predators or prey. In particular, copepods contribute significantly to the total zooplankton communities in different aquatic systems and are often a major prey item for fish (Wasserman and Froneman 2013; Deurs et al. 2015).

Several studies have shown that copepod reproduction can be affected by salinity changes in estuaries (Devreker et al. 2012; Pan et al. 2016; Souissi et al. 2016) and by pH in marine environments (Kurihara et al. 2004; Zhang et al. 2011; Cao et al. 2015). Typically, adverse effects are observed on early development stages, including egg production rates, embryonic development time, hatching success and nauplii survival (Zhang et al. 2011). Although Aguilera et al. (2013; 2015) have looked at the effects of low-pH riverine discharge on the reproduction of *Acartia tonsa*, no other studies have explored the reproductive response of estuarine copepod species subjected to changes in both salinity and pH levels. Even though species populating estuaries tend to show higher adaptability (plasticity) and tolerance to fluctuating environmental conditions (Elliott and Quintino 2007), temporal and spatial environmental changes still highly influence the distribution and abundances of copepods in estuaries (Leandro et al. 2007).

Considering the importance of copepods in estuarine systems and the potential influence of salinity and pH on their reproductive performance, we aim at exploring the reproductive response of an estuarine dwelling species to temporal variations in salinity and pH (as multiple stresses) from Coorong, South Australia. This system is susceptible to both salinity and pH variations from frequent fluctuations in freshwater discharge into the system (Hemraj et al. 2017). Freshwater discharge can contribute to low pH water during high flows or wash up acid-sulphate soils into the Coorong, therefore acidifying the system (Fitzpatrick et al. 2010). Here, we study *Gladioferens pectinatus*, a euryhaline copepod species commonly encountered in south-eastern Australia and New Zealand estuaries (Bayly 1965; McKinnon and Arnott 1985; Hall and Burn 2002). It is a pioneer herbivore that often recolonises fresh or brackish aquatic systems that have been anthropogenically or naturally modified in terms of water quality and water flow, and plays an important functional role in estuarine systems (Roennfeldt 2013). *G. pectinatus* often reaches maximum abundances in colder waters during winter and is an important food source for larval and forage fish (Roennfeldt 2013). Here, we look more specifically at reproductive response in terms of pre-naupliar data, including brood sizes, embryonic development and hatching success, as well as post-naupliar data, including percentage of nauplii that grow into copepodites and adults, mortality rate, development in relation to time, and sex ratio of copepods grown under different levels of salinity and pH. We hypothesize that, firstly, the effects of pH on the reproductive response of *G. pectinatus* will differ at different salinities. Secondly, it is expected that *G. pectinatus* will be relatively tolerant to some decreases in pH but not to very low-pH water (< 7.6). This investigation will provide new insights about the effects of a combination of salinity and pH on copepod reproduction and adaptability from estuaries.

3.3. Materials and Methods

3.3.1. Copepods and microalgae

For this study, *G. pectinatus* nauplii and adults were collected from the Coorong estuary (35°48'05.2"S 139°19'24.4"E**;** South Australia) in August 2012 (salinity 3.05; pH 8.01) and December 2013 (salinity 7.52; pH 8.34) with a 35 µm mesh plankton net. In the Coorong, *G. pectinatus* has been observed with maximum abundances around August and September in salinities ranging between 3 to 15, pH 8.0 to 8.3 and temperature 15 to 20 °C (Hemraj 2013; Shiel and Tan 2013). However, the species can be observed throughout winter and early spring with its distribution extending to the surrounding Lower Lakes (Lake Alexandrina and Lake Albert) where freshwater prevails (Shiel and Tan 2013). The copepods collected were cultured in 2 litre glass beakers. A constant temperature (17 °C) and 12:12 light and dark cycle was maintained in the culture room. The copepods were acclimatised to COMBO medium (Kilham et al. 1998: see Appendix Table 3) by dilution with filtered (10 µm) Coorong water (1:3 COMBO to Coorong water, then 1:1 COMBO to Coorong water) over three days until they could be cultured in 100% COMBO medium. The COMBO medium is a suitable culture medium for both zooplankton and phytoplankton, and has been previously used to culture *G. pectinatus* successfully (Hall and Burn 2002). It was prepared in the laboratory following instructions from Kilham et al. (1998). The copepod culture was fed daily with concentrated *Rhodomonas* sp. culture (Australian National Algae Culture Collection, CSIRO, Hobart, Tasmania) grown in Gse medium (Blackburn et al. 2001) at a salinity of 25. Gse medium was more suitable for growing *Rhodomonas* sp. than COMBO and, therefore, it was used for stock cultures to obtain a high concentration of *Rhodomonas* sp.

3.3.2. Experimental conditions

The experiment was conducted in COMBO medium at two salinities (2 and 10; based on salinity measurements in the Coorong at the time of sampling) and four pH levels. Salinity was adjusted by mixing natural filtered seawater (0.45 µm) with MilliQ water to the required salinity

prior to adding COMBO-specific nutrient solutions. In order to modify the pH of the COMBO solutions, CO_2 (prepared mixtures of 0.1% 0.2% and 0.5 % CO_2 from BOC UN, Australia) was bubbled through the medium in 2 litre clear Schott bottles (DURAN®) to obtain a stock media with corresponding pH. Bubbling of the 2 litre bottles was performed at medium flow (0.6 L/min, Gascon Systems flowmeter, Victoria, Australia) until stable pH values were obtained (approximately 10 minutes) from a Mettler Toledo probe (Seven multi, calibrated with Metler Toledo TRIS buffers and measured on a NBS scale; Table 1). Measurements of salinity and pH were taken from the stock solutions daily to monitor any change. Actual pCO_2 was calculated from pH, total alkalinity (measured by open cell potentiometric titration following Dickson et al. 2003) and salinity readings, using the CO2sys_v2.1 software (Pierrot et al. 2006) with modified constants from Millero (2010). In case of more than 10 % fluctuation in measurement, the pH was readjusted by bubbling. The Salinity of the solutions did not change.

Table 1: Mean values of abiotic parameters measured (Salinity, Temperature, pH and Total Alkalinity) or calculated (pCO_2 and HCO_3) during experimental procedure.

Salinity (PSU)	Temperature (°C)	Total Alkalinity (µmol/kg)	pH (NBS scale)	pCO_2 (µatm)	HCO_3 (µmol/kg)
2.83 ± 0.05	17	575.7 ± 12.46	7.77 ± 0.08	313.8	553.7
2.79 ± 0.06	17	594.8 ± 8.67	7.51 ± 0.11	623.6	582.3
2.88 ± 0.05	17	616.5 ± 17.68	7.29 ± 0.09	1053.1	608.6
2.83 ± 0.04	17	620.8 ± 10.78	7.18 ± 0.1	1392.6	614.7
10.51 ± 0.06	17	1159.4 ± 10.32	8.09 ± 0.11	257	1037.5
10.64 ± 0.16	17	1171 ± 24.14	7.86 ± 0.12	453.5	1093.5
10.68 ± 0.23	17	1181.3 ± 9.22	7.68 ± 0.19	733.3	1125.6
10.65 ± 0.11	17	1177.6 ± 9.51	7.41 ± 0.14	1704.3	1155.9

3.3.3. Experimental procedure

Prior to starting the reproduction experiment, male and female copepods were separated and acclimatised to the experimental treatments for three days. Acclimatisation involved placing the copepods (15 males and 15 females), in COMBO medium in each of the different treatment combinations (salinity 2 and 10 at four pH levels each), totalling 120 males and 120 females (Fig. 12). Males and females were separated and acclimatised separately. Only unfertilised mature females were selected and acclimatised to ensure fertilisation only occurred under experimental conditions. After acclimatisation, one male and one female were put together as single pair into 100 mL plastic containers (SARSTEDT, Adelaide, Australia), filled with respective treatment solutions, and allowed to reproduce. The containers were filled to maximum capacity to reduce any air – liquid CO_2 flux. Daily measurements of salinity and pH were taken from a small subsample of the treatment solutions, from each replicate, to monitor and ensure that each treatment conditions were constant. The treatment solutions were also replaced with fresh medium daily to prevent any possible changes to the treatment conditions due to accumulation of excrements. The male was removed once the female had egg sacs. If no egg sacs appeared after three days, the male was replaced by another one. For each treatment, eight replicates were studied, totalling to 64 individual pairs studied. The number of eggs produced per female (brood size) was counted under a dissecting microscope (Nikon C-LEDS) by carefully transferring the female in a well-plate with corresponding treatment medium.

The embryonic development time (EDT) was provided as a function of the number of days. If all the eggs had not hatched simultaneously, the embryonic development time was counted until the last eggs had hatched. The number of eggs that hatched per day was counted as the daily naupliar production. The hatching success was calculated from the total number of nauplii hatched from a brood as a percentage of the total number of eggs in the brood. Once all eggs had hatched, the female was removed and the number of individuals was counted daily. Counting was done in a plastic petri dish under the dissecting microscope. The stages

were grouped as nauplii (all N1 to N6), copepodites (all C1 to C5) and adults. The number of individuals developing from nauplii to copepodite and adults were monitored daily. The percentage development of nauplii to copepodite/adult was calculated from the maximum number of copepodite as a percentage of the maximum number of nauplii from the same clutch. Once all copepods reached adulthood, they were preserved with 5% formalin, staged and the sex ratio was determined.

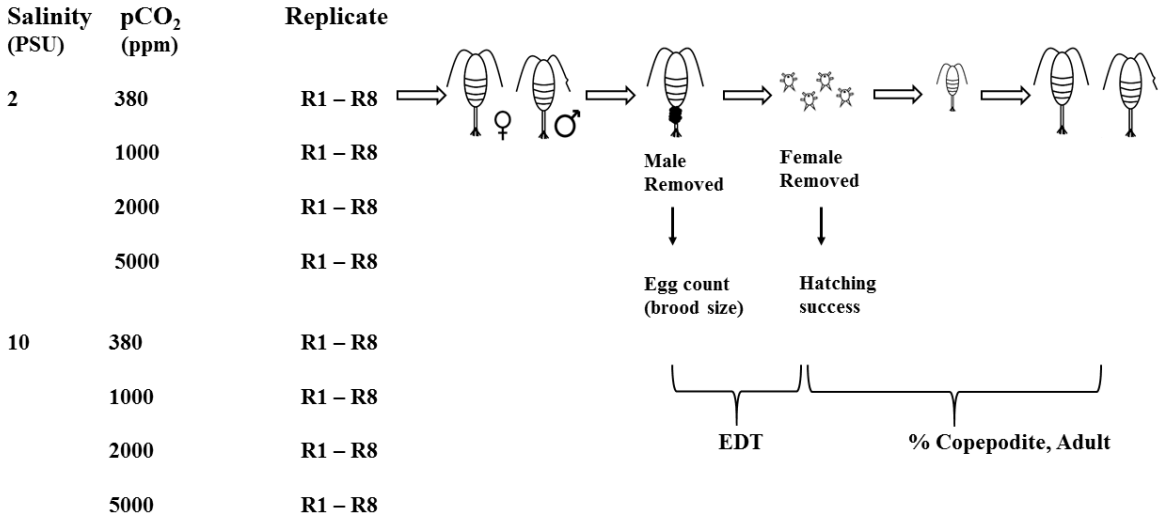

Figure 12: Schematic diagram showing experimental protocal developed and followed in chapter 3.

3.3.4. Data analysis

The variability in reproductive parameters between replicates was calculated using a coefficient of variation:

CV = (σ/μ) x 100, where μ is the mean and σ is the standard deviation (Devreker et al. 2009). The average mortality rate of individuals developing from nauplii to copepodite, and copepodite to adults, in terms of individual per day (ind/day), was calculated using the formula: Mortality rate = $(N_m – N_{m+1})/D$, where N_m is the maximum number of individuals of stage 1(e.g. nauplii or copepodite), (N_{m+1}) is the maximum number of individuals in stage 2 (e.g. copepodite or adult) and D is the number of days taken for all individuals of stage 1 to change to stage 2

or die. This accounts for all dead individuals from the start of a stage to the end of that stage. To look at development from one stage to another in relation to time, the median development time (MDT) was estimated from stage frequency data (Landry 1975, Peterson & Painting 1990). This was achieved by plotting a cumulative frequency curve of each stage against time, and determining the time when 50% of the copepods had reached that stage by least-squares linear regression. The regression was performed on the curves excluding the 10% and 90% tails (Campbell et al (2001). Stage duration (SD) was calculated as the time between the MDT of two successive stages. The development from eggs to adulthood was measured from when eggs were produced to MDT of adults (50% of adults).

Data were analysed for normality using Kolmogorov-Smirnov test (Zar 1996). As the data was not normally distributed, non-parametric tests were used for further analysis. Correlations between reproductive data and treatment data (measured pH values) were analysed using Spearman's correlation coefficient (Zar 1996). Differences in reproductive response between treatments were tested using Kruskal-Wallis ANOVA (Zar 1996). Statistical analyses were performed using IBM SPSS 22.0.

3.4. Results

Treatment of the culture media with CO_2 resulted in different pH values at salinity 2 compared to salinity 10. Therefore data at salinity 2 and salinity 10 were analysed separately.

At salinity 2, no significant difference in brood size, EDT and hatching success was observed between pH treatments ($p > 0.05$; Fig 13). Moreover, no difference was observed in the percentage of nauplii that developed into copepodites ($p > 0.05$). On the other hand, the percentage of copepodites developing into adults decreased with declining pH values (Spearman's $\rho = 0.604$, $p = 0.000$; Fig. 14) and, therefore, the total percentage of nauplii that eventually developed into adults significantly declined with lower pH (Spearman's $\rho = 0.640$, $p = 0.000$; Fig. 14). At pH 7.51, 7.29 and 7.18, no individuals developed into adults, except for

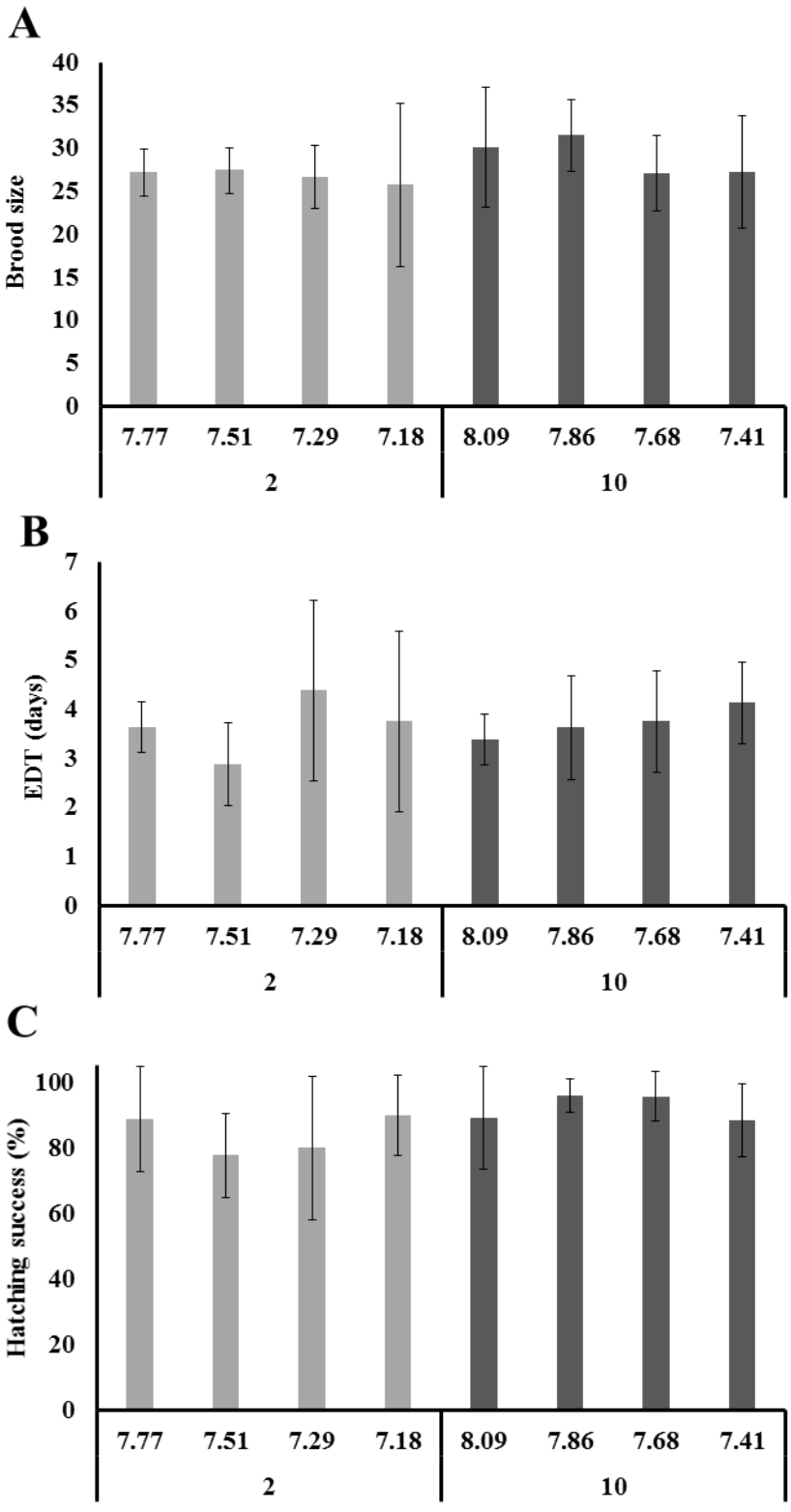

Figure 13: Bar graphs of mean pre-naupliar data representing (A) brood size, (B) embryonic development time and (C) percentage hatching success for two salinities and four pH levels. Mean for 8 replicates were calculated (n=8) and error bars represent standard deviation.

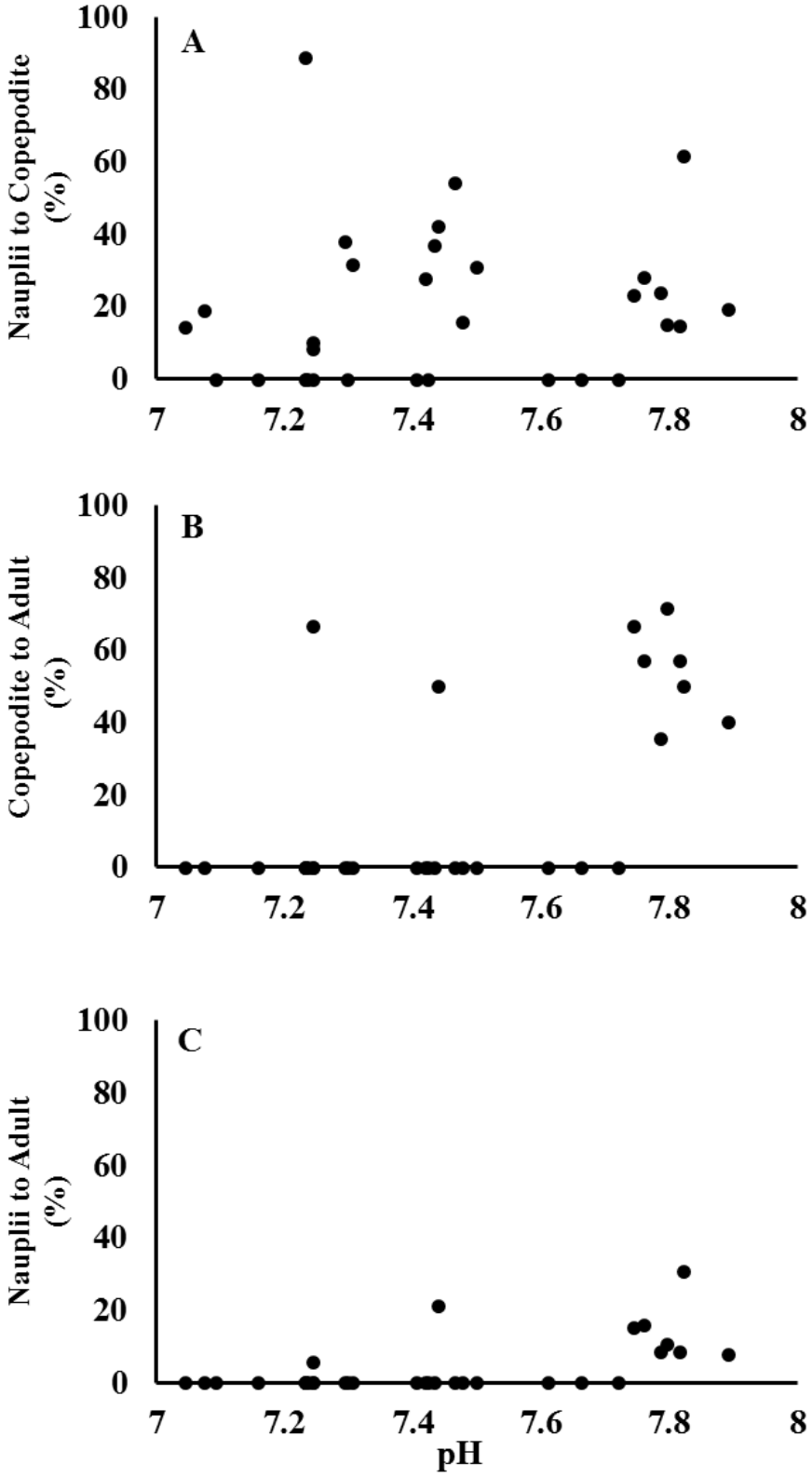

Figure 14: Percentage of individuals from salinity 2 treatments that successfully developed from (A) nauplii to copepodite, (B) copepodite to adults and (C) nauplii to adult against pH values measured during experimental procedure. At salinity 10, no differences were observed

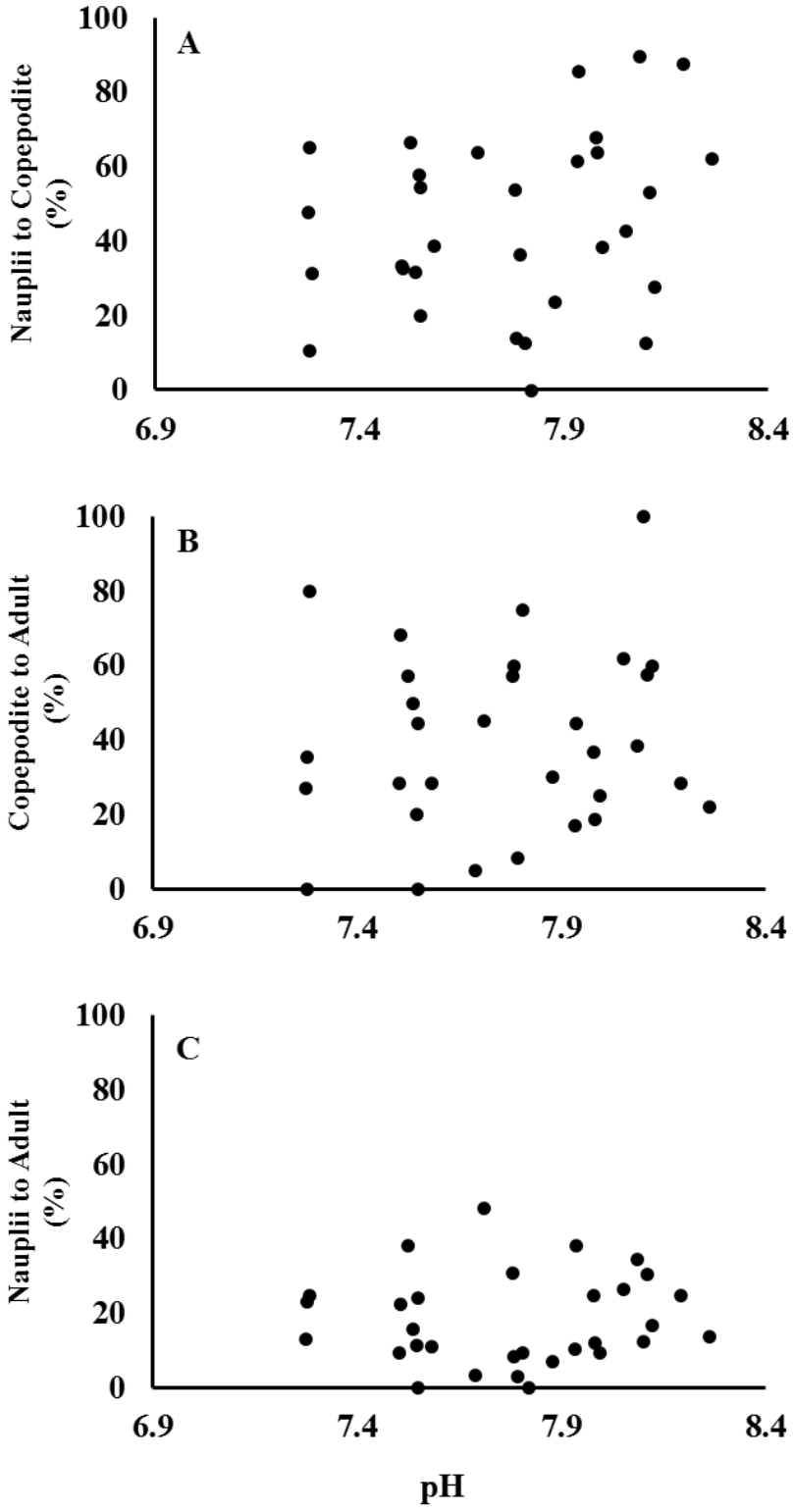

FIgure 15: Percentage of individuals from salinity 10 treatments that successfully developed from (A) nauplii to copepodite, (B) copepodite to adults and (C) nauplii to adult against pH values measured during experimental procedure.

two replicates at pH 7.51 and at pH 7.29. No significant difference and high CV (> 25%) were observed in the rates of mortality from nauplii to copepodite and copepodite to adults, across pH treatments ($p > 0.05$) MDTs for nauplii and copepodites at each pH treatment at salinity 2 had a high CV (> 25%), except for pH 7.77, and were not significantly different across pH treatments ($p > 0.05$; Fig. 17). As for stage duration for nauplii and copepodites, no significant differences were observed for the different pH treatments.

At salinity 10, no differences were observed in brood size and hatching success across pH treatments ($p > 0.05$; Fig.15). The EDT showed an increasing trend with lower pH values (Spearman's $\rho = -0.398$, $p = 0.024$). However, no significant differences were found when EDT at individual pH treatments were compared ($p > 0.05$). No trends were observed across pH treatments for post-naupliar growth (Fig. 15). The rates of mortality were not different across pH treatments and had high CV (> 25 %) and were not different (Fig. 16). No difference in MDTs and stage durations for nauplii and copepodites were observed ($p > 0.05$; Fig. 18). However, high CV were obtained across pH treatments (> 25 %), especially at pH 7. 86, 7.68 and 7.41. Finally, the sex ratios at all pH treatments were female biassed (< 1), except for pH 7.68 where it was male oriented (> 1).

3.5. Discussion

Estuarine dwelling organisms are exposed to a variety of environmental stresses that affect their physiology and reproduction. Salinity has previously been shown to be a major influence on the reproduction of several copepod species (Hall and Burn 2002; Devreker et al. 2009; Pan et al. 2016). However, many estuaries are also subjected to temporally low pH levels based on water discharge, and the effects of the resulting chemical change onto estuarine copepods are relatively unknown. Here, our results show that low salinity, coupled with low pH levels, detrimentally affects the recruitment of *G. pectinatus*, while at higher salinity there were no significant effects of low pH. *G. pectinatus* is a euryhaline species. However, the ability of this species to tolerate high salinities is also related to temperature, whereby higher

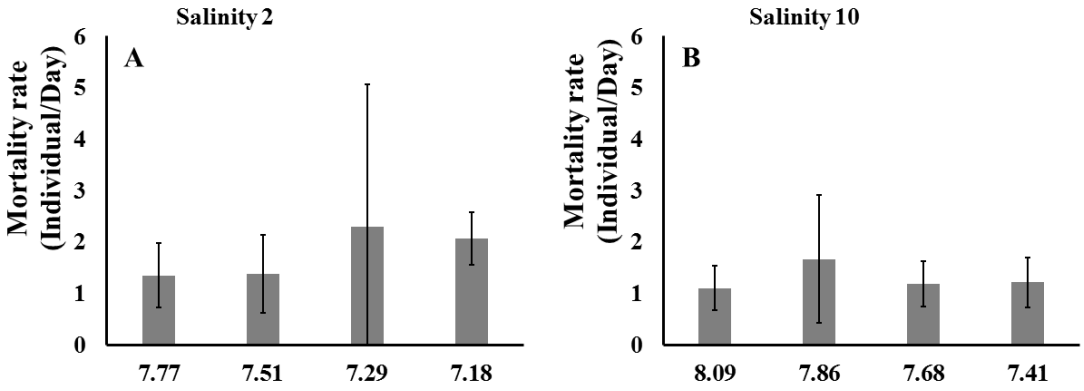

Figure 16: Overall rates of mortality (individuals per day) from nauplii to adult for each pH treatments at (A) salinity 2 and (B) salinity 10. Mean for 8 replicates were calculated (n=8) and error bars represent standard deviation.

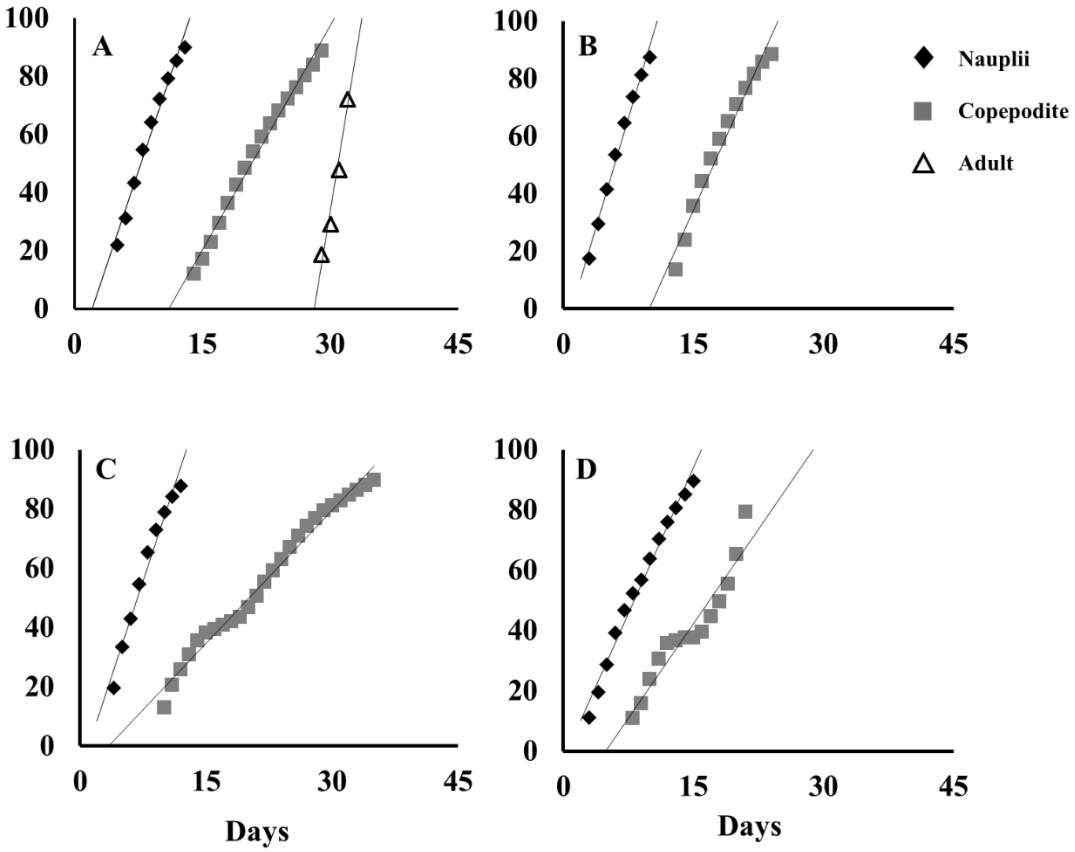

Figure 17: Cumulative frequency plot of nauplii (N1-N6), copepodites (C1-C5) and adult at salinity 2 and pH (A) 7.77, (B) 7.51, (C) 7.29, and (D) 7.18. The plots exclude the <10 % and >90 % tails.

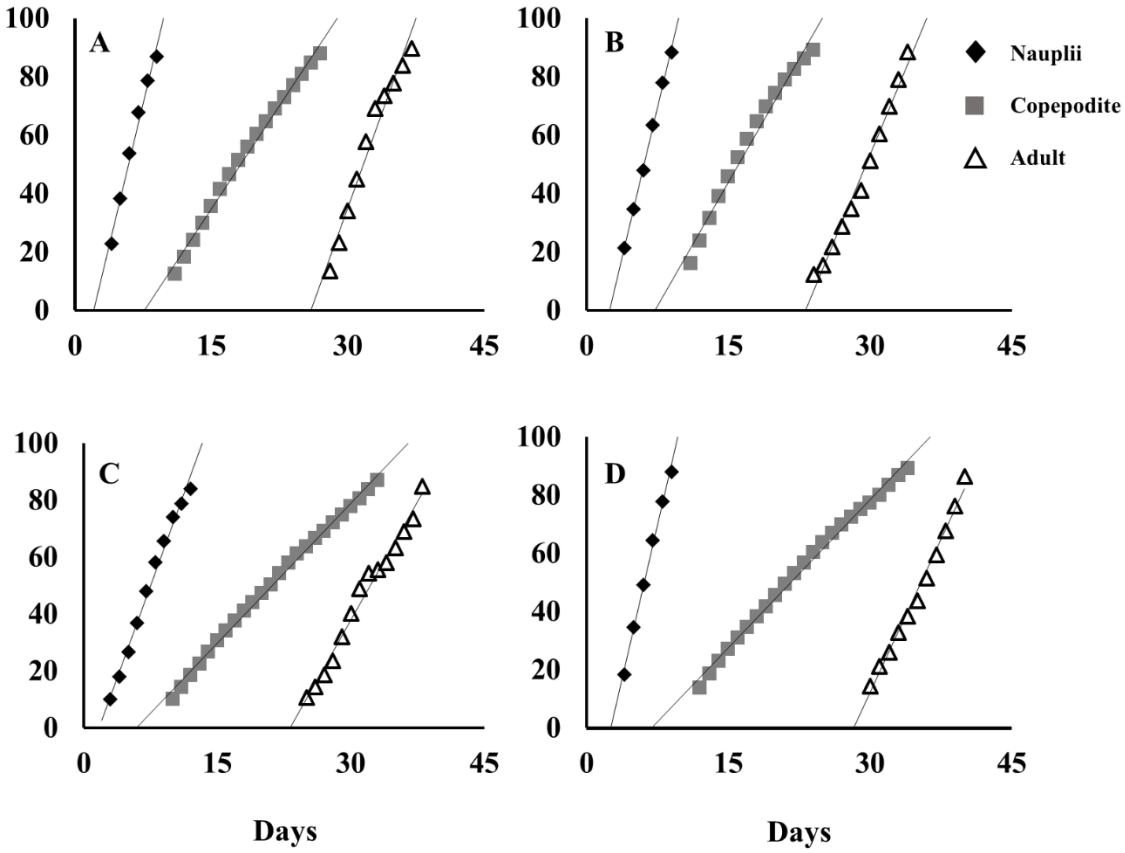

Figure 18: Cumulative frequency plot of nauplii (N1-N6), copepodites (C1-C5) and adult at salinity 10 and pH (A) 8.09, (B) 7.86, (C) 7.68, and (D) 7.41. The plots exclude the <10 % and >90 % tails.

salinities (> 25) can be tolerated at lower temperatures (< 20 °C; Bayly 1965; Hall and Burn 2002). This implies that combinations of different parameters produce variable reproductive responses in the species. Similarly, here we observe that combinations of different salinities and pH levels cause varying responses in the recruitment of *G. pectinatus*.

As shown by Calliari et al. (2006) and Svetlichny et al. (2012) the effects of salinity on reproductive success differ across a wide variety of copepod species, however, the major processes affected tend to be egg production, hatching success, and survival (Devreker et al. 2009; Pan et al. 2016; Souissi et al. 2016). Change in salinity imposes stress onto organisms by affecting their osmoregulation. Copepods accumulate organic osmolytes, such as free amino acids like proline, alanine and glycine, in response to osmotic stress (Lauritano et al. 2012). Moreover, excessive energy is commonly spent on adjusting their metabolism and regulating their intercellular solute concentrations (Kimmel and Bradley 2001), therefore reducing the energy budget for reproductive processes (Pan et al. 2016). The *G. pectinatus* population from the Coorong, displayed tolerance of a range salinities (salinity 2 to 10) and no difference in egg production, survival or development at salinity 2 control treatment compared to salinity 10 treatments.

In comparison, Hall and Burn (2002) have shown that freshwater was reproductively more favourable in a *G. pectinatus* population of New Zealand, whereby at low brackish salinities (1.5 to 5) no eggs were produced and survival was significantly altered. The results suggest a significant differentiation in salinity tolerance between these two populations. Similar results have previously been shown in estuarine dwelling *Eurytemora affinis* populations from Canada and France (Beyrend-Dur et al. 2009), whereby a Canadian population displayed greater fitness (egg production and survival) in high salinities compared to the French population. Such differences are a likely result of population genetic differentiation and phenotypic plasticity. Beyrend-Dur et al. (2009) argue that exposure to wider salinity ranges contribute to the phenotypic plasticity, therefore, enabling survival at higher salinities. In the Coorong, *G. pectinatus* is exposed to rapid changes in salinity due to the hydrodynamic complexity of

freshwater mixing with marine water, by tidal flow, and hypersaline water from the coastal lagoon (Hemraj et al. 2017). Such conditions have likely favoured the tolerance of a wider range of salinity in the Coorong population.

As well as acidifying the water, increase in CO_2 levels can impose an additional stress onto organisms by diffusing into intracellular fluids or imparing exchange of respiratory CO_2 exchange (Zhang et al. 2011; Whiteley 2011). This can eventually cause hypercapnia and react with intracellular fluid, forming H^+ and HCO_3^- (Zhang et al. 2011). Several organisms deal with such situations by passive internal buffering, ionic exchange and transport, or metabolic suppression (Fabry et al. 2008). However, dealing with excess CO_2 is potentially energetically expensive and can alter energy budgets. Therefore, is dependent on the extent of excess CO_2 and the species-specific ability to deal with hypercapnia (Fabry et al. 2008). The resulting effects of acidification and high CO_2 produce varying reproductive responses among copepod species. Almén et al (2016) have observed no effect of lowered pH on the offspring production of *Eurytemora affinis* and concluded that the species is better adapted to changes in pH due to its natural exposure to pH fluctuations. On the other hand, Cripps et al (2014) found severe impairment in nauplii survival and recruitment in *Acartia tonsa* under lower pH levels Moreover, Vehmaa et al (2016) showed a alleviation of negative effects in *Acartia* sp. up to a certain threshold of CO_2, after which egg hatching success and nauplii development were impaired. They argue that although acidification may not affect egg production, it is still likely to affect hatching and further development of nauplii. Our findings at salinity 2 are supported by both Vehmaa et al (2016) and Cripps et al (2014), in that reduced naupliar survival and adult recruitment is seen at a reduced pH. The lack of impairment at salinity 10, suggests that a combination of responses to both salinity and pH influence the survival and recruitment in *G. pectinatus*.

Several authors have argued that the mechanisms involved as a response to stress and change in energy demands increase the need of energy reallocation, which is a very important factor when dealing with certain stresses (Fitzer et al. 2012; Li and Gao. 2012; Sokolova et al.

2012; Vehmaa et al. 2012; Aguilera et al. 2013; Thor et al. 2015). Although many authors have shown adverse response in terms of egg production and hatching success in copepods (Kurihara et al. 2004; Devreker et al. 2009; Zhang et al. 2011; McConville et al. 2013), when exposed to considerable stress, energy is actually preferably allocated to stress response mechanisms and reproductive output rather than larval growth or cuticle composition (Calliari et al. 2006; Fitzer et al. 2012). This has been suggested as the likely explanation for decline in development and growth in different copepod species (Devreker et al. 2009; Aguilera et al. 2013) and could explain a decrease development under a change in salinity and additional pH stress observed in this study.

Growth and development of copepods require molting of stages within naupliar and copepodite, as well as metamorphosis from nauplii to copepodite (Andersen et al. 2001). Hormones, such as ecdysteroids, need to be produced for molting or metamorphosis. Ecdysteroids, primarily associated with molting, are present in crustaceans and the patterns of variation is relative to molt cycle phase, as observed by Johnson (2003) in the copepod *Calanus pacificus*. The reallocation of energy towards physiological stress response or reproductive output can possibly have a negative impact on energy required for molting or the production of molting hormones. Therefore, it can affect development and molting, as previously suggested in the case of Antarctic krill (Flores et al. 2012). Similarly, reallocation of energy to deal with a combination of osmoregulatory response and low pH derived stresses is the likely impairment to further development and molting in copepodites from treatments of low pH at salinity 2. Moreover, as shown by Fitzer et al. (2012) for the copepod *Tisbe battagliai*, the composition of cuticle may have also been compromised (increase in the proportion of carbon compared to oxygen) due to the exposure to low pH in *G. pectinatus*, therefore reducing survival.

Studies on the combination of salinity and low pH are scarce. Aguilera et al. (2013) conducted an examination of body length of *Acartia tonsa* copepods collected in situ and experimentally determined the egg production and hatching success when exposed to low pH riverine

freshwater input into an estuarine and coastal system. They showed that the physio-chemical characteristics attributed to low pH freshwater discharges affected the morphology and reproductive performance of the species. However, Aguilera et al. (2015) compared the ingestion rate and stress gene expression in *Acartia tonsa* from estuarine and coastal systems in relation to pCO_2. They showed a lower ingestion rate and eventual higher adaptability to high pCO_2 in copepods from the estuary, while coastal copepods had significantly higher stress gene expression. These results show that while some aspects of growth and reproduction may be altered, phenotypic plasticity in relation to fluctuating parameters plays a highly important role in estuarine copepod populations. In our experiment, copepods grown at salinity 10 did not show any major impairment in reproduction and development when exposed to low pH. While specific gene expressions were not tested, it can still be determined that, at certain salinity levels, adaptability, in terms of plasticity, to low pH levels is extant in *G. pectinatus*. Similarly, the variabilities observed in brood size, EDT and hatching success at salinity 2 and lower pH values may be linked to individual variability in adaptability and phenotypic plasticity, and therefore their ability to sustain stress and produce clutches.

While some copepod species have been shown to be affected by stress related to salinity or pH, several others have shown little or no effects (Kurihara et al. 2004; Devreker et al. 2009; Zhang et al. 2011; McConville et al. 2013; Hildebrandt et al. 2014). Moreover, variances in response from different species and populations of the same species have also been to be highly important when studying the effects of water acidification on copepods in either marine or estuarine systems (Beyrend-Dur et al. 2009; Aguilera et al. 2015). Such disparities in stress response among populations or species point out the importance of understanding inter- or intraspecific resilience, multigenerational adaptability or phenotypic plasticity in copepods, as for other organisms (Dupont and Thorndyke 2009). Furthermore, examining the cumulated effects of multi-stressors is imperative in light of further understanding the response of organisms to natural occurring or future fluctuations in water parameters.

CHAPTER 4

Population structure of *Acartia fancetti* in relation to hyperhaline and thermal stresses; a neritic species in a hypersaline lagoon

This chapter has been submitted to the journal Limnology and Oceanography on the

9th June 2017

4.1. Abstract

Phenotypic plasticity and adaptation are common traits of organisms living in highly dynamic and changing environments. Several copepod species, especially estuarine dwelling species, have demonstrated high phenotypic plasticity and adaptability to variations in environmental conditions. Copepods play a critical role in aquatic foodwebs and their population structure is closely linked to water quality fluctuations, especially salinity and temperature. Here, we examine the population structure, egg production and hatching success of *Acartia fancetti* in relation to hypersalinity (30, 40, 50 and 60) and temperature (15 °C and 20°C). *A. fancetti* is a neritic species occupying a key niche in the hypersaline areas of the Coorong, South Australia. Our results show a significant influence of salinity and temperature on the population size, nauplii production, copepodites production and adults of *A. fancetti*. The maximum numbers of nauplii, copepodites and adults were at salinity 30 and 40 at both 15 °C and 20 °C. However, substantial population sizes were also observed at salinity 50 and 60 in 15 °C treatments. At 20 °C, no individual was observed at salinity 60. This study indicates that the population of *A. fancetti* in the Coorong is tolerant to hypersalinity at lower temperatures but that the combined effects of haline and thermal stress significantly reduces the ability of this species to survive at higher temperatures. The distribution of *A. fancetti* in the Coorong is also affected by the environmental change related to anthropogenic activities.

4.2. Introduction

Calanoid copepods play a critical role in aquatic foodwebs as the prevailing mesozooplankton, especially in temperate and polar regions. The population and community response of copepods to changes in environmental conditions form a crucial link between the biogeochemical impacts of environmental conditions on primary production and the foodweb that follows (Banas et al. 2016). Copepods respond quickly to variations in environmental factors, especially salinity and temperature. As such, salinity and temperature are significant factors affecting the reproduction, life cycle, population and community response of copepods (Devreker et al. 2009; Horne et al. 2016; Werbrouck et al. 2016).

Copepods are ectothermic organisms, therefore their recruitment and size tend to shift following seasonal cycles due to the effect of temperature on their ontogeny (Horne et al., 2016). In particular, temperature has a direct influence on survival and development rates of copepods whereby increases in temperature tend to increase survival and development rates (Milione and Zheng 2008; Rhyne et al. 2009). Moreover, several studies have shown differences in egg sizes, egg hatching rates, nauplii survival and reproductive output in relation to temperature changes (Rhyne et al. 2009; Hansen et al. 2010; Escribano et al. 2014; Liu et al. 2015). However, as for metabolic rates, the positive influences of temperature on overall reproductive output, development and survival of copepods diminish after a certain threshold. Although copepod ontogeny and population are affected by changes in temperature, several studies have also shown that plasticity and adaptation to the influences of temperature play an important role in acclimatisation and resilience (Hansen et al. 2010; Souissi et al. 2016).

Similarly to temperature, salinity tolerances of copepods are dependent on their survival and ability to reproduce under various salinity levels. The major impact of salinity on copepods is the instigation of osmotic stress (Devreker et al. 2009). Under less, or more, than optimum, salinity, copepods endure an energy imbalance and stress gene and protein response to cope with osmoregulation. This, therefore, affects other biological traits, such as eggs production, developmental time, survival and recruitment (Lee et al. 2017). As a result, copepod

population and community structures are subjected to substantial variations in relation to fluctuations in salinity.

Physiological stress is a common effect of changed environmental conditions. However, many copepod species are able to show certain levels of adaptation and plasticity, especially between different populations of the same species. For example, Beyrend-Dur et al. (2009) have shown clear differentiation in clutch size and longevity between two transatlantic populations of *Eurytemora affinis* under similar salinity treatments. Moreover, Schoville et al. (2012) have shown dissimilarities in temperature tolerance of different populations of *Tigriopus californicus*, whereby possible evolutionary divergences in gene expression and regulatory pathways were observed. Such differentiation in responses shows the adaptability of copepods to changing habitat conditions, and points towards the importance of understanding population-specific variations in lifecycle and population structure in relation to habitat specific changes in environmental conditions.

In this study, the aim is to identify the reproductive response and population structure of *Acartia fancetti* to salinity and temperature variations. *A. fancetti* is a neritic calanoid copepod. *A. fancetti* was first observed and described in Westernport and Port Phillip Bays, South Eastern Australia (McKinnon et al. 1992). It is an allopatric sibling species of the *Acartia* genus. This species has been observed in typical marine waters of South Eastern Australia (McKinnon et al. 1992). However, only recently, this species has also been encountered in the hypersaline regions of the Coorong (South Australia), a coastal lagoon undergoing health restoration from past degradation, and morphologically been identified as being similar. This species has been observed at nauplius, copepodite and adult stages in salinities ranging from 40 to 65 (Hemraj et al. 2017), which are much higher than the salinities in Port Phillip Bay (Lee et al. 2012). Here, we look more specifically at the egg production and population dynamics of this species under a variety of hypersaline and temperature manipulations to gain a better understanding of the effects of seasonal and anthropogenic derived changes on the population structure. Such information provides an insight into the possible explanations in

copepod population change in the Coorong, which is highly important for managing the health of the system. This study also provides a comparative base for understanding variations in reproductive response between the Coorong and Port Phillip copepod populations.

4.3. Materials and Methods

4.3.1. Copepods and microalgae

For this study, *A. fancetti* nauplii, copepodite and adults were collect from the Coorong at Parnka Point (35.9017° S, 139.3961° E, South Australia) in November 2016. The water salinity and pH at time of sampling were 52.45 and 8.33, respectively. Quantitative copepod samples were taken using a plankton net (35 µm mesh). *A. fancetti* has been observed in high abundance at Parnka Point at salinity 40 to 60 (Hemraj et al. 2017). Copepods were cultured in 10 L Nalgene bottles (Thermo Fisher Scientific, Australia). Cultures were maintained in a culture room at 20 °C, with a photoperiod of 12:12 h light and dark cycle. After 5 days, cultures were mixed with COMBO medium (Kilham et al. 1998; see Appendix Table 3) to achieve a ratio of 1:3 (COMBO: Coorong water). The use of COMBO eliminates the need to undertake trips for obtaining water from the Coorong, while being a highly appropriate medium for culturing zooplankton and is easily manipulated to change salinities. This was repeated every five days, each time reducing the amount of Coorong water (1:1 and 3:1), until copepods could be reared in 100% COMBO medium. The COMBO medium was used as it is a good culture medium for zooplankton and, especially, due to the difficulty of obtaining freshly filtered Coorong water at salinity 50. Preparation of COMBO medium at salinity 50 was done by mixing 10 µm-filtered seawater (salinity ~37) with sodium chloride (Chem Supply Pty Ltd) to achieve the desired salinity. A 25% change of the culture volume was performed every three days to provide fresh conditions and copepods were fed with 100 mL of an *Isochrysis galbana* culture at stationary growth stage (176 ± 49 x 10^5 cells/mL algae culture), grown in f/2 medium at salinity 37.

4.3.2. Experimental conditions and procedure

Experimental conditions included four different salinities (30, 40, 50 and 60; measured with a Metler Toledo probe; Table 2) and two temperatures (15 °C and 20 °C), representing environmental conditions at Parnka Point and Jack Point where *A. fancetti* were collected in the Coorong. Prior to experimentation, male and female copepods were separated from the stock cultures and acclimatised for three days at different conditions. 30 males and 30 females were acclimatised under each of the eight different conditions, summing to a total of 480 individuals. To look at population structure under each condition, 6 males and 6 females were placed in 2 L of COMBO medium of the relevant salinities. Three replicates of each condition were prepared, totalling to 288 breeding adults.

Copepods were cultured for 21 days (Fig 19). Each culture bottle was aerated using air stones to provide continuous water movement and mixing. A 25% change of the culture volume was performed every three days by mixing (to resuspend any deposited faecal pellets) and filtering 500 mL of each replicate through a 50 µm sieve. Organisms caught on the sieve were then placed back into the culture and 500 mL of fresh medium was added. 20 mL of *I. galbana* concentrated culture (176 ± 49 x 10^5 cells/mL) was added to the copepod cultures. After 21 days, organisms were preserved in 3% formalin (by volume), staged on a gridded Greiner square petri dish (120 x 120 mm; Sigma-Aldrich) and counted using an inverted microscope (Nikon Eclipse TS100). The total number of nauplii, copepodites and adults was recorded.

To examine at the daily egg production, a copepod pair (1 random male and 1 random female) was placed in a well-plate (24 wells) containing 1.5 mL of COMBO medium with 15 µL of concentrated *I. galbana*. Acclimatised males and females were used and placed in well-plates with COMBO of respective salinities. Four replicates were prepared for each treatment. The number of eggs produced and the number of hatched eggs were counted daily, for 5 days, using an inverted microscope (Nikon Eclipse TS100). After 5 days the adults were removed

and the remaining eggs were allowed to hatch. If no eggs were produced after 3 days, the male was replaced. Hatched nauplii were carefully removed with a Pasteur pipette.

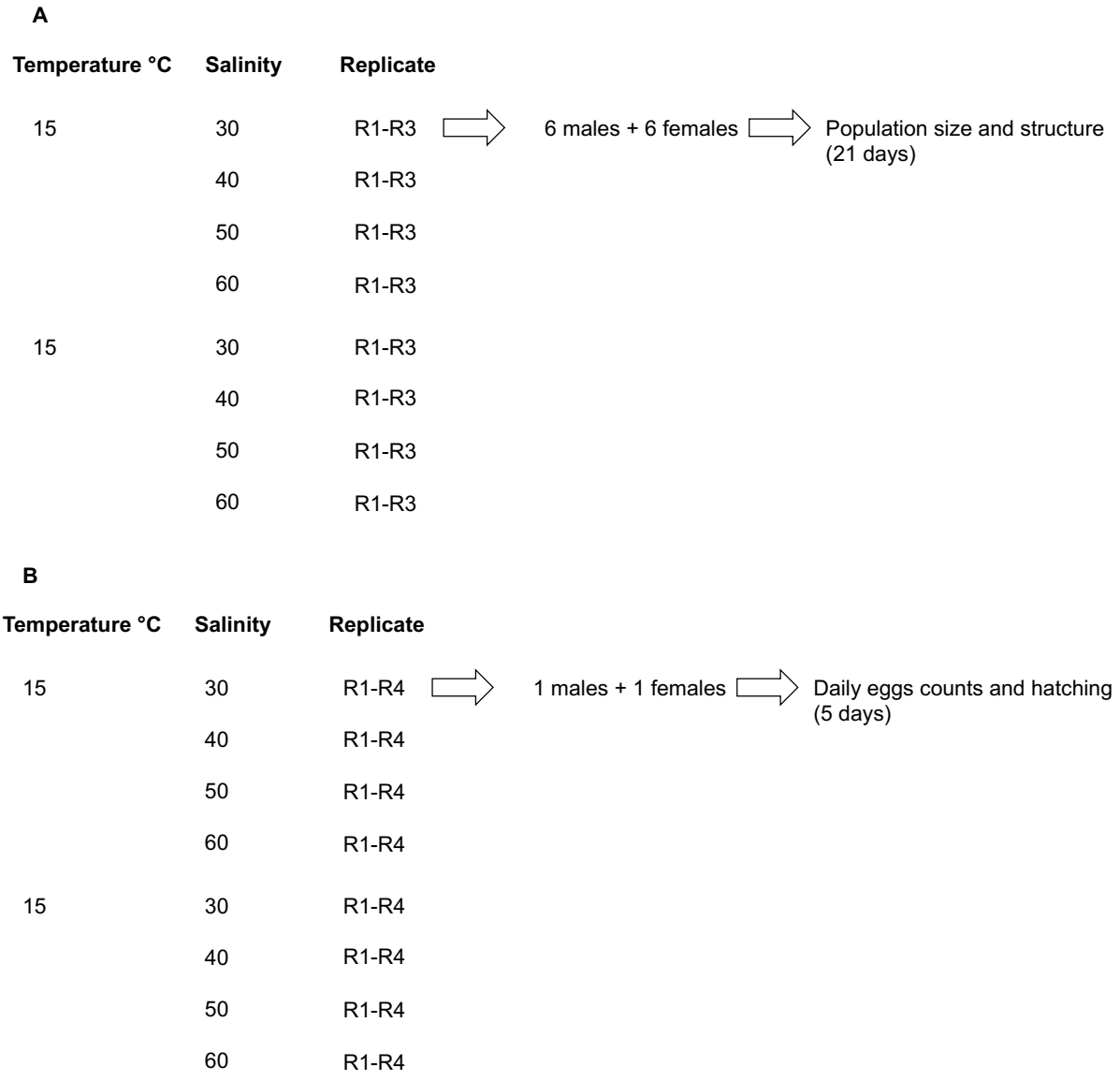

Figure 19: Schematic diagram of population experiment (A) and egg production experient (B) developed and used for chapter 4.

Table 2: Actual salinity and pH values of prepared COMBO for experimental procedure.

Treatment **15 °C**			**Treatment** **20 °C**		
	Actual Salinity	**pH**		**Actual Salinity**	**pH**
30	30.83 ± 0.09	8.29 ± 0.06	**30**	30.73 ± 0.05	8.38 ± 0.11
40	40.85 ± 0.36	8.31 ± 0.05	**40**	41.60 ± 0.4	8.28 ± 0.04
50	52.84 ± 2.73	8.37 ± 0.09	**50**	52.45 ± 0.2	8.31 ± 0.03
60	60.01 ± 0.33	8.25 ± 0.02	**60**	62.25 ± 1.11	8.25 ± 0.07

4.3.3. Data analysis

The data collected were analysed using IBM SPSS (Version 23). Data were analysed for normality using a Shapiro-Wilk test and data that were not normal were square root transformed. Independent sample t-test and One-way ANOVA with Tuckey post hoc were then used to identify differences in population size, population structure (nauplii, copepodite and adult), sex ratio, egg production and hatching success at different salinities and temperatures. Correlations between parameters were analysed using Spearman's *ρ* correlation. Daily egg production was calculated as the mean number of eggs produced over 5 days and hatching success was calculated using the formula:

HS = (ET/NT) * 100, where, HS is the hatching success, E_T is the total number of eggs produced by a female over the study period and N_T is the total number of nauplii hatched from the eggs of that particular female over the study period.

The variability in population parameters between replicates was calculated using a coefficient of variation:

CV = (σ/ μ) * 100, where, μ is the mean and σ is the standard deviation (Devreker et al. 2009).

4.4. Results

Across the two temperature conditions, the respective actual salinity and pH values for each salinity treatments (30, 40, 50, and 60) were not significantly different (One-way ANOVA $P > 0.05$; Table 2). Therefore, the data from the two temperature conditions could be directly compared to each other.

A strong negative correlation was observed between overall population size and salinity at both 15 °C and 20 °C (Spearman's $\rho = -0.891$, N = 12, $P < 0.01$ and Spearman's $\rho = -0.867$, N = 12, $P < 0.01$, respectively; Fig. 20) and the overall population size at 15 °C was higher than that at 20 °C (Independent sample t-test $P < 0.05$). The population structure was also different across salinity levels at each temperature. At 15 °C, the total number of nauplii, was significantly higher at salinity 30 (517 ± 248 individuals; Independent sample t-test $P < 0.05$; Fig. 21A). The number of nauplii at salinity 60 was significantly lower than in other treatments (16 ± 5 individuals; One-way ANOVA $P < 0.05$; Fig. 21A), while no significant difference in number of nauplii was observed between salinity 40 and 50 (182 ± 56 individuals and 133 ± 91 individuals, respectively; One-way ANOVA $P > 0.05$; Fig. 21A).

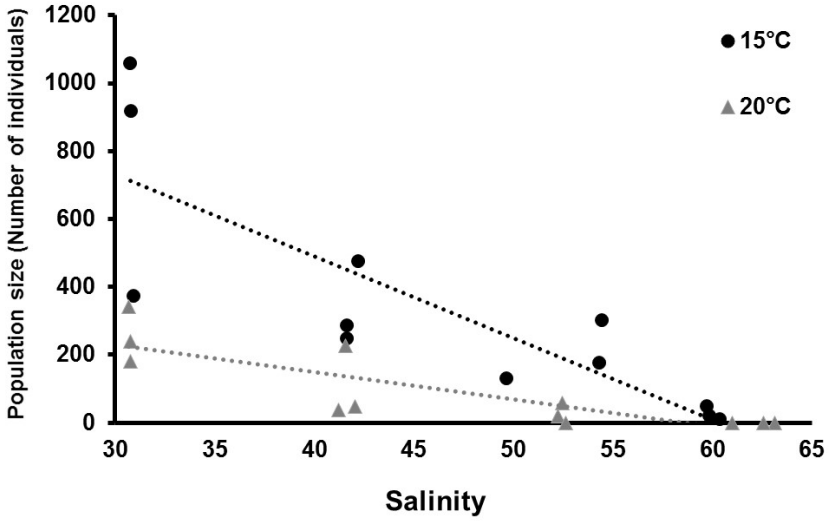

Figure 20: Population size in relation to salinity and temperature. Black dotted trendline ($R^2 = 0.6897$) represents population size at 15 °C (Spearman's $\rho = -0.891$, N = 12, $P < 0.01$) and grey dotted trendline ($R^2 = 0.6916$) represents population size at 20 °C (Spearman's $\rho = -0.867$, N = 12, $P < 0.01$).

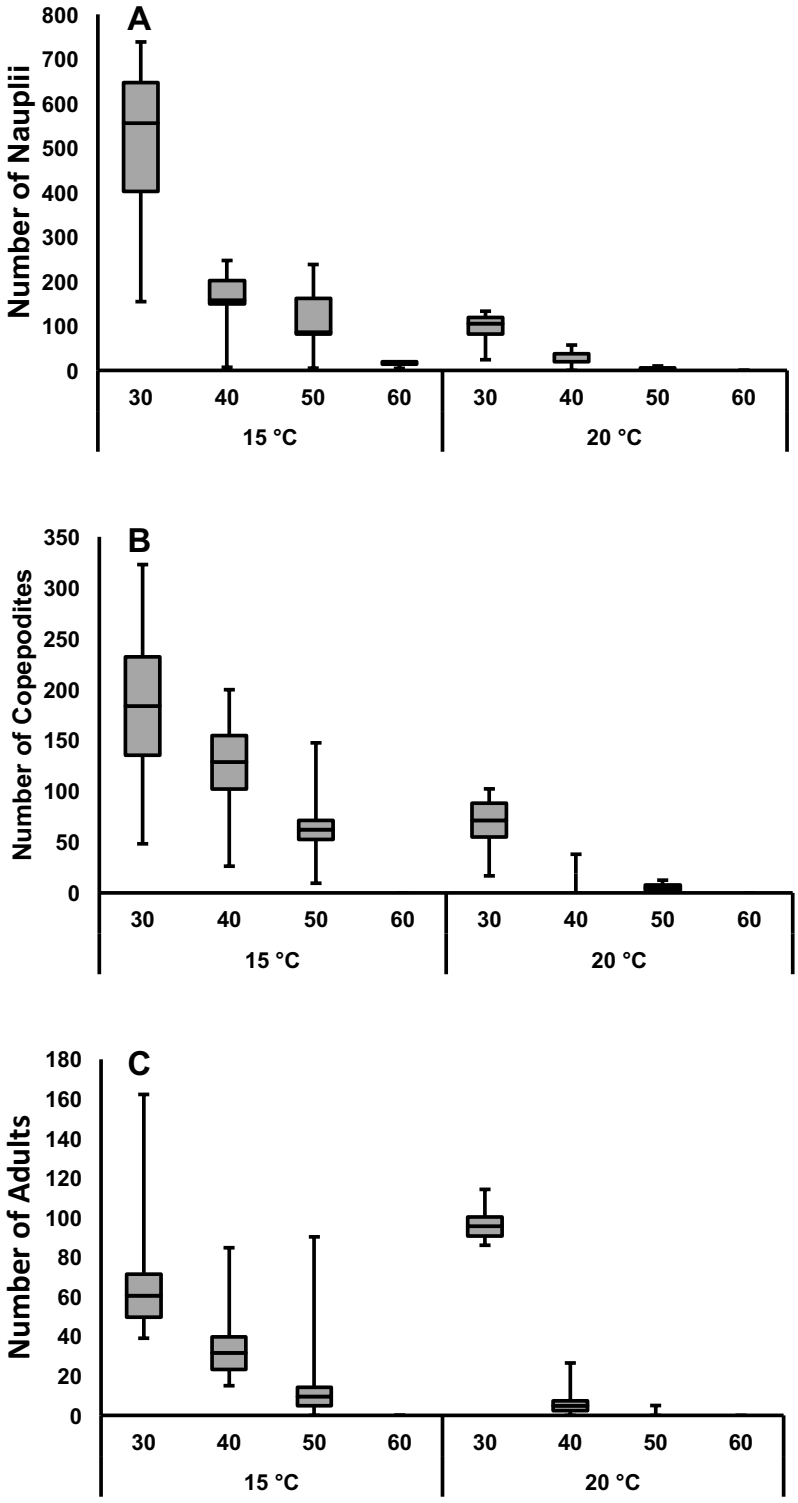

Figure 21: Box plot of data distribution for number of nauplii (A), copepodite (B) and adults (C) across salinity and temperature treatments. The lower edge of the box represents Quartile 1, the upper edges represents Quartile 3, while the middle line is the median. Error bars indicate the minimum and maximum numbers of individuals per treatment.

The number of copepodites showed a strong negative correlation with salinity at 15 °C (Spearman's ρ = -0.900, n = 12, $P < 0.01$); however, due to the variability between replicates, only the number of copepodites at 60 showed a lower average number of individuals (5 ± 8 individuals; One-way ANOVA $P < 0.05$; Figure 21B) Similarly to copepodites, the number of adults showed a strong negative correlation with salinity (Spearman's ρ = -0.843, n = 12, $P < 0.01$) and the number of adults at salinity 30 was significantly higher than other treatments (79 ± 39 individuals; One-way ANOVA $P < 0.05$; Fig. 21C).

At 20 °C, similarly to 15 °C, the total number of nauplii, copepodites and adults showed a strong negative correlation with salinity (Spearman's ρ = -0.942, -0.814, -0.802, respectively, n = 12, $P < 0.01$). The number of nauplii at salinity 30 was significantly higher than at salinity 60 (98 ± 38 individuals; One-way ANOVA $P < 0.05$) and the number of copepodites and adults at salinity 30 were, both, significantly higher than salinity 50 or 60 (70 ± 34 individuals and 86 ± 19 individuals, respectively; One-way ANOVA $P < 0.05$). Overall, the number of individuals of nauplii produced at 15 °C was higher than at 20 °C across all salinities (One-way ANOVA $P < 0.05$; Fig. 21A).

In terms of percentage composition of the population (nauplii to copepodite to adult) in each treatment, a significantly higher percentage of copepodites was observed at salinity 50 compared to salinity 30 at 20 °C (One-way ANOVA $P < 0.05$; Fig. 22). Although the percentage of nauplii seemed lower at 20 °C, no significant difference was observed (One-way ANOVA $P > 0.05$; 22). No significant difference in sex ratio was observed (1 male: 3 females; One-way ANOVA $P < 0.05$; Fig. 23), except for salinity 60 at 15 °C and salinity 50 at 20 °C. The coefficients of variance for population size, number of nauplii, copepodites and adults, and percentage composition were relatively higher (> 50%) at higher salinities (50 and 60 for both temperatures) and higher temperature (20 °C).

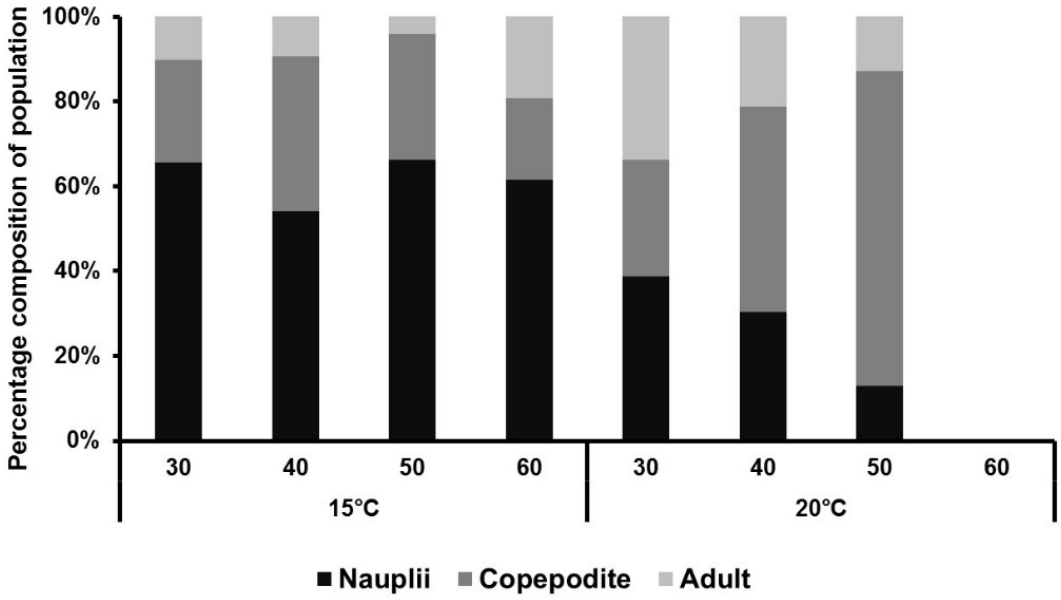

Figure 22: Percentage contribution of nauplii, copepodite and adults to the overall population structure at each salinity and temperature treatments. At 20 °C and salinity 60 no individuals were observed in the experiment.

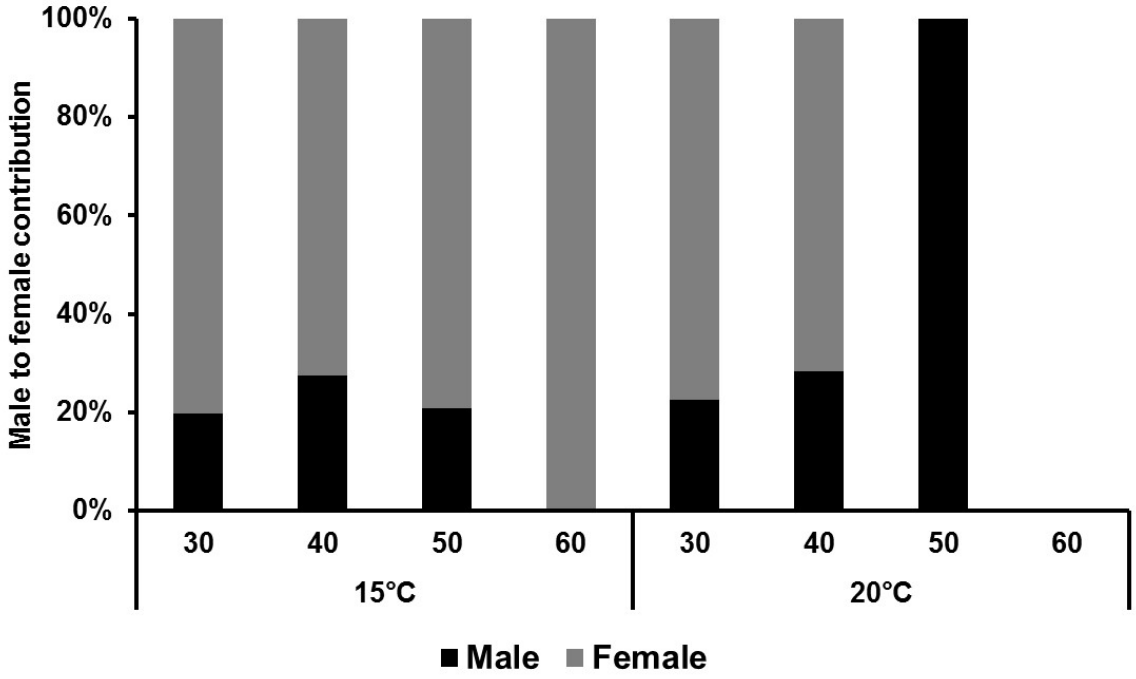

Figure 23: Male to female ratio of adult population observed at each salinity and temperature treatment. At 20 °C and salinity 60 no individuals were observed in the experiment.

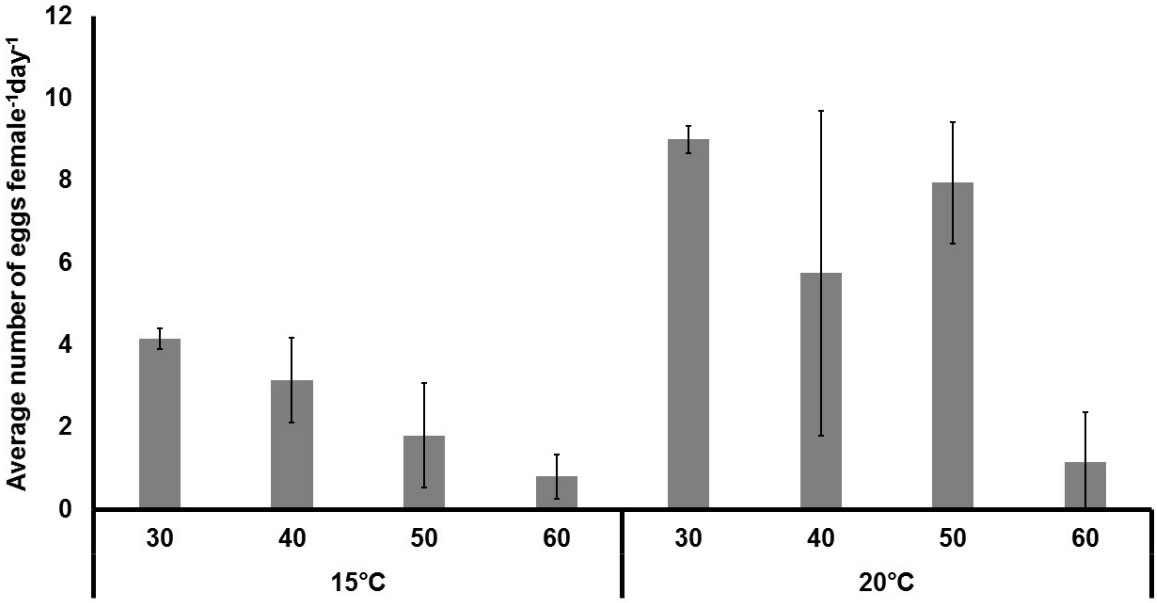

Figure 24: Average egg production per female at each salinity and temperature treatment. Error bars represent standard deviation.

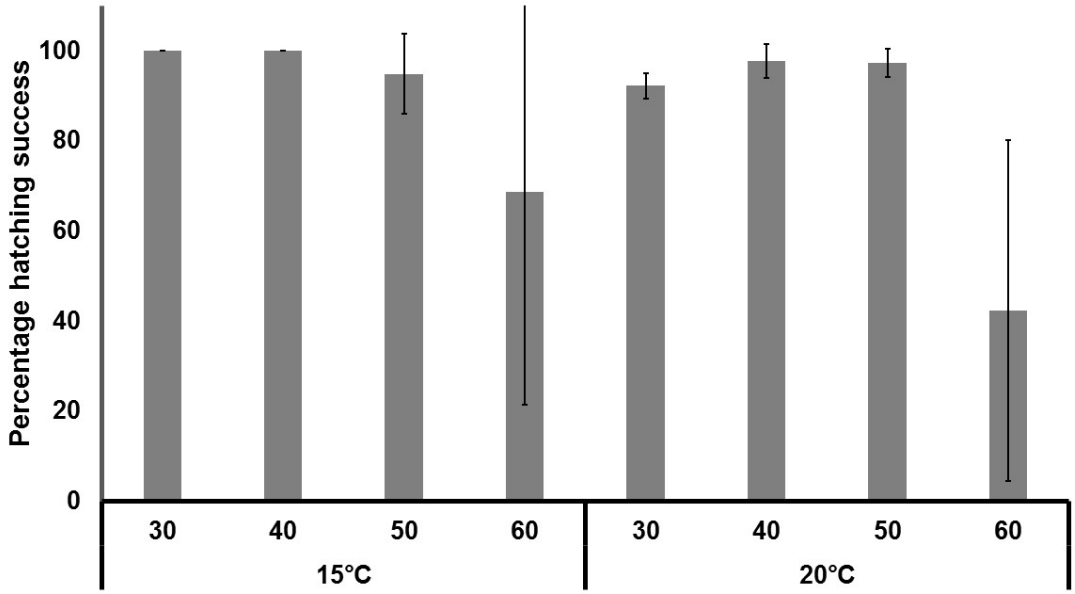

Figure 25: Average egg hatching success per treatment condition. Error bars represent standard deviation.

Overall average daily egg production was higher at 20 °C compared to 15 °C (independent sample t-test $P < 0.05$; Fig. 24). At 15 °C, daily egg production was significantly higher at salinity 30 (4.2 ± 0.3 eggs/female/day) compared to salinity 50 or 60 (1.8 ± 0.1.3 and 0.8 ± 0.5 eggs/female/day, respectively; One-way ANOVA $P < 0.05$). On the other hand, at 20 °C, the daily egg production was significantly lower only at salinity 60 (1.5 ± 1.2 eggs/female/day) compared to salinity 30, 40 and 50 (9.0 ± 0.3, 5.8 ± 3.9, and 7.9 ± 1.5 eggs/female/day, respectively; One-way ANOVA $P < 0.05$; Fig. 24). No significant difference was found in eggs hatching success across all treatments, except for salinity 60 at 20 °C, which was lower (One-way ANOVA $P < 0.05$; Fig. 25).

4.5. Discussion

The results of this study show a clear decrease in population size at high salinities, especially at 50 and 60. This corroborates with the natural distribution of *A. fancetti* in the Coorong, South Australia, as observed by Hemraj et al. (2017). In the present study, the highest population size and the number of nauplii, copepodites and adults, at both temperatures, were higher at salinity 30, except for copepodites at 15 °C. Salinity 60 consistently produced lower numbers of individuals of each stage (nauplii, copepodites and adults) at 15°C and no individuals were produced at 20 °C. This also aligns with the natural distribution of this species in the Coorong, where at salinities higher than 60, low abundances were observed (Hemraj et al. 2017). These results show a clear interaction between the effects of salinity and temperature on the population size and structure of *A. fancetti* in the Coorong. At lower temperatures and, especially, lower salinities, the population growth, in terms of total individuals produced over the 21 days, tended to respond positively. On the other hand, at higher temperature and salinities, there was a less pronounced increase in population size and high variabilities, as indicated by the coefficients of variance. These results suggest reduced and variable population growth under such conditions. Since the experimental conditions were controlled, part of such variability can be attributed to plasticity or adaptability of individuals to higher

temperature and salinity levels. As such, from the initial start-up cultures (6 males and 6 females per replicate), the high variability shows that not all individuals survived and reproduced successfully at high temperature and salinity. Moreover, at 20 °C, the dominant percentage contribution of copepodites (at salinity 50) and the lower number of individuals, suggest that the development of individuals is relatively slower compared to other treatments. This is likely an adverse effect of hypersalinity and heat stress on the development of younger individuals (Devreker et al. 2004; Pan et al. 2016). Similarly, several authors have shown an impairment in the development of younger stages (especially nauplii) when exposed to suboptimal salinities and temperatures (Anzueto-Sánchez et al. 2014; Cook et al. 2007; Devreker et al. 2007; Garzke et al. 2015; Pan et al. 2016).

The egg production experiment showed higher number of eggs per female per day at 20 °C. This corroborates several studies that have shown higher eggs production at higher temperatures (Hansen et al. 2010; Holste and Peck 2006; Milione and Zeng. 2008). Hatching success was not significantly different across treatments, except for salinity 60 at 20°C. These results are somehow unexpected, considering the higher population sizes and number of nauplii at 15 °C in the population experiment. A likely explanation for this could be associated with the results from Fitzer et al. (2012) who have shown a decrease in growth and cuticle composition but a higher naupliar production under acidity stress. These authors suggested a preferential reallocation of energy resources towards reproductive output. Similarly, our results suggest increased reproductive output under higher thermal stress. However, at a high salinity (60), it is likely that the level of osmotic stress inhibits eggs production and hatching to a very large extent. Moreover, in relation to the results from the population experiment, it would seem that, although reproductive output is relatively higher at 20 °C, osmotic and thermal stresses inhibit population growth. Nonetheless, further investigation into the effects of hypersalinity and temperature on egg latency, individual development and multigenerational development is likely to provide a better understanding on the mechanisms involving reproductive output for this.

The level of response to abiotic derived stress varies between copepod species. Typically, response to stress involves energy reallocation towards the mechanism that deals with regulating physiological characteristics (Aguilera et al. 2013; Fitzer et al. 2012; Thor et al. 2015; Vehmaa et al. 2012). In most cases, salinity stress in copepods involves an energy imbalance, where more energy is required for osmoregulation (Lee et al. 2017). Several studies have shown significant physiological changes from gene expression to reproductive output, relating to salinity derived stress. For example, Lee et al. (2017) have shown significant alterations in development, fecundity, fatty acid synthesis, lipid accumulation and variation in stress response proteins in the copepod *Paracyclopina nana* resulting from energy reallocation due to salinity stress. Moreover, Ibrahim et al. (2016) have demonstrated substantial modification in cuticle and muscle structure of *Pseudodiaptomus marinus* resulting from salinity and temperature stresses. Finally, Devreker et al. (2009), Pan et al. (2016) and Peck et al. (2015) have shown clear differences in reproductive and recruitment response, including egg production, development, survival and population growth of copepods. Our results suggest an increased stress in relation to hypersalinity in *A. fancetti* population. The reduction in population size, number of nauplii, copepodite development and egg production at high salinities suggest a decrease in reproductive output and survival in hypersaline conditions. Nonetheless, this population of *A. fancetti* from the Coorong demonstrates some level of phenotypic plasticity and adaptability to hypersalinities up to 50. Although direct comparisons of population growth to other populations of the species are not made here, this species has only recently been recorded in the Coorong, which is the first record of its existence in hypersaline waters.

Temperature has a major influence on metabolic activities, and metabolism is closely linked to growth of living organisms (Ikeda et al. 2001). Copepods, especially those inhabiting estuaries in temperate latitudes, encounter substantial seasonal fluctuations in temperature, which influence various parts of their life cycle and population dynamics. Such changes occur in relation to less than optimum temperatures, whereby organisms undergo stress responses

to cope with different intensities of either hyperthermia or hypothermia. Similarly to haline related stress, response to thermal stress involves energy reallocation and increase in energy demand. For example, Castellani et al (2005) have measured respiration rates of copepod *Oithona similis* and showed a significant increase in energy demand of 3% and 32% body-C/day at temperatuires of 4 °C and 25 °C, respectively. Several studies have also demonstrated up-regulation of genes for the production of heat shock proteins, such as HSP70, HSP90 and grp78, as a function of thermal stress response (Rahlff et al. 2017; Schoville et al. 2012). Such changes in energy allocation to different physiological mechanisms alter processes involving reproductive output, development and growth (Fitzer et al. 2012).

Interestingly, Kelly et al (2016) have recently shown that increased physiological tolerance to certain levels of thermal stress correlated with a decrease in plasticity in *Tigriopus californicus*. Their results, therefore, suggest a reduction in adaptability to wider ranges of thermal traits, as restricted by decreased plasticity. Our results partially confer with this, as shown by a lower number of nauplii at 20 °C compared to 15 °C, and higher population size at 15 °C compared to 20 °C, throughout all salinity treatments. This suggests that this species is more adapted to lower temperatures, and that a 5 °C increase causes noticeable negative influence. However, in our study, although temperature and salinity imposed some level of independent influences on the population, it is more likely that population growth, size and structure were multi-stressed under a combined influence of salinity and temperature. Our results show an exacerbated negative effect of salinity at higher temperature, whereby fewer or no individuals were observed at salinities 50 and 60 at 20 °C. Nonetheless, significant population sizes were observed at hypersalinities at 15 °C. These results suggest that the population of *A. fancetti* from the Coorong is more tolerant to hypersaline conditions at lower temperatures and that at high temperatures, the combined haline and thermal stress diminishes its ability to survive.

A. fancetti is a neritic species identified in South Eastern Australia, mainly in Westernport and Port Phillip Bays (McKinnon et al. 1992). It has recently been identified in the hypersaline

areas of the Coorong (salinity 40 – 65) and recognised as an important indicator species for the health of this system (Hemraj et al. 2017). The Coorong is an estuarine and hypersaline coastal lagoon undergoing recovery from severe health degradation (Hemraj et al. 2017; Lester and Fairweather 2009). The water quality of the Coorong is influenced by freshwater input that is anthropogenically regulated by barrages and salinity levels in the hypersaline area generally varies from 40 to 60 in colder months and 50 to 95 in warmer months (Hemraj et al. 2017). In regards to the salinity variations in the Coorong, the results of this study suggest that *A. fancetti* population likely reaches maximum in a relatively short period during colder months when salinities are lower and there are reduced heat stress. Moreover, during warmer months, individuals are unlikely to survive due to increased haline and thermal related multi-stressors, and may persist as diapause eggs until environmental conditions are favourable (Baumgartner and Tarrant 2017). Finally, due to these stresses, there is likely to be a shift in habitat, whereby populations may increase, or decrease, in relation to water quality fluctuations in different areas of the Coorong.

CHAPTER 5

ANTHROPOGENIC SHIFT OF PLANKTON FOOD WEB STRUCTURE IN A COASTAL LAGOON BY FRESHWATER FLOW REGULATION

This chapter has been published in the journal Scientific Reports.

Reference:

Hemraj, D.A., Hossain, A., Ye, Q., Qin, J.G. and Leterme, S.C., 2017. Anthropogenic shift of planktonic food web structure in a coastal lagoon by freshwater flow regulation. *Scientific Reports*, *7*, p.44441.

5.1. Abstract

Anthropogenic modification of aquatic systems has diverse impacts on food web interactions and ecosystem states. To reverse the adverse effects of modified freshwater flow, adequate management of discharge is required, especially due to higher water requirements and abstractions for human use. Here, we look at the effects of anthropogenically-controlled freshwater flow regimes on the planktonic food web of a Ramsar listed coastal lagoon that is under recovery from degradation. Our results show shifts in water quality and plankton community interactions associated with changes in water flow. These shifts in food web interactions represent modifications in habitat complexity and water quality. At high flow, phytoplankton-zooplankton interactions dominate the food web. Conversely, at low flow, bacteria, viruses and nano/picoplankton are more dominant and there is a substantial switch towards heterotrophy. This can be associated with excess organic matter loading, decomposition of dead organisms, and synergistic and antagonistic interactions. We suggest that a lower variability in flow amplitude, along with water discharge over longer time period could be more beneficial for sustaining water quality and plankton interactions seasonally, while improving ecosystem health of systems facing similar stresses as the Coorong.

5.2. Introduction

Direct modification of aquatic systems by anthropogenic pollution, or development, has caused diverse impacts on ecosystem functioning and food web interactions (Estes et al. 2011; Wollrab et al. 2012). Some of the most important modifications have involved bottom-up or top-down regulated trophic cascades, significantly influencing trophic interactions in food webs (Möllmann et al. 2008; Wollrab et al. 2012). Such alterations are often followed by non-equilibrium and degradation (i.e., system recovery is slower than the frequency of disturbance) in ecosystems, therefore, creating instability in ecosystem state (Lester and Fairweather 2009; Hudon et al. 2014). Unstable systems are often more susceptible to further deterioration in overall ecosystem functional integrity due to quick shifts in environmental conditions and climate change (López et al. 2013; Watson et al. 2013).

In aquatic systems, the microbial loop plays a highly important role in the regulation of nutrient and carbon cycling, as well as bottom-up control of the food web in an ecosystem (Kent et al. 2006; Sanders et al. 2015; Àvila et al. 2016; Schmoker et al. 2016). Primary producers are the base of the food web and are therefore closely linked to environmental changes, especially nutrient loading and salinity variations (Pickney et al. 2001; Caffrey et al. 2007; Riemann et al. 2016). Sudden changes in water quality, such as high nutrient loading, or temperature, are known to shift aquatic food webs by increasing bacteria, viruses and nano/picoplankton activities and biomass (Caffrey et al. 2007), more often causing harmful eutrophic environments than boosting productivity along the food web.

Consequences of food web alterations can be severe for ecosystems, and even more so for those in a non-equilibrium that require longer recovery time. Moreover, food web alteration can affect ecosystem functions, thus weakening both food web diversity and economic output (Braat and de Groot 2012). Such situations are typical of many estuaries, where coastal development and pollution have altered ecosystem functioning (Barbier et al. 2011), and is especially true for the Coorong, South Australia. The Coorong is a shallow saline coastal lagoon of approximately 110 km long which provides a range of commercial and recreational

ecosystem services (Colloff et al. 2015). It is situated at the end of Australia's largest river basin, the Murray-Daling basin, and is part of a Ramsar listed wetland area (Ramsar Convention on Wetlands, an intergovernmental treaty for the conservation and adequate use of wetlands; www.ramsar.org) called the Coorong, Lower Lakes and Murray Mouth region. The system is only connected to the sea at the mouth of the Murray River and the main source of freshwater is through barrages built to prevent seawater intrusion into the lakes and to regulate freshwater input into the Coorong (Dittmann et al. 2015; Leterme et al. 2015).

Freshwater is released into the Coorong in high volumes around colder months and in much lower volumes around warmer months. However, water discharge from the lakes is controlled primarily for the benefit of human use and is dependent on water levels in the lakes. From 2002 to 2009, the system was affected by a severe drought where no water was released and the floral and faunal communities were severely negatively affected (Paton and Bailey 2013; Dittmann et al. 2015; Leterme et al. 2015). Moreover, the ecosystem health of the Coorong was significantly degraded (Lester and Fairweather 2009). Since 2010, freshwater release has resumed, but the system remains relatively unstable and under recovery. Moreover, the salinity levels in the southern part of the lagoon remains hypersaline. The availability of freshwater for the Coorong is variable because of natural seasonal fluctuations, but also because of significant water extraction higher up the Murray River for human use. Therefore, the adequate management of water available for release into the system is of high importance for improving the rate of recovery, biodiversity and overall ecosystem health.

In this study, we investigate the effects of freshwater water release on the planktonic food web of the Coorong, to understand the effects of fluctuations in flow regimes on the overall food web interactions of coastal lagoons. We first look at the communities present in the system along water quality gradients and then examine changes in community interactions to provide an insight into the understanding of the microbial and zooplankton food web, based on flow regime. Due to the high difference in water release into the system, we hypothesise that the difference in flow regime will cause shifts in trophic interactions. With climate change

increasing environmental variability, managers will be challenged to balance freshwater needs of humans and wetlands (Middleton and Souter 2016). This study uses a food web network approach rather than single trophic levels to gain better understanding of ecosystem functioning. This approach was used for the first time on microbial and zooplankton communities in estuaries and coastal lagoons, and it provides more applicable information for government agencies to manage water flow and ecosystem health of coastal lagoons such as the Coorong.

5.3. Materials and Methods

5.3.1. Study area

The Coorong is separated from the ocean by sand dunes and the only connection to the sea is at the Murray Mouth (Fig. 26). A natural shallow and narrow channel is present at Parnka Point that separates the system into two lagoons, the North and South lagoons. From November 2013 to October 2014, monthly samples were taken at six sites along the Coorong. Sites 1 to 3 were situated in the North lagoon and Site 4 was situated at Parnka Point, while Sites 5 and 6 were in the South lagoon.

5.3.2. Water quality and environmental parameters

Salinity (PSU), pH, temperature (°C) and turbidity (NTU) were measured using an AquaRead multi-parameter probe (Aquaprobe AP-800). Dissolved oxygen (DO) was measured using a Thermo Scientific portable meter (Orion Star A323 RDO/DO). Triplicate water samples (10 mL) were collected at each site for the determination of dissolved inorganic nutrients concentrations including silica (SiO_2), ammonia (NH_3), orthophosphate (PO_4^{3-}) and nitrate/nitrite (NO_x^-) and analysed following Leterme et al. (2015).

Flow data for the barrages and Salt Creek were sourced from the WaterConnect website (www.waterconnect.sa.gov.au) and from the Department of Environment, Water and Natural Resources (DEWNR).

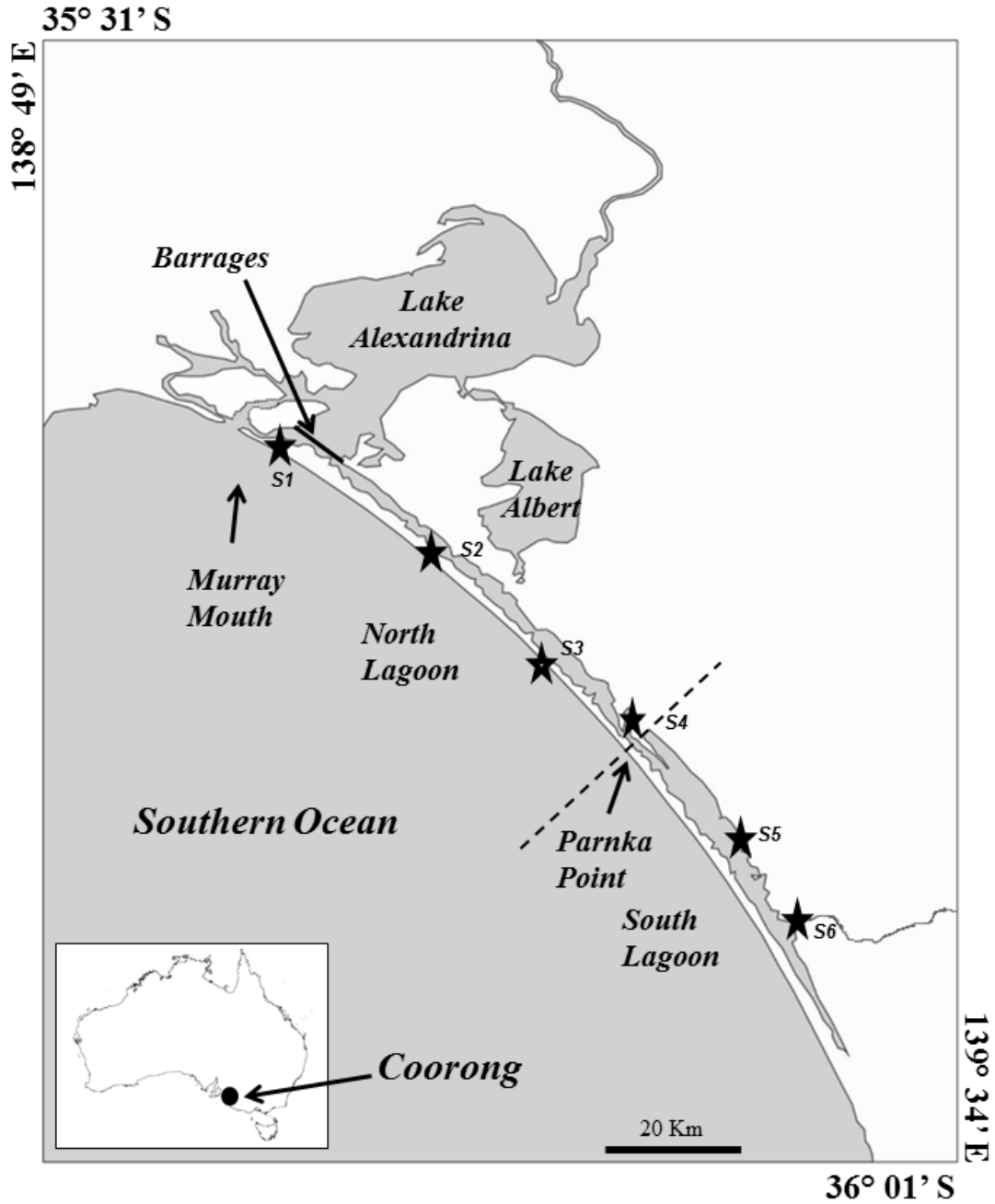

Figure 26: Map of the Coorong and Lower lakes showing lagoons separation and the Murray Mouth. Sampling sites are represented by stars. Basemap was created using ArcMap (version 10.3.3; www.arcgis.com) and Coreldraw X7 (http://www.coreldraw.com/en/product/technical-suite-education-edition/).

5.3.3. Bacteria, viruses and nano/picoplankton

Triplicate samples of 1 mL were taken at each site for the analysis of bacteria, virus and nano/picoplankton populations. Bacteria and virus samples were preserved with 2 % glutaraldehyde and nano/picoplankton samples were preserved with 12.5 % paraformaldehyde. Samples were frozen in liquid nitrogen and stored at -80 °C until analysis. The analysis of samples were carried out using a BD FACScanto 2 flow cytometer following the protocol by Marie et al (2010).

5.3.4. Phytoplankton and zooplankton

Phytoplankton samples were collected using a Niskin bottle and preserved in 5% Lugol's solution. Identification and enumeration of samples were carried out by microscopy at Microalgal Services in Ormond, Victoria as described by Leterme et al. (2015). Zooplankton samples were collected using a modified Schindler-Patalas trap. Samples were preserved in 2 to 5 % formaldehyde. Identification to the lowest taxonomic level and enumeration were carried out by inverted microscopy.

5.3.5. Statistical analysis

Data analysis was carried out using SPSS and Primer + Permanova. All data were tested for normality prior to analysis. Correlations coefficients between biotic and abiotic data were calculated using Spearman's correlation. Significance in difference in data were tested using Kruskal-Wallis one-way ANOVA. A Principal Component Analysis (PCA) was used to explore the water quality parameters contributing to the differentiation between the North and South lagoons. Biotic data were log (X+1) transformed and a Distance Based Redundancy Analysis (dbRDA) was used to explore the effects of environmental parameters on differentiating the planktonic communities along the system. A Spearman's correlation threshold of 0.6, was applied to plot the most highly correlating variables. Moreover, two separate dbRDAs were

used to look at the community of the North and South lagoons, separately, in relation to barrage flow. Finally, a BEST (BIOENV) test was used to identify the most important environmental parameters influencing the plankton communities in the North and South lagoons separately.

Analysis of species interaction in the planktonic food web was carried out by network analysis using the free software package Cytoscape (http://www.cytoscape.org/; Shannon et al. 2003; Kara et al. 2013). Networks were created to identify community interactions under a high or a low freshwater input into the Coorong, using Bray-Curtis similarity. A threshold of 100,000 GL was used to differentiate between high and low freshwater inputs. Also, a Bray-Curtis similarity threshold of 0.6 was used for all networks to capture the more significant community interactions. The threshold allowed for the exclusion of outliers. Analysis of network was done using the Network Analyser application (Assenov et al. 2008). Closeness centrality and edge-betweenness were implemented in the layout of network (Closeness centrality and edge-betweenness are network specific). Closeness centrality denotes the probability of a node to be functionally relevant for several other nodes, therefore can be central for the regulation of other nodes. Closeness centrality values range from 0 to 1, where 0 is low and 1 is high. Edge-betweenness denotes the importance of the interaction between two nodes for the network functional organisation. The higher the edge-betweenness, the more important the interaction is for organising other processes in the network (Newman 2007; Scardoni et al. 2009).

5.4. Results

Over the study period, monthly freshwater discharge pulsed from 428,472 ML/Day to 18,376 ML/Day. In the North and South lagoons of the Coorong (Fig. 26) salinity and nutrient levels were the main environmental parameters driving the chemical and biological processes. Flow through the barrages was an important forcing factor of both salinity and nutrient levels. In particular, flow levels were negatively correlated to salinity (Spearman's ρ: - 0.164, $p < 0.05$),

especially in the South lagoon (Spearman *rho*: - 0.375, $p < 0.05$), and NH_3 (Spearman's ρ: - 0.329, $p < 0.05$), and positively correlated to NO_x^- (Spearman's ρ: 0.309, $p < 0.05$). Additionally, the North and South lagoons were significantly different in terms of chemical composition (Fig. 27). The North lagoon varied from low brackish to high marine salinity depending on barrage flow, and distance from the barrages and Murray Mouth. The North lagoon also had significantly higher PO_4^{3-} and NO_x^- levels than the South lagoon (Kruskal-Wallis one-way: $p < 0.05$). Moreover, higher spatial and temporal variability in water quality was observed. On the other hand, the South lagoon was hypersaline throughout the year and it reached up to 98 around summer and autumn. It was also characterised by higher turbidity, ammonia levels and total phytoplankton biomass (Kruskal-Wallis one-way: $p < 0.05$).

Different plankton communities were observed between the North and South lagoons of the Coorong (Fig. 28), with water quality being the main driving factors for changes in the communities (Fig 28). In both the North and South lagoons, barrages flow, salinity and temperature were the most important contributors to the fluctuations in overall plankton community (BEST test, $\rho > 207$ and > 0.462, respectively).

In particular, plankton abundances significantly increased with increasing salinity along the system (Spearman's $\rho > 0.400$, $p < 0.05$), except for zooplankton. In addition, bacteria, virus, nano/picoplankton, *Prochlorococcus* and *Synechoccoccus* total abundances were positively correlated to NH_3 levels (Spearman's $\rho > 0.280$, $p < 0.05$), however, phytoplankton biomass was not correlated to nitrogen loading from barrages release. Finally, there was a substantial difference in community structure in relation to barrages flow in both the North and the South lagoons (Fig. 29).

Food web network structures were considerably different between the two lagoons, and especially the community interactions between high and low freshwater input regimes Fig. 30 and 30). In the North lagoon, at high freshwater input, the central part of the plankton food web (Bray-Curtis > 0.6) was driven by phytoplankton-zooplankton interactions (Fig. 30a).

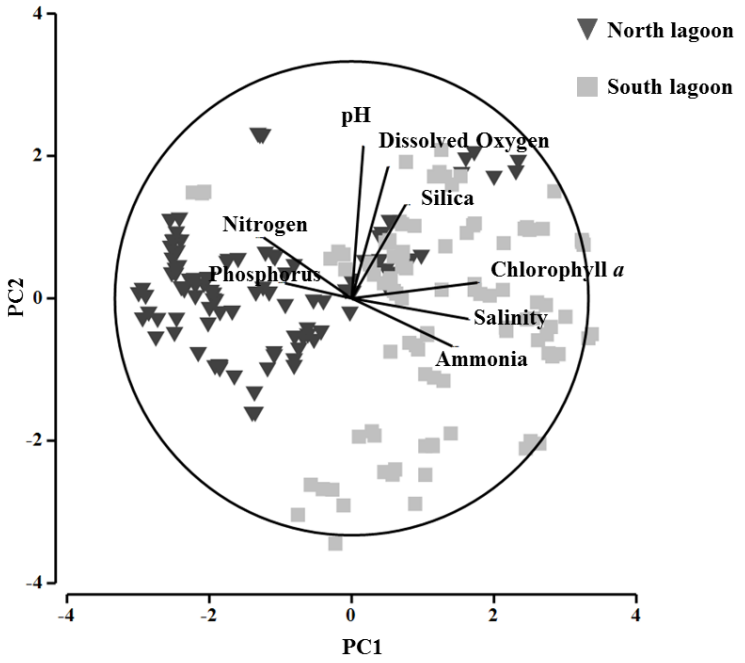

Figure 27: Principal Component Analysis of water quality parameters of the Coorong showing differentiation between the North and South lagoons. The vectors represent the highest contributing parameters (Spearman's correlation $\rho > 0.2$).

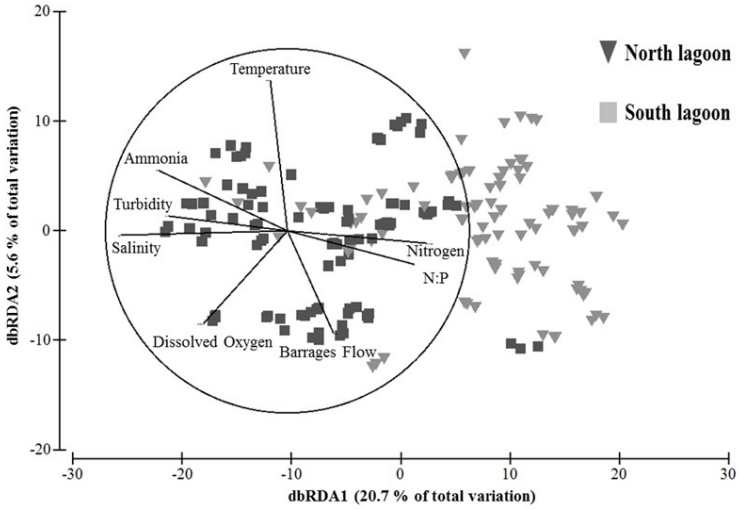

Figure 28: Distance Based Redundancy Analysis of plankton community differentiation in relation to water quality changes across the Coorong. The vectors represent the highest correlating water quality parameters (Spearman's correlation $\rho > 0.6$).

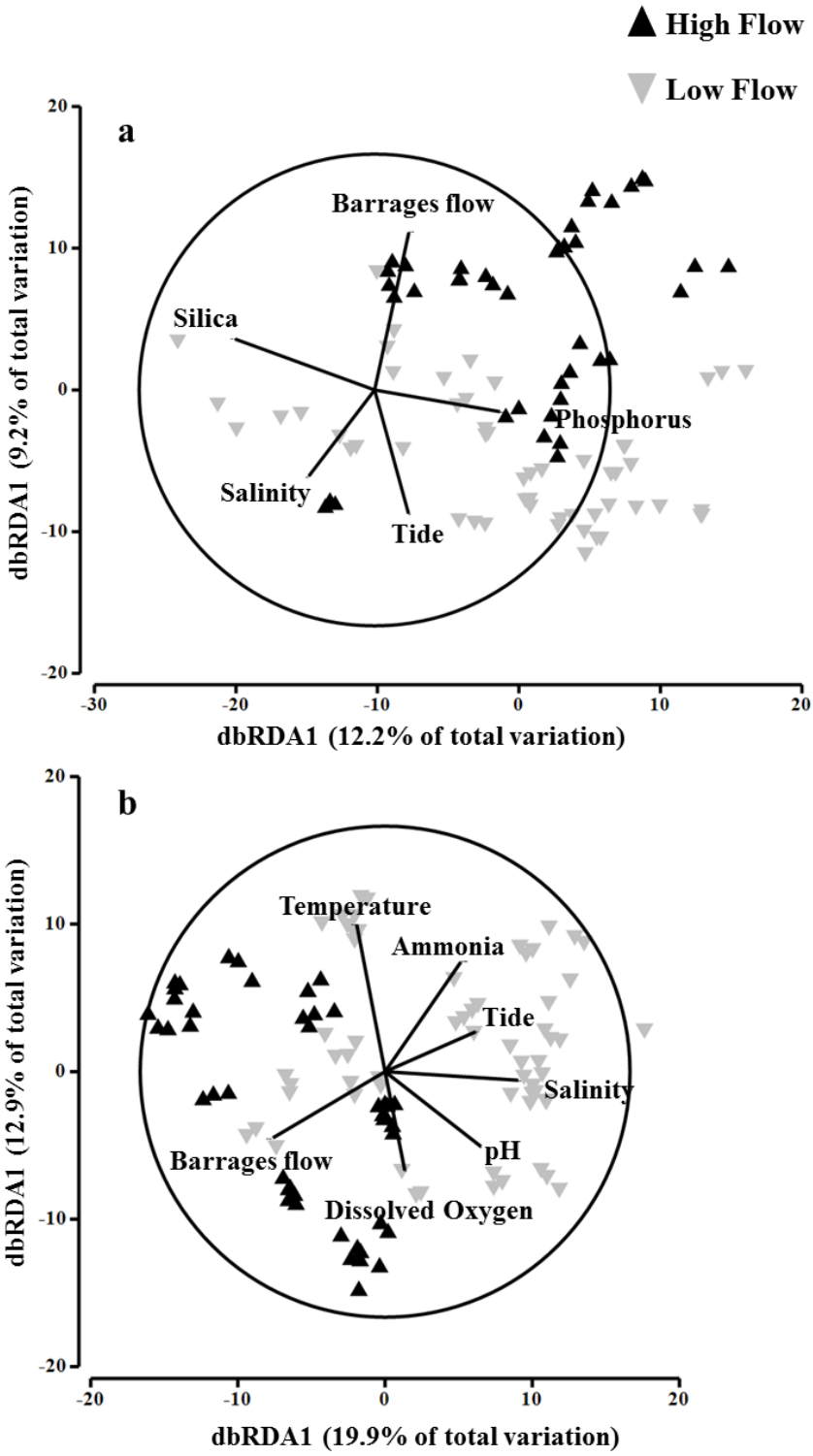

Figure 29: Distance Based Redundancy Analysis of plankton communities in the (a) North lagoon and (b) South lagoon in relation to water quality parameters and barrage flow as factors. The vectors represent the highest correlating water quality parameters (Spearman's correlation $\rho > 0.3$).

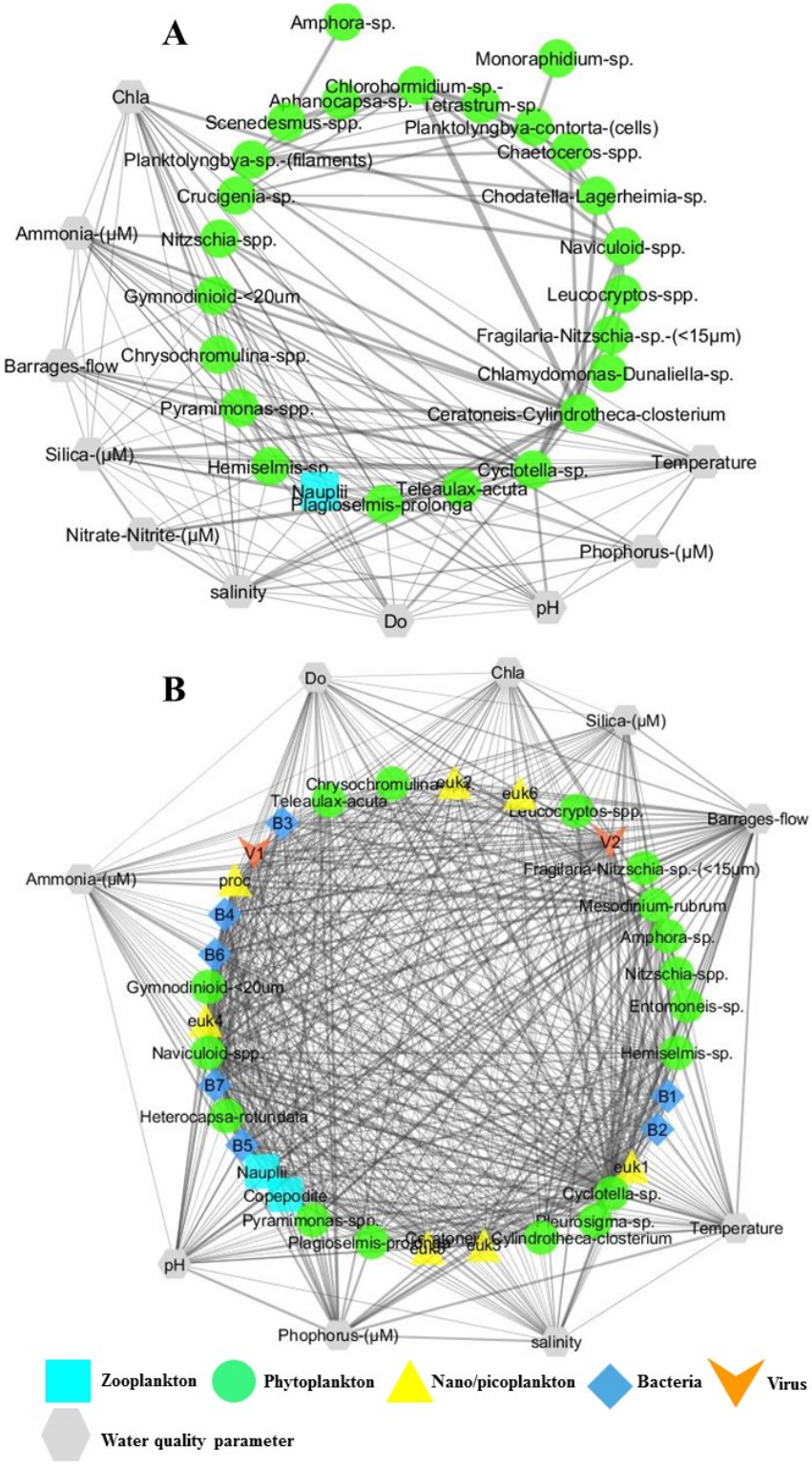

Figure 30: Network of water quality and plankton interactions for the North lagoon of the Coorong under high (a) and low (b) barrage flow. Edges are undirected and the thickness represent edge-betweenness centrality. A Bray-Curtis similarity threshold of 0.6 was applied to capture the most significant interactions.

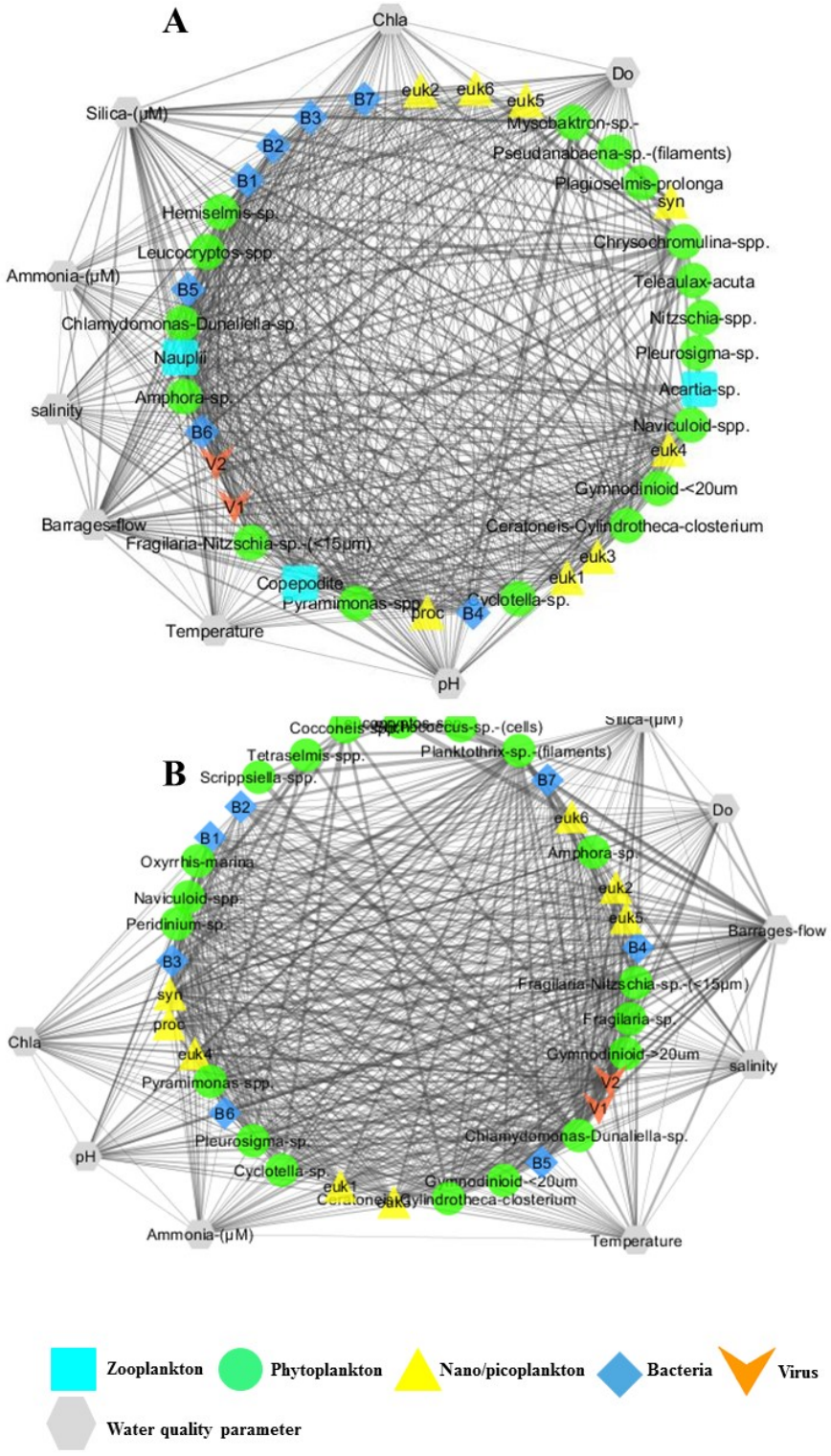

Figure 31: Network of water quality and plankton interactions for the South lagoon of the Coorong under high (a) and low (b) barrage flow. Edges are undirected and the thickness represent edge-betweenness centrality. A Bray-Curtis similarity threshold of 0.6 was applied to capture the most significant interactions.

Moreover, a large number of species were involved (~ 50% of total number of species observed in the North lagoon throughout the year), all part of either phytoplanktonic or zooplanktonic groups. Closeness centrality values were generally low and spread (0.2 – 0.6), showing no major control of a particular species on the network. However, copepod nauplii were highly linked to several phytoplankton species (Clustering coefficient: 0.91; Steele et al. 2011). Co-occurrence and/or competition for resources between phytoplankton species was also prominent as it's shown by a large number of linkages between several phytoplankton species in the network. As edges in the network were undirected, the inference of the exact type of interaction is not possible. At low freshwater flow, a significant shift in plankton web interactions was observed (Fig. 30 b). Virus, bacterial and nano/picoplanktonic groups were major interactors in the network (~ 50% of nodes). Closeness centrality values were also high (0.35 – 0.87) with virus, bacteria and nano/picoplankton being integral for the network (Closeness centrality > 0.76).

On the contrary to the North lagoon, at high flow in the South lagoon, the main interactions initially involved bacteria, viruses and nano/picoplankton communities (Fig. 31a) However, copepod nauplii, copepodite and several phytoplankton species (*Ceratoneis* sp., *Hemiselmis* sp., *Pyramimonas* sp., and *Naviculoid* spp.) still played the central role in the functioning of the network in the South Lagoon (Closeness centrality > 0.81; Network Closeness centrality values: 0.34 – 0.84). On the other hand, at low flow, similarly to the North lagoon, the food web shifted towards bacteria, viruses and nano/picoplankton dominated interactions (Fig. 31b).

The Closeness centrality values of all bacteria, viruses and nano/picoplankton were over 0.86 (Network Closeness centrality values: 0.48 – 0.89). Moreover, no involvement of zooplanktonic organisms was detected in the network.

5.5. Discussion

Freshwater flow from the barrages is highly important and has a significant impact on the chemical and biological properties of the Coorong. It greatly reduces salinity and NH_3 levels by encouraging flushing out of the system. Direct flushing mostly occurs for water between the barrages and the Murray Mouth (Webster 2010). Most of the flushing for the entire system, however, is a consequence of scouring of the Murray Mouth at higher barrage flow, which then facilitates sea water intrusion and increased water levels, enhancing exchange and mixing between the South and North lagoons (Webster 2010). Our results show that the high peak and low trough in freshwater flow through the barrages is highly influential on the changes in water quality. High flow contributes to nitrogen loading, especially in the North lagoon. Nutrient loading into a system alters the balance of nutrient flux and is often linked with blooms of algal species (Schmoker et al. 2016). In the Coorong, however, neither biomass nor abundance of phytoplankton were observed to significantly increase with higher freshwater flow regime and direct nitrogen loading. Excess nitrogen loading through high flow from the barrages increases the nitrogen to phosphorus ratio and intensifies phosphorus limitation, which is likely to prevent excessive algal biomass in periods of higher flow. Moreover, no contribution from benthos phosphorus to the phosphorus levels in the water column was observed by Haese et al. (2009). This corroborates Aldridge & Brookes' observations that in the Coorong, phosphorus was low compared to nitrogen in relation to the nutrient requirements of Coorong organisms (Aldridge and Brookes. 2011). Phosphorus limitation during high flow regime, as well as temporal and spatial variations in phosphorus levels, would explain the high importance of fluctuations in nitrogen to phosphorus ratio on driving the plankton community in the North lagoon. Moreover, nitrogen input from barrages does not seem to have an immediate effect on plankton but a rather accumulative and long term effect in case of low flushing out rate.

The South lagoon is composed of different chemical and biological characteristics compared to the North lagoon (Fig. 27). Salinity and NH_3 levels are the principal driving factors of plankton communities in the South lagoon. Salinity fluctuations in the South lagoon are

principally affected by freshwater flow from the barrages that forces exchange and reduces the salinity levels, whereas that of the North lagoon is also influenced by tidal exchange through the Murray Mouth. On the other hand, the high NH_3 levels are mainly derived from ground water seepage (Haese et al. 2009). However, dissimilatory nitrate reduction to ammonium (DNRA) by bacterial populations is also a likely important source. DNRA is an important nitrogen cycling pathway in aquatic systems such as estuaries and salt marshes, and is highly predisposed to a higher carbon to NO_3^- ratio, sulphide level, temperature and salinity (Song et al. 2014; Robertson et al. 2016). Such conditions are prevalent in the South lagoon, therefore presenting a highly suitable environment for DNRA. Moreover, bacterial degradation of organic matter is also a likely source (Clark et al. 2008). As it is shown by Figure 25, the prevalence of different environments in the Coorong drives the community change of plankton. Similarly, a split in phytoplankton communities, whereby diatoms dominated the South lagoon, was observed by Leterme et al. (2015). Moreover, a distance-driven prokaryote community in the system due to higher variations in water exchange around the Murray Mouth and North lagoon compared to further towards the South lagoon can be observed in the system (Blazano et al 2015).

Differences in plankton communities imply variations in food web structure. Figures 30 and 31 clearly represent the differences in food web structure between the North and South lagoons. The abrupt change in water quality, related to the variation from a very high flow to a low flow regime, significantly influences the plankton communities and seems to drive food webs towards heterotrophy (Fig. 30 and 31). In the North lagoon, freshwater input at high flow likely contributes to direct importation of high numbers of freshwater planktonic organisms. Moreover, it contributes to significant organic matter loading (Haese et al. 2009) and freshening of the lagoon, therefore modifying the environment (Aldridge and Brookes. 2011). High freshwater pulses have previously been shown to be linked to increased sedimentation, rapid salinity changes, displacement of organisms and shift in community structure (Sklar and Browder 1998; Alber 2002; Aldridge and Brookes 2011). Sudden changes to the environment,

and to water quality, also create modification in habitat complexity and biotic communities, therefore modifying the degree of species interactions (Didham et al. 2007; Kovalenko et al. 2012). At high freshwater flow, in both North and South lagoons, the food web is primarily dominated by primary and secondary producers. However, once the flow decreases, shift in the species interaction can be observed, whereby the bacteria, viruses and nano/picoplankton become the major part of the web.

Higher involvement of bacteria, viruses and nano/picoplankton in the aquatic food web is often indicative of bottom-up control (Àvila et al. 2016) and heterotrophy. Moreover, a shift in food web structure can be linked to several processes including response to organic matter loading, bacteria related decomposition, synergistic and antagonistic bacteria and virus interactions, virus-induced prokaryote mortality and predation on prokaryotes by nanoflagellates (Steele et al. 2011; Di Poi et al. 2013; Chow et al. 2014; Tsai et al. 2015). For example, loading of organic matter has been shown to significantly decrease primary production and cause a shift towards heterotrophic production based food webs, highly influenced by heterotrophic nanoflagellates (Forsström et al. 2013). Moreover, a stimulation of heterotrophic interactions by loading of high amounts of dissolved organic matter, but no such effect with moderate loading, whereby primary production was still stimulated has been demonstrated (Sanders et al. 2015). Several other studies have shown changes in specific interactions between bacteria, viruses and nano/picoplankton due to changes in different environmental conditions (Steele et al. 2011; Chow et al. 2014; Tsai et al. 2015). Although organic matter loading is likely to enhance bacteria, viruses and nano/picoplankton activity, a very high flow is likely to cause substantial change towards heterotrophy, but also light limitation of phytoplankton and benthic algae (Sanders et al. 2015), therefore also reducing phytoplankton production. Our results also show that, along with changes in salinity and nutrients caused by freshwater discharge, water temperature has an effect on the plankton community interactions. Increased temperature contributes to higher growth rates of bacteria, viruses and nano/picoplankton and, therefore, contribute to the higher populations observed at low flow. However, although a reduction in

freshwater flow into the Coorong occurs around warmer months, the regulation of flow is independent of temperature, but dependent on water levels in the river and lakes. As these tend to be lower during warmer months, we argue that a combination of higher temperature, high organic matter loading and a quick change in water quality due to very low freshwater flow contribute to the change towards a highly heterotrophic system where bacteria and viruses are dominant.

Hydrologic alterations have affected estuaries and aquatic systems globally, especially through reduction of freshwater discharge (Poff et al. 2010). The reintroduction of higher freshwater flow is of significant importance for the conservation or restoration of affected ecosystems. However, management of adequate freshwater flow regimes is of equal importance and often neglected in favour of total volume (Poff et al. 2010). Sudden transition from low to very high inputs of freshwater, have been linked to changes in net ecosystem metabolism, leading to changes in ecosystem function (Russell et al. 2006; Bruesewitz et al. 2013). In the Coorong, freshwater release through the barrages is vital for flushing of high salinity and nutrient levels, however, as it's shown by the networks of species interactions, high and low freshwater inputs are linked to strikingly different food web structures due to the associated changes in environmental conditions. A reduced difference in amplitude between high and low freshwater flow regimes, which is sustained over an extended period of time, but yet reflecting seasonal fluctuations, is likely to be more beneficial to sustaining the ecosystem's health. We suggest that such conditions are likely to provide more mixing and flushing out of the system, as well as follow natural seasonal fluctuations in water levels and mixing. Moreover, an extended period of flow is likely to provide a transitional period of change from wet/cold season to dry/hot season, as well as better recovery from the effects of fluctuating water levels and quality. Alternatively, we suggest that a higher monthly minimum water discharge is also likely to increase mixing and flushing out of the system while still following natural seasonal water level fluctuations. This would also reduce the magnitude of stress related to water quality change. Similarly, several authors have argued that increased

water discharge could contribute to balance of processes at spatial and temporal scales in systems affected by altered flow regimes, but that each system is likely to behave differently (Bianchi et al. 2011; González-Ortegón et al. 2012). However, a higher monthly minimum water release volume would imply an increase in the total volume of water available for the Coorong, which is possibly harder to achieve due to increasing human demands. For the Coorong, although increased water discharge, in terms of a higher monthly minimum volume, is likely to be beneficial, the adequate management of the currently available water may be of similar, if not better, significance. Finally, the implementation of overall network analysis of species interaction in understanding variations in ecosystem function can be a significant tool for managing ecosystems that are anthropogenically modified.

Our results show that pronounced amplitude differences between high and low water discharges may not be beneficial in terms of ecosystem function and the health of the Coorong. Our suggested amendments to water discharge are likely to be highly beneficial for mixing, transitional periods and flushing out of the system, thus improving the recovery rate and ecosystem health of the Coorong. However, further study and monitoring should be encouraged, especially in relation to the hydrodynamic effects and change in overall community interaction that might follow. Finally, with global change influencing environmental variability, managing freshwater needs of humans and aquatic systems is likely to be highly complex. Therefore, adequate use and distribution of freshwater towards the health of aquatic ecosystems under freshwater flow restrictions, or stress, should be further looked into.

CHAPTER 6

GENERAL DISCUSSION

6.1. Overview and major findings

Planktonic organisms are integral parts of aquatic food webs and form its base by being the major primary and secondary producers. Several planktonic organisms are essential players in nutrient and carbon cycling in aquatic systems and are also highly responsive to changes in water quality. As such, plankton represents an invaluable source of information for understanding spatial and temporal changes in water quality, ecosystem functioning and ecosystem health. The overall aim of this study was to provide the first comprehensive account of the plankton communities and their ecological importance subject to the water quality fluctuations of the Coorong in order to understand the functioning of this system. This in turn would provide critical information to better manage the recovery process of the degraded ecosystem. To this end, planktonic indicator species, changes in zooplankton lifecycle (The two copepod species studied are from separate habitats and likely have different ecological niches. One is only observed in the North Lagoon and the other in the South lagoon.) and shifts in plankton trophic dynamics in response to fluctuations in water quality have been explored.

Several key results were found during this study. These are as follow:

1) The water quality in the Coorong was significantly different between the two lagoons (North and South). In the North lagoon, lower salinities were observed with higher concentrations of nitrate and phosphate. The South lagoon was highly saline and contained high concentrations of ammonia. However, seasonal fluctuations in water quality along the system were prominent, especially around Parnka Point and Noonameena where, during warmer months, these areas show water quality levels typical of the South lagoon.
2) Two distinct communities of phytoplankton and zooplankton were identified in the Coorong. The North lagoon had relatively higher phytoplankton taxonomic diversity compared to the South lagoon, which was highly dominated by diatoms and chrysophytes. The first comprehensive account of zooplanktonic organisms for the

whole Coorong was provided. Although there was high seasonal variability in species distribution along the system, they tended to be more abundant and diverse in the North lagoon. Moreover, the marine species *Acartia fancetti* was observed residing in hypersaline waters of the South lagoon.

3) Salinity and nutrient levels were the main influencers of plankton community changes. Both phytoplankton and zooplankton species richness correlated negatively to salinity, while phytoplankton biomass was positively correlated to salinity.
4) Based on the calculated indicator values, both positive and negative indicators of water quality and ecosystem health state were identified for the North and South lagoons. In the North lagoon, gastropod and polychaete larvae were found to be good positive indicators for water quality while the diatom *Rhizosolenia* spp. was representative of high salinity and ammonia levels in the North lagoon. On the other hand, in the South lagoon, *Acartia fancetti* adults were only present at moderate hyper salinity while the diatom *Cyclophora* sp. and the dinoflagellate *Scrippsiella* sp. were only present at very high ammonia and salinity levels. The distribution and abundances of these organisms are highly likely indicative of the water quality and habitat quality in the Coorong and, therefore, can be used as indicators for monitoring the health of the system.
5) In terms of salinity and pH levels, the copepod *Gladioferens pectinatus*, collected from the Coorong, showed developmental and recruitment response to pH levels at different salinities. At salinity 2, no individual developed into adults at lowered pH levels (> 7.77), while at salinity 10, there were no major effects of lowered pH (> 8.09). This shows that adverse responses to multi stressors may be more likely than negative responses to single stressors. Therefore, changes in water quality, such as that caused by high freshwater input, are likely to influence the lifecycle of these organisms, and others, in the Coorong.
6) The neritic copepod, *Acartia fancetti*, was identified in the hypersaline sections of the Coorong. The experiment examining the tolerance of this species to hypersalinity and temperature changes revealed that the species was adapted to certain levels of

hypersalinity. At lower temperatures, the species was able to reproduce in salinity up to 60. However, at higher temperatures, the ability of this copepod to reproduce and survive was significantly reduced with increased salinity. These results show a multi-stressor effect of salinity and temperature on the organism, indicating possible population changes in relation to freshwater release in the Coorong and seasonal changes in environmental factors.

7) The current anthropogenically controlled freshwater flow regime was shown to create significant shifts in water quality, habitat structure, plankton interaction and ecosystem function.
8) Bacteria and viruses were highly abundant in the South lagoon in comparison to the North lagoon, and were an integral part of the overall planktonic foodwebs. However, at higher freshwater flow in both parts of the system, the planktonic interactions network mainly involved primary and secondary production, including high numbers of phytoplankton and zooplankton interactions.
9) During the extended period of low freshwater flow in the Coorong, planktonic interaction networks were shown to be highly dominated by bacteria, viruses and nano/picoplankton interactions and the system shifted towards high heterotrophy. This shows that the current freshwater release regime in the Coorong is not highly beneficial to the recovery of the system. A prolonged period of high flow is required to initiate more flushing and mixing, maintain adequate water quality, and stimulate production.

6.2. Knowledge advancement and significance of each section

6.2.1. Chapter 2

The ecosystem health of the Coorong, which is part of Australia's largest river basin and a Ramsar listed wetland, has been degraded throughout the years by anthropogenic modification of its hydrodynamics and, especially by the extended period of severe drought

(2001-2010). There have been major changes in water quality, communities and productivity along the system, resulting from the environmental degradation. Improving the health of the system requires a clear understanding of the changes in water quality and how they link with biological communities and ecological processes. Planktonic organisms are closely linked to water quality and therefore are good natural indicators of changes in water quality and ecosystem health (Albaina et al., 2009; Amengual-Morro et al., 2012). Chapter 2 provided the first full description of zooplanktonic communities along the Coorong, particularly for the South lagoon.

Moreover, the seasonal variations in both phytoplankton and zooplankton community dynamics in relation to water quality were studied. Using both phytoplankton and zooplankton, this chapter was the first study to identify robust positive and negative indicators along the Coorong that directly indicate water quality, habitat and ecosystem health states along the system. These can be used as a significant tool to monitor and manage the changes in habitat quality and health of the Coorong. The methods and organisms studied can be used to monitor, predict and manage ecosystem health in other estuaries and different aquatic systems.

6.2.2. Chapter 3

Estuarine systems are highly complex in terms of physiochemical parameters. The most pronounced complexity involves the changes in salinity gradient along estuaries in relation to freshwater input and tidal forcing. This poses a significant level of stress on estuarine dwelling organisms and, therefore, affects their distribution, population dynamics and community structures. However, due to freshwater input and *in situ* respiration levels, estuaries are also likely affected by variations in pCO_2 and pH levels, yet the influences of these parameters have often been disregarded.

This study is among the first ones to examine the reproduction and recruitment of estuarine copepods in function of both salinity and pH stressors. It has shown that estuarine dwelling copepods are likely resilient to certain combinations of stressors, but are significantly negatively impacted by others. Moreover, the importance of understanding disparities in stress response among populations or species and inter- or intraspecific resilience, multigenerational adaptability or phenotypic plasticity in copepods, has been pointed out. Furthermore, this section stresses on the need to examine the cumulated effects of multi-stressors to further understand the response of organisms to naturally variability, anthropogenically influences, habitat changes or future changes in water parameters.

6.2.3. Chapter 4

Phenotypic plasticity and adaptability are common traits of many neritic and estuarine copepods. These organisms are often exposed to changes in environmental factors, especially salinity. Seasonal changes in environmental factors drive population and community structures of planktonic organisms depending on their ability to adapt to or tolerate changes. Anthropogenic-related interruption of natural seasonal variations changes the functioning of systems and, therefore, the population and community structures of organisms that reside in these systems. Such is the case for the Coorong. Few studies have explored the influences of a combination of hypersalinity and temperature on the population structure of copepods.

In this study, the influence of hypersalinity and seasonal temperature changes on the population, egg production and hatching success of *A. fancetti* was examined to understand the possible changes in population structure in relation to anthropogenically controlled freshwater flow that drives water salinity. This is the first study that examines the population structure of *A. fancetti*. The results revealed that the species is likely more abundant in the Coorong at lower salinities and lower temperatures, and is heavily negatively impacted by hypersalinity at higher water temperatures. This study shows the influences of multi-stressors

on copepod population structure and reproduction, and provides valuable information support freshwater flow management for the Coorong in order to maintain healthy copepod populations in the system. Moreover, similar population based studies can be applied for other estuarine systems to gain a better understanding of ontology and phenology of copepod populations in relation to changes in environmental stressors.

6.2.4. Chapter 5

Understanding and managing the recovery of a degraded system is unlikely achievable with limited knowledge of the system functioning and the processes involved. The interactions between organisms are highly indicative of ecological processes. This final chapter examined the global ecosystem functioning in relation to water quality and freshwater flow into the Coorong. It is the first study to apply network analysis of species interaction in estuarine systems to understand total community and interaction shifts in relation to water quality changes brought by regulated freshwater flow regime. As well as incorporating new methods, it has shown that the current flow regime is likely not enhancing the recovery of the Coorong. An extended period of low flow altered water quality and boosted microbial heterotrophic interactions throughout the system. This global change in ecosystem functioning indicates that a longer period of high flow rather than a pulse of high volume of freshwater is necessary to enhance the ecosystem health and increase primary and secondary production to support the foodweb along the Coorong.

6.3. Plankton trophic dynamics in estuaries and use of plankton for estuarine health management

Plankton foodwebs support trophic links within aquatic ecosystems. Changes in planktonic community structure and desynchronization between predator and prey interactionsinfluence the functioning of the overall ecosystem (Chevillot et al. 2017). Over the past few years,

plankton interactions and their effects on trophic dynamics and phenology have been subject to many research studies, especially in relation to climate change biology (Rice et al. 2015; Brown et al. 2016; Poloczanska et al. 2016; Marchese et al. 2017). However, very few studies have addressed the changes in trophic interactions and ecosystem functions resulting from anthropogenic modification of aquatic systems (Niquil et al. 2014; Tecchio et al. 2016). The close interaction between physical, chemical and biological processes in estuaries show the complexity of estuaries and the cascade effects that the modification of one of these processes can have on an estuarine ecosystem.

Using planktonic interaction studies to understand the effects of such modifications is particularly beneficial because planktonic organisms are highly responsive to water quality. Mapping the changes in plankton community interaction is likely to provide, both a rapid assessment of water quality, habitat structure and ecosystem health, and an indication of possible changes along higher trophic interactions of aquatic systems. This study shows that human related alterations in hydrodynamics and natural mixing significantly impact the plankton community structure and trophic linkages throughout the Coorong. The alterations in water quality and habitat structure resulting from variations in freshwater flow regime threaten organism recruitment and population structure, and change overall community structure and diversity. Moreover, these alterations shift foodweb interactions towards lower trophic levels, increasing heterotrophy, likely involving detritivory and carbon recycling.

Using plankton communities, this study has revealed the major changes in habitat structure and ecosystem functioning along a degraded estuarine system and provided useful information for improving the management of this system. Considering the importance of planktonic organisms in driving higher trophic linkages, this study also points out a lack of knowledge in the link between planktonic organisms and higher predators' recruitment and phenology, and most importantly, how these interactions are affected by freshwater flow and water quality. As such, it is suggested that future research in the Coorong and other estuaries should involve developing a comprehensive understanding of the connectivity between

changes in organisms' trophic linkages in relation to alterations in habitat structure, either naturally or, especially, from anthropogenic sources.

6.4. Estuarine ecosystem and Coorong health management

The management of estuaries and coastal lagoons is highly complex and involves the understanding of the various factors that influence system functioning. This study, although being focused on the Coorong, has presented a variety of methods and strategies that can be applied in various estuaries, and perhaps even other types of aquatic systems, around the world to gain a better understanding of how ecosystems function, and the processes and interactions that are involved. The Coorong has experienced ecological degradation since the system was modified in 1940, with the building of barrages to separate the Lower lakes, creating a major ecological barrier in connectivity and leading to a significant reduction in flow throughout the system. The impact was especially marked by a severe degradation between 2001 and 2010 due to an extended drought period in the Murray-Darling basin. Managing the recovery of the ecosystem health involves a variety of factors relating to policies on water availability, recreational use, commercial use, and ecological traits, among others.

This study focused on the changes in ecological characteristics and biological communities in relation to environmental fluctuations in the Coorong. The results show that the system is prone to significant seasonal changes in environmental parameters, community structure and ecosystem functioning throughout a year, related to both natural and anthropogenic factors. In relation to these fluctuations, this study has identified key organisms that indicate the health state of the system, examined changes in population dynamic and recruitment of organisms and provided a comprehensive understanding on the alterations in organism interactions and ecosystem functioning in relation to the management of water release into the Coorong. The results of this study indicate that increases in salinity, especially during warmer months, significantly affect the plankton population structures and communities throughout the Coorong. For example, as shown by chapters 4 and 5, increases in salinity and temperature during lower freshwater input considerably decreases the population size of *A. fancetti* and

also changes the overall plankton interactions. Moreover, the drastic seasonal changes in ecosystem functioning may not be entirely beneficial to the recovery of the Coorong. Therefore, modifications to the timing and volume of freshwater released into the system could reduce the drastic seasonal variability, promote and sustain larger populations of key copepods, especially during summer months, follow a more natural-like seasonal fluctuation and promote productivity throughout the Coorong.

6.5. Future Research

This study has provided some important insights into the plankton populations and community interactions of a complex estuarine system, in relation to environmental fluctuations. Nonetherless, it has also opened up pathways for further studies regarding changes in plankton communities in the Coorong and other estuaries, including the following:

1. Although freshwater release into the Coorong is highly important, there is a lack of knowledge of the extent of organic matter loading and gas flux along the system. These are key elements that influence changes in water quality and microbial communities. Further research should explore the changes in hydrology involved with freshwater release and its effects on the distribution of organic matter and humic substances along the system. Moreover, changes in gas flux in relation to organic matter loading, humic substances and environmental fluctuations can further be examined. Finally, the implementation of more sites along the system, especially in the North lagoon, is likely to give a more informative and precise data set.
2. Avenues for regulation of freshwater release have been suggested in this study based on changes in plankton community interactions. The study clearly shows a necessity of adjusting the freshwater release into the system and can be initiated by managers. More detailed research on the specific effects of adjusting water flow on water quality and community interactions is likely to provide an even more optimum water release

regime. This is likely to increase the ecosystem health recovery rate and maintain a healthy ecosystem state.

3. This study pointed out the importance of understanding the combined effects of multi-stressors onto planktonic organisms. Future research should further explore the effects of such multi-stressors on multi-generations to examine the accumulated stress or adaptations of planktonic organisms to changes in environmental parameters. Moreover, the influences of multi-stressors on the genotypes, gene expression and co-expression, and plasticity should be further explored to gain a better understanding of the the influences of environmental parameters on regulating physiological changes towards reproductive behaviour, feeding and growth of estuarine planktonic organisms.
4. This study applied network analysis to study the interactions between planktonic species in relation to environmental stresses throuthout the Coorong. Further research should also explore the energy pathways, incorporating benthic organisms and higher trophic levels (planktivorous fish, higher predators...etc) into the foodweb network to gain more in-depth knowledge on the changes of planktonic communities in relation to top-down influences and of the overall aquatic community in relation to environmental fluctuations and freshwater flow regimes of the Coorong and other estuaries. Moreover, methods including stable isotope analysis and, especially, DNA analysis of foodweb and energy pathways should be incorporated.

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APPENDIX

Table 1. List of phytoplanktonic organisms observed in the Goolwa Channel and the Coorong between November 2013 and October 2014.

(OTUs)	*Goolwa Channel*	*North Lagoon*	*South Lagoon*
Diatoms			
Acanthoceras sp.	x		
Achnanthes sp.		x	x
Amphora sp.	x	x	x
Anaulus australis		x	
Aulacoseira granulata		x	
Asterionellopsis glacialis	x	x	x
Ceratoneis/Cylindrotheca closterium	x	x	x
Chaetoceros spp.	x	x	x
Cocconeis spp.		x	x
Coscinodiscus spp.		x	x
Cyclophora sp.			x
Cyclotella sp.	x	x	x
Dactyliosolen sp.	x		
Encycoma sp.			x
Entomoneis sp.		x	x
Eucampia zodiacus		x	
Eunotia sp.			x
Fragilaria sp.	x	x	x
Fragilaria/Nitzschia sp. (<15µm)	x	x	x
Fragilariopsis sp.			x
Grammatophora sp.		x	x
Guinardia flaccida		x	
Guinardia striata		x	
Guinardia delicatula		x	
Gyrosigma spp.			x
Leptocylindrus danicus	x	x	
Licmophora sp.		x	x
Lioloma sp.		x	
Melosira sp.	x		
Minidiscus trioculatus		x	
Minutocellus sp.		x	
Minutocullus scriptus			x
Naviculoid spp.	x	x	x
Nitzschia spp.	x	x	x
Nitzschia sigmoidea		x	
Ondotella sp.	x		
Paralia sulcata		x	
Pleurosigma sp.	x	x	x

Pseudo-nitzschia delicatissima group		x	
Pseudo-nitzschia pungens/multiseries		x	
Pseudo-nitzschia multristriata		x	
Rhizosolenia spp.	x	x	
Rhizosolenia setigera		x	
Rhopalodia sp.			x
Skeletonema costatum/pseudocostatum	x	x	
Surirella sp.	x		
Thalassiosira cf. mala		x	x
Thalasiossira sp.	x	x	
Tabellaria sp.	x	x	
Thalasiosema sp.			x
Dinoflagellates			
Amphidimium sp.			x
Alexandrium pseudogonyaulax	x		
Alexandrium margalefi		x	
Cochlodinium sp.	x		
Diplopsalis lenticula			x
Diplopsalid sp.		x	x
Dinophysis acuminata		x	
Gonyaulax spp.		x	
Gymnodinioid <20um	x	x	x
Gymnodinioid >20um	x	x	x
Gyrodinium spp.	x	x	x
Heterocapsa spp.		x	x
Heterocapsa rotundata	x	x	x
Noctiluca scintillans		x	
Ostreopsis sp.	x		
Oxyrrhis marina	x	x	x
Peridinium sp.	x	x	x
Polykrikos schwartzii		x	x
Propocentrum triestinum		x	
Prorocentrum micans			x
Preperidinium meunieri		x	
Protoperidinium spp.		x	x
Scrippsiella spp.		x	x
Chrysophyte			
Apedinella spinifera			x
Dinobryon sp.		x	
Mallomonas sp.	x		
Paraphysomonas sp.		x	
Unidentified ochrophytes	x	x	x
Primnesiophytes			
Chrysochromulina spp.	x	x	x
Emiliana huxleyi		x	
Prymnesium patellifera			x
Cryptophyte			
Campylomonas reflexa	x	x	
Hemiselmis sp.	x	x	x

Komma sp.	x	x	
Leucocryptos spp.		x	x
Plagioselmis prolonga	x	x	x
Rhodomonas salina	x	x	x
Rhodomonas sp.		x	
Euglenophyte			
Eutreptiella spp.	x	x	
Ploeotia sp.			x
Chlorophyte			
Ankistrodesmus sp.	x	x	
Chlamydomonas/Dunaliella sp.	x	x	x
Chlorella sp.	x	x	x
Chlorohormidium sp.	x	x	
Chodatella/Lagerheimia sp.	x	x	
Closteriopsis sp.	x		
Crucigenia sp.	x	x	
Dictyosphaerium sp.	x	x	x
Dimorphococcus sp.	x		
Fusola sp.	x	x	
Keratococcus sp.	x	x	
Kirchneriella sp.	x	x	x
Koliella sp.	x	x	x
Monoraphidium sp.	x	x	x
Oocystis sp.	x	x	x
Pediastrum sp.	x	x	
Scenedesmus quadricaudus	x		
Scenedesmus spp.	x	x	
Schroderia sp.	x		
Staurastrum sp.	x	x	x
Stichococcus sp. (cells)	x	x	x
Tetrastrum sp.	x		x
Prasinophyte			
Nephroselmis sp.	x	x	
Pseudoscourfieldia marina		x	
Pyramimonas spp.	x	x	x
Tetraselmis spp.		x	x
Cyanoprokaryota			
Anabena sp. (cells)	x		
Aphanizomenon sp.	x	x	
Aphanocapsa sp.	x	x	
Aphanothece sp. (cells)	x	x	
Arthrospira sp. (cells)		x	
Chroococcus sp.		x	x
Cuspidothrix sp.	x		
Cylindrospermopsis raciborskii	x	x	
Gloeothece sp.			x
Nodularia spumigena	x	x	
Oscillatoria sp.		x	
Limnothrix sp.			x
Merismopedia sp.			x
Mysobaktron sp.		x	x

Planktolyngbya sp.	x	x	x
Planktolyngbya subtilis	x	x	
Planktolyngbya contorta (cells)	x	x	x
Planktothrix sp. (filaments)	x	x	x
Pseudanabaena limnetica	x	x	x
Pseudanabaena sp. *(filaments)*		x	x
Trichodesmium erythraeum (cells)		x	x
Other			
Actinomonad sp.			
Ebria tripartita		x	
Unidentified bodonids	x	x	
Unidentified heterotrophic flagellates		x	x
Amoeba sp.	x	x	
Mesodinium rubrum	x	x	x
Telonema subtilis			x
Picoplanktonic cells	x	x	x

Table 2: List of zooplanktonic organisms observed in the Goolwa Channel and the Coorong between November 2013 and October 2014. * organisms observed only once at Salt Creek (S7) in August 2014.

(OTUs)	*Goolwa Channel*	*North Lagoon*	*South Lagoon*
Protista			
Indet foraminifera	x	x	x
cf. *Noctiluca* sp.		x	x
Tintinnids	x	x	
Indet ciliate 1	x		
Indet ciliate 2	x		
Rotifera			
Branchionus novaezealandiae	x		
Cephalodella sp.	x	x	
Filinia australiensis	X		
Filinia pejleri	x	x	x*
Filinia saltator		x	
Filinia terminalis	x		
Keratella australis	x	x	x*
Keratella procurva	x		
Keratella tropica	x	x	
Testudinella sp.	x	x	x
Syncheata pectinata	x	x	
Syncheata sp.	x	x	x
Cladocera			
Bosmina meridionalis	x		x*
Ceriodaphnia sp.	x		
Daphnia lumholtzi	x	x	x*
Daphnia carinata	x		
Copepoda			
Nauplii	x	x	x
Copepodite	x	x	x
Acartia cf. *fancetti*			x
Boeckella triarticulata	x	x	x*
Calamocia ampulla	x		
Mesochra sp.	x	x	x
Quinquelaophonte sp.		x	x
Indet Cyclopoid 1	x	x	
Indet Cyclopoid 2	x	x	x
Ostracoda			
Indet Ostracod	x	x	x
Macroinvertebrate			
Indet Amphipod	x	x	
Chironomidae larvae			x
Mollusc larvae	x	x	
Indet Nematode	x	x	x
Indet Oligochaete		x	
Indet Polychaete larvae 1	x	x	
Indet Polychaete larvae 2		x	
Indet Decapod (crab) larvae	x	x	
Other			
Indet Appendicularia		x	

Table 3: Chemical composition of COMBO medium as per Kilham et al. (1998).

Compound	*Stock (g/L)*	*Final medium (mg/L)*
Seven major stocks		
$CaCl_2\ 2H_2O$	36.76	36.76
$MgSO_4\ 7H_2O$	36.97	36.97
K_2HPO_4	8.71	8.71
$NaNO_3$	85.01	85.01
$NaHCO_3$	12.60	12.60
$Na_2SiO_3\ 9H_2O$	28.42	28.42
H_3BO_3	24.00	24.00
KCL	7.45	7.45
Algal trace elements (ATE)		
$Na_2EDTA\ 2H_2O$	4.36	4.36
$FeCl_3\ H_2O$	1.00	1.00
$MnCl_2\ 4H_2O$		0.18
$CuSO_4\ 5H_2O$		0.001
$ZnSO_4\ 7H_2O$		0.022
$CoCl_2\ 6H_2O$		0.012
$NaMoO_4\ 2H_2O$		0.022
H_2SeO_3		0.0016
Na_3VO_4		0.0018
Animal trace elements (ANIMATE)		
LiCl		0.31
RbCl		0.07
$SrCl_2\ 6H_2O$		0.15
NaBr		0.016
KI		0.0033
Vitamins (VIM)		
B_{12}		0.00055
Biotin		0.0005
Thiamin		0.1