MONASH University

Linking flow, nutrients and fish: an integrated approach to estuary management

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A thesis submitted for the degree of *Doctor of Philosophy* at
Monash University in 2018

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Abstract

Coastal regions are the most densely populated areas worldwide and are subjected to increasing urbanisation and intensive agricultural activities. As a result, estuaries often receive reduced freshwater flows due to diversions for human use, and elevated nutrient inputs from anthropogenic activities. The impacts of such changes include estuarine habitat degradation, algal blooms, reduced food web productivity and declining fish populations. This thesis investigated how altered flows and nutrient inputs affected the physical structure and biogeochemical processes controlling the estuarine food web. Two field studies were undertaken in two estuarine systems of contrasting human impacts, both known spawning grounds for the black bream, *Acanthopagrus butcheri* (Munro) which are endemic to Australia. A laboratory bioassay experiment was also undertaken to examine how changes in bioavailable nitrogen (ammonium; NH_4^+ and nitrate; NO_3^-) concentrations influenced uptake, growth and abundances of phytoplankton in the two estuarine systems.

Fortnightly longitudinal studies of the Werribee estuary (40 km southwest of Melbourne, Australia) and the upper Mitchell estuary (280 km east of Melbourne) were undertaken in the springs of 2014 and 2015; respectively. In both studies, a range of water quality, nutrient, phytoplankton, zooplankton and fish larvae community data were collected. In the Werribee, reduced freshwater flows and high NO_3^- concentrations (10 to 220 µmol L^{-1}) resulted in minimal stratification, high residence times and algal blooms. An environmental flow release (EFR) alleviated these conditions, created a more stratified water column and increased productivity in the form of prey for *A. butcheri* larvae. This highlighted the importance of stratification, and provided preliminary information as to the timing and magnitude of EFRs to manage human impacts on the Werribee estuary.

In contrast, nutrient concentrations in Mitchell estuary were low (<5 μmol L^{-1}), and unregulated freshwater flows resulted in the periodic removal of the salt wedge during high flows. Salt wedge intrusion was found to facilitate benthic nutrient recycling and NH_4^+ accumulation, which promoted increased phytoplankton biomass. Mixing at the fresh/saltwater interface aided accumulation of phytoplankton at the halocline, where phytoplankton could access surface water NO_3^-, which further stimulated productivity. However, stratification also caused decreased bottom water dissolved oxygen, which was hypothesised to delay the spawning of estuarine fish and caused the absence of *A. butcheri* larvae.

To further explore how nutrient dynamics controlled phytoplankton assemblages, nitrogen was added to algal samples from both estuaries in the form of NH_4^+ and NO_3^-. Preferential NH_4^+ uptake occurred before NO_3^- uptake, which increased once NH_4^+ concentrations were low. Treatments with NO_3^- addition stimulated diatom and chlorophyte productivity, while the addition of NH_4^+ to samples with high background NO_3^- also stimulated flagellate phytoplankton. This reflected the conditions after the EFR in the Werribee, when NH_4^+ accumulation occurred under more stratified conditions and resulted in increased phytoplankton and zooplankton productivity.

In combination, these results allowed for a comparison of estuarine productivity. Overall, the main controls on phytoplankton biomass were nutrient loads, stratification strength, residence times and grazing pressure by zooplankton. This research highlighted the complex ways in which hydrodynamics and biogeochemical processes interlink, and the need to understand them when considering remediation practises.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: 

Print Name: **Caitlyn McNaughton**

Date: **12/10/2018**

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 1 original paper submitted to a peer reviewed journal and 2 unpublished publications. The core theme of the thesis is the role of hydrodynamics and biogeochemical processes in driving estuarine productivity. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Water Studies Centre, School of Chemistry, Faculty of Science under the supervision of A/Prof Perran Cook.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of *Chapters 2-4* my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2	Environmental flows stimulate estuarine productivity by altered salinity structure and enhanced nutrient recycling	Submitted to Marine Ecology Progress Series	Conceived, designed and performed the field experiment. Performed the laboratory work, analysed the data and wrote the manuscript (70%)	Wei Wen Wong Manuscript edits and cover letter (2%) Ryan Woodland Conceived and designed the experiments, performed field work, manuscript edits (5%) Wayne Koster Conceived and designed the experiments, performed field work and experiments (9%) Paul Reich Manuscript edits (1%) Gregory P. Jenkins Manuscript edits (1%) Ian Cartwright Conceived and designed the	No No No No No No

				experiments, manuscript edits (4%) John Beardall Conceived and designed the experiments, manuscript edits (3%) Perran L.M. Cook Conceived and designed the experiments, input into manuscript (5%)	 No No
3	Salt wedge intrusion as a driver of spring productivity in the upper estuary of an unregulated river system	Not Submitted	Conceived, designed and performed the experiments. Analysed the data and wrote the manuscript (90%)	Gregory P. Jenkins Conceived and designed the experiment, field work, input into manuscript (5%) Perran L.M. Cook Conceived and designed the experiment, input into manuscript (5%)	No No
4	The response of natural phytoplankton populations from two estuarine systems to nutrient enrichment using bioassays	Not Submitted	Conceived, designed and performed the experiments. Analysed the data and wrote the manuscript (90%)	John Beardall Conceived and designed the experiment, input into manuscript (5%) Perran L.M. Cook Conceived and designed the experiment, input into manuscript (5%)	No No

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signatur [redacted] **Date: 12/10/2018**

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work.

Main Supervisor signature: [redacted] **Date: 12/10/2018**

Acknowledgements

There are so many people to thank for their incredible support during the completion of my PhD. My utmost gratitude goes to my supervisors; Assoc. Prof. Perran Cook, Dr. Ryan Woodland, Prof. John Beardall and Prof. Ian Cartwright.

First and foremost to Perran, I sincerely thank you for your guidance and support throughout my studies. Your knowledge, continual input and the push you gave me to find the answers substantially improved the quality of this project. I am grateful for your patience when reading countless drafts and for the time you set aside to focus on my work. You taught me to think more critically and consider the bigger picture when I got locked in the nitty gritty.

Ryan, John and Ian, your expertise was invaluable. Ryan, thank you for all your help in the field and lab when I was just starting out. Even when you moved back to the US you were always a support and your excitement was contagious. John and Ian, I greatly appreciated your willingness to provide advice and feedback throughout my studies. John, thank you for your help deciphering the masses algal data. Ian, thank you for help interpreting the role of groundwater.

To everyone in the Water Studies Centre, thank you for your assistance in all parts of this project, from the lab to the field, and for just being there when I needed to decompress. I'd like to extend a particular thank you to Wei Wen Wong for essentially being my fifth supervisor given the amount of support you gave me. Thank you for your advice, help in the lab, help on my drafts, and for just being a relaxing presence in general. I am also

very appreciative of David Brehm, without whom Chapters 3 and 4 would not have happened. Your help in the field, boating expertise and fish knowledge was fantastic. Kerrie Browne and Vera Eate, thank you for your help in the lab and showing me how things work around the WSC. To my office buddy Erinn, it was great to have you there to bounce ideas off. To Steph, thanks for getting that song in my head. To everyone in the WSC - Cami, Adam, Doug, Kellie, Sharlynn, Keryn, Mike, Tina, Michael and Zahra - thanks for making this a fun four years, full of laughs, coffee, netball and racquetball. You all helped me maintain my sanity throughout my PhD.

To Steve, my fiancé, thank you for keeping me grounded and being so patient as I took my time finishing this thesis. You mean the world to me. Finally to my parents, Liz and Don, thank you for always believing in me and giving me everything I needed to be able to achieve this goal. You sparked an interest in science in me from an early age, and encouraged me to follow my own path. I hope I have made you proud.

Thank you everyone!

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Chapter 1: General Introduction

Estuaries are critical coastal ecosystems that provide ecological habitat, iconic recreation areas and generate millions of dollars in revenue for the tourism industry. They form an important part of the coastal environment by providing a link between riverine, marine and subsurface environments. The mixing of these environments and the sheltered nature of estuaries, makes them an optimal feeding, spawning and nursery site for a wide range of freshwater, brackish and marine aquatic lives. Spawning fishes are very active in estuaries as temperatures increase in spring and in response to increased phytoplankton and zooplankton biomass. This estuarine food web is driven by nutrient inputs, which enter via riverine and marine sources, runoff, groundwater and also internal processes that recycle nutrients. However, increasing population and land use intensity places a strain on these ecosystems, through increased nutrient inputs and reduced river flows, which can lead to algal blooms, loss of habitat and reduced fish recruitment.

Throughout this chapter, these processes will be further introduced and defined in order to highlight the hydrodynamic and biogeochemical processes that control nutrient cycling and estuarine productivity and how they are impacted by human activities. This chapter will also introduce the estuarine food web and highlight the complex interactions between each trophic level, and within the estuarine habitat. Finally, the specific context of this study will be outlined and the key research questions and thesis structure will be stated.

1.1 A general overview of estuarine environments

The degree of salt and freshwater mixing in estuaries plays an important role in the cycling and fate of nutrients, phytoplankton, zooplankton and fish larvae. The strength of both freshwater flows and tidal forces strongly control whether an estuary is well-mixed, partially mixed or a salt wedge estuary. A salt wedge estuary receives high freshwater inputs that flow over the encroaching salt water, resulting in the freshwater/seawater interface known as the halocline. The halocline has been widely studied as a zone of accumulation of nutrients (Correll 1978, Cauwet 1991, Geyer 1993), phytoplankton (Holligan et al. 1984, Viličić et al. 1989), zooplankton (Harder 1968, Newton 1996, Lougee et al. 2002) and fish larvae (Newton 1996, Jenkins et al. 2010). Accumulation occurs due to the mixing forces and friction of the opposing fresh and salt water flow, density differences between the fresh and salt water layers, and in the search for food or refuge (Correll 1978, Viličić et al. 1989, Cloern 1991, Tiselius 1992, Cloern 2001, Williams et al. 2013). Therefore, the halocline is a highly productive zone in salt wedge estuaries, which exerts a particularly strong control on phytoplankton productivity. Many studies have found that periods of stratification in estuaries are linked to phytoplankton biomass increases (Cloern 1984, Malone et al. 1988, Cloern 1991, Lucas et al. 1998, Kasai et al. 2010). However, a more in depth look at phytoplankton productivity and species composition has mainly resulted in a discussion of phytoplankton assemblages above and below the halocline (Ueda et al. 2005, Burić et al. 2007, Kasai et al. 2010, Watanabe et al. 2014). Additionally, few studies have been undertaken to determine how the halocline and stratification strength affects nutrient cycling, phytoplankton, zooplankton and fish larvae communities simultaneously. These dynamics are

increasingly important as humans continue to alter estuarine flow regimes and increase nutrient loads.

The strength of water column stratification and the halocline is highly dependent on freshwater flows (Kurup et al. 1998, Kimmerer 2002b). The construction of dams and weirs to regulate riverine flows and divert water for human use has a significant impact upon the amount of freshwater estuaries receive. In a highly regulated catchment, reduced riverine flow often results in a partially to well-mixed estuary, dominated by tidal forces and subject to high residence times. These conditions can lead to increased algal growth, which can negatively affect estuarine health and higher trophic levels. Therefore, a common remediation technique is the release of an environmental flow, in order to restore parts of the natural flow regime and help maintain suitable salinity conditions (Richter et al. 2006). Environmental flows are implemented for a number of purposes; to increase connectivity and exchange of materials; to provide a resource or habitat; to act as a disturbance in the hopes of resetting ecological communities (Sponseller et al. 2013). They are also commonly aimed at the maintenance of target species populations, however there are relatively few examples of this being tested and achieved in practice, and the nutrient and food web dynamics that determine trophic level responses are often overlooked (Rolls et al. 2012, Rolls et al. 2017). In one such study an environmental flow release (EFR) was used to extend floodplain inundation in the Murray River (Australia), which when compared to two previous years of little flooding, was found to increase the spawning and recruitment of several fish species (King et al. 2009).

The study of these dynamics is also important in an unregulated catchment as it may provide a baseline to inform environmental flow requirements in other systems. In

contrast to a regulated catchment, an episodic rainfall event within an unmodified catchment may remove the saline layer entirely and result in decreased residence times. Therefore salt wedge intrusion and re-establishment of a stratified water column will play an important role in estuarine productivity. Salt wedge intrusion has been found to greatly affect biological production over seasonal time scales (Kasai et al. 2010, Watanabe et al. 2014), however the short term effects of salt wedge intrusion requires a greater focus in the study of many biogeochemical processes.

One of the most important biogeochemical processes focussed upon throughout this thesis is nutrient cycling, as nutrients form the basis of the estuarine food web and have a strong control on phytoplankton biomass. Nutrient inputs, particularly in the form of nitrogen (N), are increasing in coastal environments due to anthropogenic activities (Vitousek et al. 1997). Wastewater and agricultural inputs are two major sources of nitrate (NO_3^-) and ammonium (NH_4^+) to estuarine waters (Vitousek et al. 1997, McClelland & Valiela 1998, Valiela & Bowen 2002). In previous studies, submarine groundwater discharge (SGD) has been found to be an important source of N to coastal and estuarine waters (D'elia et al. 1981, Lapointe et al. 1990, Valiela et al. 1990, LaRoche et al. 1997, Paerl 1997, Dowling et al. 2004, Hu et al. 2006). However, SGD is often overlooked in studies on estuarine productivity and only a few direct links to phytoplankton biomass have been reported (Basterretxea et al. 2010, Lee et al. 2010, Su et al. 2014). More common in estuarine studies, is a focus on the role of watershed N inputs, delivered as runoff after rainfall and high winter flows (Malone et al. 1988, Holmes et al. 2000, Valiela & Bowen 2002, Watanabe et al. 2014). These inputs introduce high agricultural N loads to coastal waters, dominated by NO_3^-, which are strongly linked to increased phytoplankton biomass in spring (Malone et al. 1988, Holmes et al. 2000, Watanabe et al. 2014). When

watershed N loads are depleted as flows decrease in summer, a second biomass peak often occurs, supported by regenerated N, dominated by NH_4^+ (Malone et al. 1988, Alpine & Cloern 1992, Valiela et al. 1992, Holmes et al. 2000, Watanabe et al. 2014). This highlights the importance of nutrient cycling and recycling in the removal (denitrification, phytoplankton assimilation) and accumulation (decomposition, remineralisation, nitrification, N-fixation) of estuarine N.

Nutrient cycling and recycling processes are strongly influenced by the degree of freshwater flow and salinity stratification. When residence times are high or mixing forces at the halocline are strong, phytoplankton accumulate and nutrient assimilation increases. When nutrients become depleted, phytoplankton die and settle to the bottom where decomposition begins in the sediments. This causes dissolved oxygen (DO) levels to decrease in the salt wedge tending towards hypoxia, which has been linked with greater release of remineralised N and P from the sediments in the forms of NH_4^+ and PO_4^{3-} respectively (Margalef 1978, Cloern 1984, Davis & Koop 2006, Wilson 2008). The release of NH_4^+ from estuarine sediments has also been linked to increased pore water salinity, when salt wedge intrusion occurs after periods of high freshwater flow (Haedrich 1983, Ketchum 1983, Milton & Arthington 1985, Paerl et al. 2001, Weston et al. 2010, Parker et al. 2012). Nitrification may then occur, whereby NH_4^+ is oxidised to NO_2^- then NO_3^-, which can cause an accumulation of NO_3^- or a loss of N via coupled denitrification. However, in low DO conditions, NH_4^+ released from the sediments will accumulate in the bottom water as the potential for nitrification decreases. Accumulation of nutrients in the bottom water is also facilitated by the lack of mixing between layers in stratified estuaries (Cloern et al. 1983, Cauwet 1991, Ueda et al. 2005, Cook et al. 2010).

Increased nutrient loads and altered nutrient ratios can lead to harmful algal blooms (HABs), which are a poor source of nutrition and can degrade seagrass beds and lead to fish kills (Cloern 2001). This paradigm has been intensively studied in coastal waters since the 1970s, however there are still many important links between nutrient biogeochemistry, phytoplankton growth and fish ecology that remain poorly understood.

1.2 Description of the estuarine food web of focus within this thesis

1.2.1 Phytoplankton

One of the main primary producers within the estuarine water column are phytoplankton. Also known as microalgae, phytoplankton are photosynthetic microorganisms that require sunlight and inorganic nutrients such as NO_3^-, NH_4^+ and PO_4^{3-} to grow. They range from single celled algae to multicellular floating plants and form two main groups: cells with or without flagellates. Diatoms (cells without flagellates) are one of the most abundant groups of phytoplankton, found in a range of marine and freshwater ecosystems. Flagellate phytoplankton including dinoflagellates, cryptophytes and some chlorophytes, are named for their small whip like structure/s that allow propulsion of the cell. Phytoplankton motility can play an important role in estuaries, where turbulence and mixing forces vary according to freshwater flows, tidal influences and the degree of stratification. As turbulence decreases due to decreased freshwater flows and intrusion of the salt wedge, flagellate phytoplankton are among the first to recolonise the water column. This is because motility allows them to migrate vertically (3-5 m) to access nutrient-rich water (Hamilton et al. 1997). The location of diatoms within the water column is strongly controlled by mixing forces. Strong stratification has been found to negatively affect diatoms if turbulence is not great enough to mix them into the euphotic zone (Margalef 1978). However, if mixing and friction between the freshwater/salt water interface is sufficient diatoms can be entrained into the nutrient-rich halocline (Correll 1978, Holligan et al. 1984, Viličić et al. 1989, Cauwet 1991, Geyer 1993).

Nitrogen is generally considered to be one of the most important nutrients controlling phytoplankton productivity within estuarine systems as it often has the greatest potential to limit biomass accumulation (Twomey et al. 2005). The two dominant forms of N utilised by phytoplankton are NH_4^+ and NO_3^-, which are key drivers of phytoplankton abundance and diversity (Lomas & Glibert 1999a, Berg et al. 2003). The preferred source of N for phytoplankton is NH_4^+, as its assimilation requires the least energy, while NO_3^- must be reduced within the algal cell in a process requiring significant energy expenditure (Syrett 1956, 1981). Additionally, NO_3^- assimilation has been found to be inhibited in the presence of NH_4^+ (Dortch 1990, Dugdale et al. 2007). More specifically, it has been found to inhibit the enzyme nitrate reductase, which is required to reduce NO_3^- to NH_4^+ within the algal cell so that it can be utilised. A number of studies have found that concentrations of NH_4^+ as low as 1 - 4 µM inhibit NO_3^- uptake by phytoplankton (Dortch 1990, Domingues et al. 2011). This means that in order for NO_3^- to be utilised during the spring bloom, NH_4^+ concentrations need to be reduced initially, by dilution and uptake by phytoplankton (Dugdale et al. 2007). However, Lomas and Glibert (1999a) have found that this inhibition phenomenon is neither as universal nor as severe as previously thought. The degree of NH_4^+ inhibition and the effects on NO_3^- uptake and storage have been found to vary widely as outlined by Glibert et al. (2016). According to Lomas and Glibert (1999a), NH_4^+ never fully inhibits NO_3^- assimilation, and NO_3^- storage can still occur under high NH_4^+ concentrations (Dortch 1990). Regardless, the relative availability of NH_4^+ and NO_3^- does exert an important control on phytoplankton community composition (Glibert el al. 2016).

In NO_3^- enriched systems, phytoplankton biomass peaks are commonly dominated by diatoms, which assimilate NO_3^- at much faster rates than dinoflagellates (Vanni 1987,

Goldman 1993, Lomas & Glibert 1999a, Torres-Valdés & Purdie 2006, Wilkerson et al. 2006, Domingues et al. 2011). In contrast, estuaries with increased NH_4^+ concentrations, generally have phytoplankton communities dominated by mixotrophic algae such as dinoflagellates and cyanobacteria (LaRoche et al. 1997, Glibert et al. 2001, Berg et al. 2003, Rothenberger et al. 2009). Mixotrophic algae are not only phototrophic but also phagotrophic (Nygaard & Tobiesen 1993, Burkholder et al. 2008). Phagotrophy is a method of nutrient uptake that involves engulfing a food particle such as nanoplankton or bacteria and ingesting it in a phagotrophic vacuole. This process is more likely to occur in a light and/or nutrient poor habitat, particularly under P limitation, as bacteria have high P content (Nygaard & Tobiesen 1993, Burkholder et al. 2008). Flagellates including crytophytes, chrysophytes, prymnesiophytes and dinoflagellates are the main groups capable of mixotrophy (Andersson et al. 1989, Bockstahler & Coats 1993, Sanders et al. 2001).

1.2.2 Higher Trophic Levels

Phytoplankton are primary producers that form the basis of the estuarine food web, providing food for zooplankton, which provide food for fish of all life stages (Fig. 1.1). Zooplankton biomass responds quickly to phytoplankton blooms, particularly in spring and summer and after increased freshwater flows (Kimmerer 2002a). Copepods, a group of small crustaceans, generally form the majority of zooplankton biomass in marine systems (Longhurst 1985), and are an important link from primary producer to higher consumers. The dominant pelagic copepods are calanoid copepods and cyclopoid copepods, which are found in a wide variety of aquatic environments. In the environments studied within this thesis the dominant calanoid copepods belong to the

genera *Acartia*, *Gladioferens* and *Paracalanus*, while the dominant cyclopoid genera is *Oithona*. *Acartia* have a strong tolerance for a wide range of salinities but are most abundant when salinity increases to >20 ppt (Rippingale & Hodgkin 1974), *Gladioferens* occur in estuaries with a wide salinity range of 1 to 30 ppt (Arnott et al. 1986), *Paracalanus* are a marine/brackish genera, common in coastal waters and *Oithona* can be abundant in estuaries where salinity is >5 or 6 ppt (Bayly 1965, Johnson & Allen 2012).

Different spawning strategies are employed by the two copepods groups, which can influence their dominance throughout the year. Most calanoid species spawn in spring and summer, in synchrony with blooms of larger phytoplankton, while cyclopoid egg production is spread across the year in response to temperature and food level (Fransz & Gonzalez 1995, Nielsen & Sabatini 1996). Once spawned, calanoids tend to release their eggs, which sink to the sediment where they are vulnerable to predation and low DO bottom waters. In order to avoid these vulnerabilities, *Oithona* carry their eggs until hatched (Nielsen & Sabatini 1996). During adulthood, the small size of *Oithona* allows them to better exploit low oxygen environments than calanoid genera, due to their lower metabolic rate (Boto & Bunt 1981, Williams & Muxagata 2006). These factors suggest that *Oithona* is better adapted to strongly stratified estuaries, where high bottom water residence times lead to DO depletion. However, strong stratification is important for copepods in general because copepods congregate at the halocline where primary productivity is high and phytoplankton biomass peaks are observed (Cloern et al. 1983, Kimmerer 2002a, Williams et al. 2013, Jenkins et al. 2015). As a result, copepods are exposed to a wide range of phytoplankton from which to select their optimal prey.

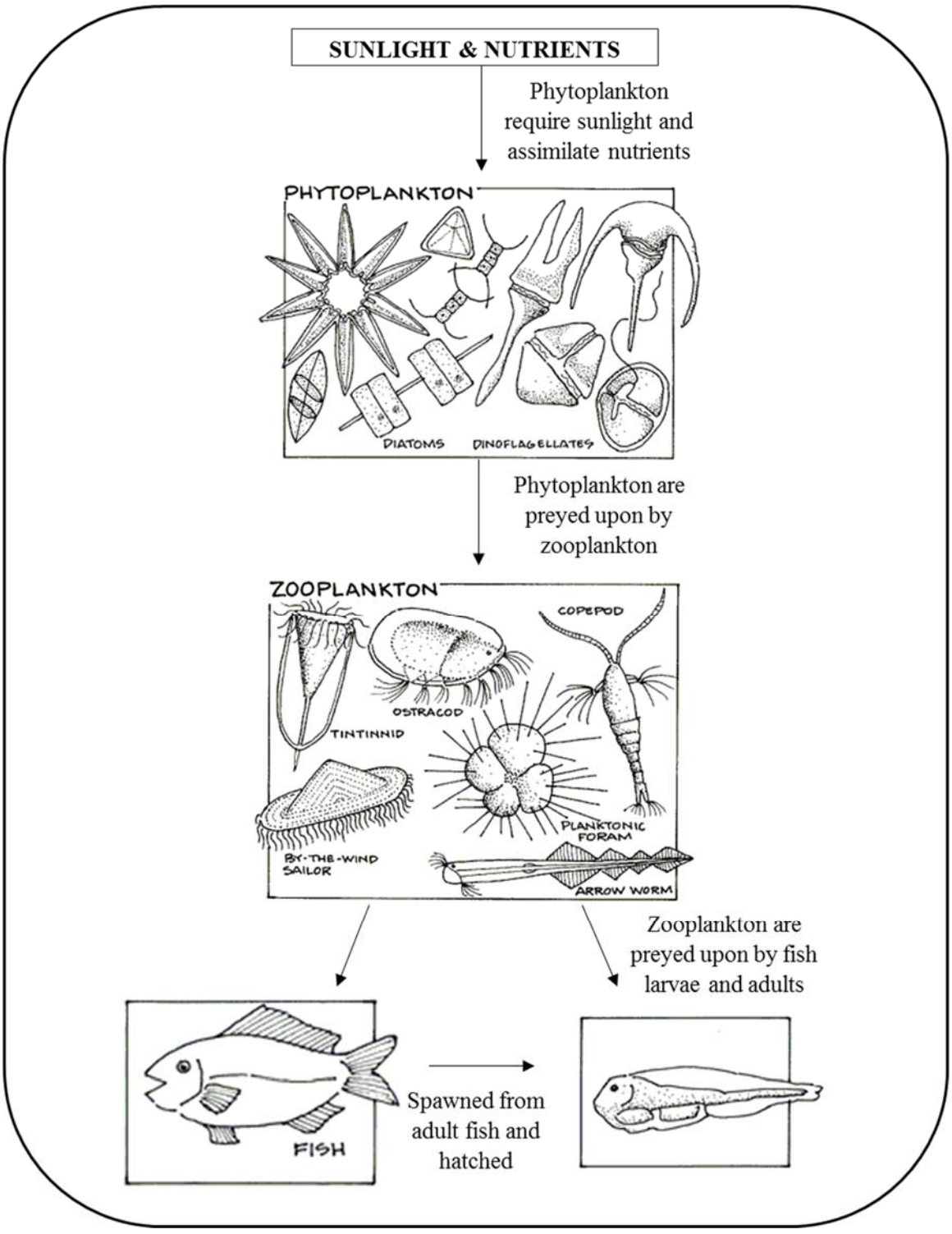

Figure 1.1: A simple diagram of the estuarine food web of focus within the thesis

In the early literature (ca 1900 to 1992) copepods were thought to be either non-selective grazers or size-selective grazers based on the size of their feeding appendages, as discussed by Kleppel (1993). Many studies focussed on measuring copepod biomass in relation to increasing phytoplankton cell size and abundance (Hargrave & Geen 1970, Frost 1972, Frost 1977, Cowles 1979). More recently, studies have focussed on the complex behavioural responses copepods exhibit when exposed to different food stimuli, which are outlined by Cowles et al. (1988). Copepods are now known to be capable of actively searching for, capturing and choosing which food particles to ingest or reject (Kleppel 1993). The age and growth rate of the algal cell are two important factors for copepod ingestion. Copepods are able to identify living and dead algal cells and selectively ingest the living ones (Starkweather & Bogdan 1980, Paffenhöfer & Van Sant 1985). But most important to food selectivity by copepods is the presence of toxins and the nutritional adequacy of the cell (Gulati & Demott 1997, Koski et al. 1998, Shin et al. 2003). Copepods are incapable of synthesising long-chain polyunsaturated fatty acids (PUFAs), amino acids and lipids (sterols), which along with vitamins and proteins, are essential for copepod survival and reproduction (Breteler et al. 1990, Jónasdóttir 1994, Kleppel et al. 1998, Müller-Navarra et al. 2000, Broglio et al. 2003). Therefore copepods must obtain these fatty acids and micronutrients from their diet (Jónasdóttir 1994, Breteler et al. 2005). Brown et al. (1997) found that the amino acid content of all algal groups is relatively similar, while sugar and vitamin content varies between species and the fatty acid content is widely different between taxonomic groups.

Fleming (1939) identified diatoms as one of the principal components of copepod diets, and much of the early literature assumed that the structure of the food web was a simple diatom-copepod link. However, more recent studies have found that, although diatoms

promote somatic growth of copepods, they can be deleterious to copepod egg hatching success and naupliar survival (Gifford & Dagg 1988, Kleppel 1993, Chaudron et al. 1996, Ban et al. 1997, Turner et al. 2001, Ianora et al. 2003, Paffenhöfer et al. 2005, Lauritano et al. 2012). There has since been increasing evidence that post-diatom blooms of mixed phytoplankton are linked to the bulk annual recruitment of copepods (Kleppel 1993, Runge & Lafontaine 1996, Jones et al. 2002). As discussed previously the post-diatom bloom is dominated by mixotrophic algae (including many flagellates) that can ingest nanoplankton and bacteria, which leads to a transfer of previously unavailable energy to copepods (Sherr et al. 1985). However many dinoflagellates are rejected by copepods as they may have harmful or toxic effects (Kleppel 1993, Carlsson et al. 1995).

Ultimately, copepods are omnivorous and within natural environments a wide variety of prey are available for selection, including diatoms, flagellates, ciliates and nauplii (Dolan 1991, Fessenden & Cowles 1994, Turner et al. 2001, Zeldis et al. 2002, Tan et al. 2004). However the motility and size of the food particles does play a role when comparing calanoid and cyclopoid diets. It has been found that *Oithona* prefer motile prey as evidenced by their feeding technique. *Oithona* wait motionless within the water column until they encounter food. The movement of this food particle provides a hydrodynamic signal that *Oithona* quickly detects using receptors on their antennule (Jonsson & Tiselius 1990, Atkinson 1995, Kiørboe & Visser 1999). Therefore diatoms do not form part of *Oithona*'s preferred diet (however they can make up a fraction of it) and a preference for motile flagellates and ciliates is shown (Atkinson 1996, Zamora-Terol et al. 2014). The small size of this prey is also optimal for the smaller *Oithona*, which have been found to ingest smaller particles ranging from 8-10 µm up to 35-40 µm for adults and 2-5 µm for nauplii (Eaton 1971, Drits & Semenova 1984). Calanoids on the other hand, ingest

particles up to 200 µm in size but are inefficient grazers on smaller phytoplankton (<10 µm) (Berggreen et al. 1988, Nielsen & Sabatini 1996, Nakamura & Turner 1997). As slower-moving suspension feeders, calanoids can take advantage of non-motile diatoms (Tiselius & Jonsson 1990).

Copepods progress through several nauplii and copepodid life stages before reaching adulthood. Fish larvae initially feed on copepod nauplii and then copepodites due to their size (Payne & Rippingale 2001). As fish larvae grow and become more mobile, larger prey become available. Estuaries are important fish nurseries where many marine, estuarine and freshwater fish spawn throughout spring and summer. A number of estuarine spawning fish have ubiquitous spawning strategies, meaning they spawn multiple times throughout a spawning season to increase the likelihood of successful recruitment to the adult life stage (Newton 1996). This strategy is utilised by gobies, gudgeon, estuary perch and anchovy species to ensure their larvae are present when plankton blooms occur (Melville-Smith 1980, Townsend 1983, Newton 1996). Other fish such as black bream are synchronous spawners that spawn only when certain water temperatures and salinities are reached, generally after a flow event (Newton 1996). This occurs in late spring/early summer and is a strategy to synchronise spawning with peak plankton biomass (Willis et al. 1999).

The fish larvae discussed throughout this thesis include several goby and gudgeon species including the flathead gudgeon *Philypnodon grandiceps*, as well as Australian smelt *Retropinna semoni* and black bream *Acanthopagrus butcheri*. *P. grandiceps* is a benthic freshwater species, however it is found in upper estuarine tidal habitats (Llewellyn 2007), and is known to spawn in estuaries (Newton 1996). Spawning is likely to occur between

October and April when water temperatures are between 18 and 28 ºC, and has been found to be initiated by increased food biomass (Llewellyn 2007). *R. semoni* is a freshwater fish that begins spawning in winter when water temperatures are ~11 to 15 ºC (Milton & Arthington 1985). This life history strategy is aimed at avoiding the impacts of summer storms and fluctuating water levels, which is an adaptive value in seasonally unstable environments such as coastal waterways (Milton & Arthington 1985). However, high winter flows and spring episodic events can flush this species into estuarine environments.

A. butcheri is an estuarine fish endemic to the south coast of Australia (Fig. 1.2). It is an important recreational and commercial fishery species and a highly suitable single species indicator of estuarine condition as described by (Valesini et al. 2017). Spawning typically occurs from August to December when salinity is >10 psu, dissolved oxygen (DO) is >2 mg L^{-1} and is triggered by temperatures >15°C (Haddy & Pankhurst 1998, Sarre & Potter 1999, Walker & Neira 2001, Nicholson et al. 2008, Williams et al. 2012, 2013). *A. butcheri* spawning is most successful when freshwater flows are sufficient for the development of strong salinity stratification and high productivity at the halocline (Jenkins et al. 2010, Williams et al. 2012, 2013). In these conditions eggs occur throughout the saline layer, with the highest concentrations in the halocline, where they are positively buoyant (Williams et al. 2013). Therefore, once hatched, there is a spatial and temporal overlap of larvae with copepod nauplii prey, which has been suggested to maximise larval survival (Williams et al. 2013).

A. butcheri larvae are very selective consumers at early life stages. Larvae <9 mm selectively consume calanoid copepod nauplii, while larvae and juveniles between 9 and

40 mm selectively consume calanoid copepod nauplii and copepodites (Willis et al. 1999). This selectiveness is one reason why *A. butcheri* recruitment success varies from year to year and why certain environmental factors trigger spawning in adults in synchrony with the spring bloom of copepods (Newton 1996, Norriss et al. 2002). Increased freshwater flows and the presence of the halocline have been linked with year class strength of *A. butcheri* (Jenkins et al. 2010, Sakabe et al. 2011). Therefore, variations in freshwater flows is another important reason why recruitment success varies.

Figure 1.2: The distribution of *Acanthopagrus butcheri* (orange shading) (Russell et al. 2014)

1.3 Site specific context

The research presented in this thesis aims to provide a comprehensive case study of two estuarine systems, subjected to very different hydrodynamic and biogeochemical processes. These systems, the Werribee River estuary and the Mitchell River estuary, Australia, were chosen because of their contrasting flow regimes and nutrient loads, and because both are known as spawning sites of *Acanthopagrus butcheri*. Therefore there is potential to compare, contrast and develop new knowledge about the internal and external processes driving productivity within these systems.

1.3.1 Study site one: the Werribee River estuary

The Werribee catchment lies west of Melbourne, Australia and covers approximately 2715 km^2 (Melbourne Water 2013). Within the catchment runs the Werribee River which rises from the Wombat State Forest on the southern slopes of the Great Dividing Range, and drains into Port Philip Bay. Annual rainfall is highest in the headwaters of the Werribee River (~1000 mm) and lowest on the plains near Werribee (~450 mm) (Melbourne Water 2013). Flows within the Werribee River and its tributaries have been highly regulated by the Melton reservoir (14360 ML capacity) and diversion weirs in order to supply water to two major irrigation districts at Bacchus Marsh and Werribee as well as private diverters. Therefore the natural flow of the river has been altered significantly and only 25% of the catchment retains natural vegetation (Melbourne Water 2013). In the lower Werribee River, a ford near Werribee Park marks the beginning of the Werribee River estuary. The estuary is 8.25 km long and ends at the mouth of the river, located about 40 km southwest of Melbourne (Melbourne Water 2013). The Werribee

River estuary is a shallow partially-mixed estuary, which runs through the internationally listed Ramsar site at the Western Treatment Plant (WTP) and is therefore a key habitat for migratory water birds and fish. On the eastern shore of the estuary lies a golf course, agricultural lands (mainly lettuce production) and stormwater drainage. This estuary has previously been the subject of an intense groundwater study, where groundwater was discovered to be a major source of NO_3^- to the estuary (Wong et al. 2013). As a result, the Werribee River estuary is one of Victoria's most highly nutrient laden systems (~22 mol m^{-2} y^{-1} nitrogen; Wong et al. (2013)), yet there have been no recorded HABs and it is known to support high fish productivity, including *A. butcheri* (Byrnes 1986, Hassell 2009).

The complex spawning behaviour of *A. butcheri* and the narrow diet of their larvae means that successful recruitment to the adult life stage is very sensitive to altered hydrodynamics and biogeochemistry. In the Werribee River estuary, the *A. butcheri* population is dominated by few year classes. This is thought to reflect the hydrodynamics of the region, which have been highly modified for human use and therefore freshwater flows may not be sufficient to promote stratification of the water column. However, there is potential to experimentally manipulate flow in this system, where its small size is suitable to elucidate the details of the relationships between flow, stratification and *A. butcheri* recruitment. This would also allow an exploration of the nutrient dynamics within the Werribee River estuary, where increased NO_3^- inputs have an unknown effect on primary productivity and therefore food web effects may contribute to the infrequent recruitment of *A. butcheri* within this system.

1.3.2 Study site two: the Mitchell River estuary

The Mitchell River catchment lies west of Melbourne and covers approximately 1365 km^2. Within the catchment runs the Mitchell River which begins in the Victorian Alps and drains into Lake King of the Gippsland Lakes. The Gippsland Lakes are a large series of interconnected coastal lagoons that flow into Bass Straight via a permanently open artificial sea-entrance constructed in 1889. The three main lakes, Lake Wellington, Lake Victoria and Lake King occupy 354 km^2 and with the exception of Lake Wellington are usually salinity stratified. Increased salinity has led to changes in the Gippsland Lakes ecology, including fish and seagrass species change and loss of fringing vegetation (Harris *et al.* 1998). Saline water from the Lakes intrudes into the Mitchell River estuary as far as a rock sill, 25 km upstream, creating a salt wedge estuary below this point. The Mitchell River is the largest river system in Victoria that is unregulated (Southern Rural Water 2014), however it does provide irrigation water to the Lindenow valley farmers and also urban water users including Bairnsdale and Paynesville (Southern Rural Water 2014). In wetter years diversions remove approximately 1.5% of total inflows and approximately 4.5% in drier years (Southern Rural Water 2014). The remaining flows provide important freshwater inputs into the Gippsland Lakes, listed as a Ramsar site in the Convention in 1982 (BMT WBM 2011). The Mitchell River drains relatively intact watershed except for the lower flats where high intensity agriculture surrounds the lower river and estuary. Nitrogen loads to the estuary have not been quantified, however the Mitchell River contributes 35% of mean annual inflow to the Gippsland Lakes, where Lake King is known to receive low to moderate loading rates (~0.4 mol m^{-2} y^{-1} nitrogen; Cook and Holland (2012)). Therefore, the Mitchell River estuary is likely to receive

similarly low N loading, and although there have been no recorded HABs in this estuary, the Lakes suffer from recurring blooms of the cyanobacteria *Nodularia spumigena*.

Significant progress has been made in understanding the factors that lead to the initiation of cyanobacterial blooms in Lake King (Cook et al. 2010, Cook & Holland 2012, Holland et al. 2012), however, no studies have been undertaken within its main tributary, where low nutrient concentrations must produce a food web capable of supporting larval *A. butcheri* populations (Williams et al. 2012, Williams et al. 2013). As an unregulated system, the salinity structure produced must play an important role in driving this estuarine productivity. Exploration of these dynamics would further our understanding of the function of the halocline and the role of salt wedge intrusion after high flow events.

Table 1.1: The main similarities and differences between the Werribee and Mitchell River Estuaries.

	Werribee River Estuary	**Mitchell River Estuary**
Flow regime	Regulated	Unregulated
Salinity structure	Partially mixed to stratified	Stratified
Nutrient status	High NO_3^- loads via groundwater inputs	Low NO_3^- loads, no groundwater inputs
Fish population	Known spawning ground of *Acanthopagrus butcheri*	Known spawning ground of *Acanthopagrus butcheri*

1.4 Project aims and thesis structure

A graphical outline of the research chapters in this thesis and how they inter-link is presented below in Figure 1.3. The major focus of this thesis is to determine how hydrodynamic and biogeochemical processes control the estuarine food webs of two Australian estuaries, both known spawning ground of the black bream *Acanthopagrus butcheri*. In more specific terms this thesis will focus on the role of freshwater flows, the structure and function of the halocline, nutrient inputs and cycling, and how these processes impact upon phytoplankton, zooplankton and larval fish dynamics to elucidate their inter-relationships. Therefore, this project aims to develop new knowledge as to how these internal and external processes drive estuarine productivity.

Specifically, this thesis aims to answer the following research questions:

1. How do environmental flows stimulate estuarine productivity in the highly regulated, nutrient-rich Werribee River Estuary?
2. What role does salt wedge intrusion and the strength of stratification play in driving estuarine productivity within the unmodified, nutrient-poor Mitchell River Estuary?
3. How do the natural phytoplankton communities of the Werribee and Mitchell River estuaries respond to altered nutrient ratios and concentrations in nutrient enrichment bioassays?

1.4.1 Chapter 2 – The Werribee River Estuary

This chapter discusses the investigation undertaken at the Werribee River estuary during the spring and early summer of 2014. Sampling took place approximately fortnightly, during which water quality, nutrient, groundwater, phytoplankton, zooplankton and fish larvae samples were collected at six sites along the estuarine gradient. During sampling, an environmental flow was released with the aim of increasing stratification strength in the estuary and promoting *A. butcheri* larvae survival. The resultant data were used to infer the relative importance of biogeochemical processes and hydrodynamics as drivers of estuarine productivity. It also helped inform the timing and magnitude of freshwater flows required to maximise success of *A. butcheri* larvae.

1.4.2 Chapter 3 – The Mitchell River Estuary

This chapter discusses the investigation undertaken at the Mitchell River estuary during the spring and early summer of 2015. Sampling undertaken was similar to the Werribee estuary, however only three sites were chosen in the upper estuary to target the furthest reaches of the salt wedge. This chapter aimed to determine the importance of unmodified flows on the salinity structure and nutrient recycling of the upper estuary. The resultant data were used to infer the importance of salt wedge intrusion as a driver of estuarine productivity in a low nutrient environment and help inform baseline environmental flow requirements.

1.4.3 Chapter 4 – Bioassay experiments

The fourth chapter narrows the focus of the previous two chapters and tests how the natural phytoplankton populations of both estuaries respond to nutrient addition bioassays. Typically, bioassays are used to demonstrate nutrient limitation or release of nutrients from point sources (Dalsgaard and Krause-Jensen, 2006). Bioassays are based on the concept that addition of a limiting nutrient will increase growth rate compared to that in a control with *in situ* nutrient concentrations. In practice, they often reveal what nutrient might become limiting should phytoplankton populations increase above levels in the control (Beardall et al. 2001). Here however, the bioassay approach was used to test how changes in NH_4^+ and NO_3^- ratios and concentrations influence growth and population composition of phytoplankton in the two systems. This experiment was aimed at further investigating the results and conclusion from the previous two chapters as well as simulating increased nutrient loads.

1.4.4 Chapter 5 – Conclusion

This chapter is a synthesis of the key findings of the preceding three chapters and a comparison of the two estuaries. It will focus on the different human impacts, hydrodynamics and biogeochemical processes that affected estuarine productivity and the interactions between nutrients, phytoplankton, zooplankton and larval fishes. Finally, potential avenues for future research will be discussed.

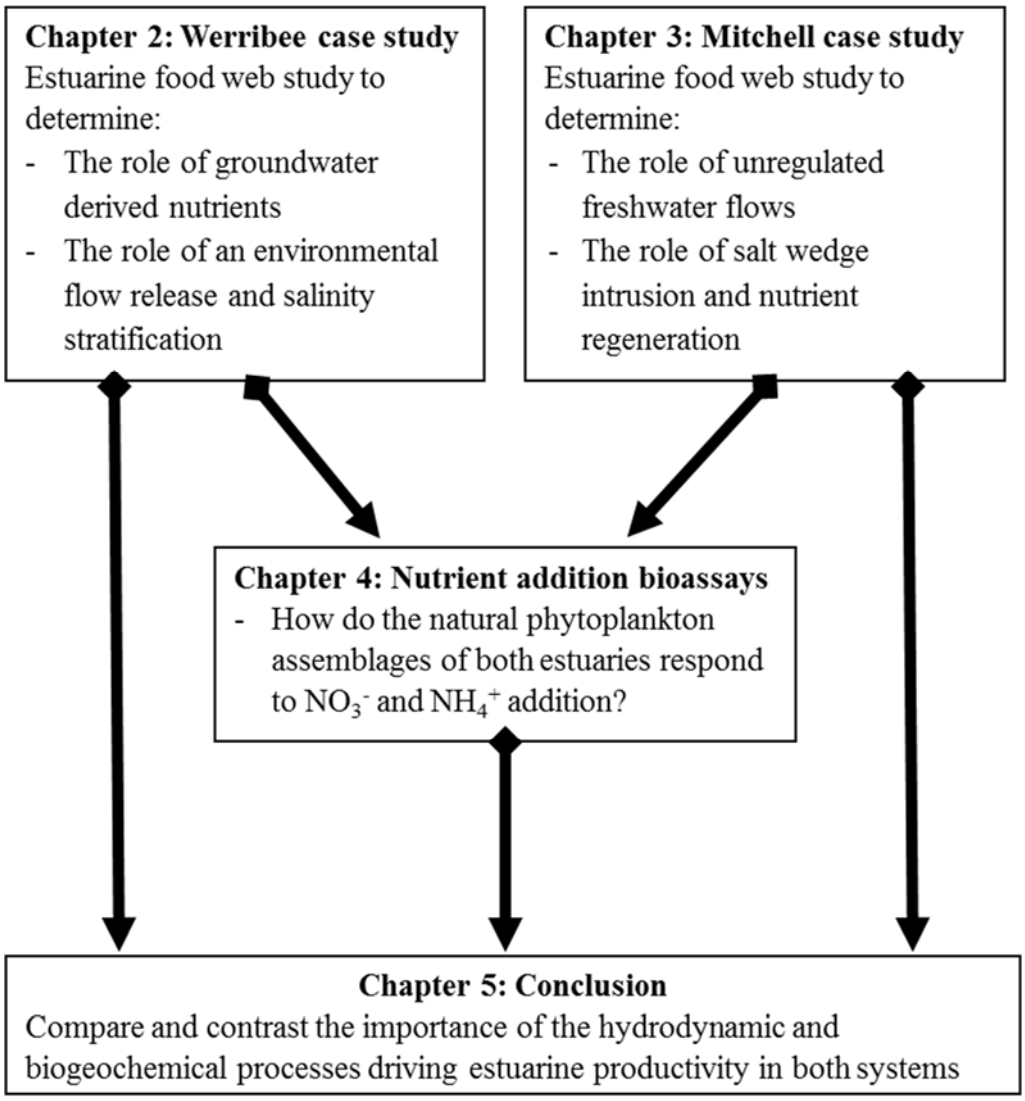

Figure 1.3: Summary of thesis

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Chapter 2: Environmental flows stimulate estuarine productivity by altered salinity structure and enhanced nutrient recycling

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The Werribee River estuary site 5. Credit birdsaspoetry
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This chapter has been submitted to Marine Ecology Progress Series for publishing

2.1 Abstract

Nutrient inputs and freshwater flows play an important role in driving estuarine productivity. In this study, we hypothesised that groundwater nutrient inputs and an environmental flow release would be strong drivers of phytoplankton and zooplankton community structure, and larval fish abundance in a highly flow regulated, partially mixed estuary. High NO_3^- concentrations were consistently observed within the estuary, while NH_4^+ concentrations fluctuated. Elevated phytoplankton biomass was found to be associated with a bloom of flagellates of the genus *Euglena*, followed by a bloom of the diatom *Cyclotella* sp. The abundance of *Cyclotella* sp. correlated significantly with ^{222}Rn ($p < 0.04$), strongly suggesting this bloom was associated with NO_3^--rich groundwater entering the estuary. An environmental flow release triggered the end of the *Cyclotella* sp. bloom and led to increased stratification strength and NH_4^+ release within the estuary. The increased NH_4^+ concentration was associated with a significant increase in the abundance of flagellate taxa ($p < 0.04$). Calanoid copepod abundances, which had declined in the presence of the *Cyclotella* sp. bloom, also responded significantly to increased NH_4^+ concentrations ($p < 0.01$) and flagellate abundances ($p < 0.03$). Calanoid copepodites and nauplii are known to be the preferred food for larval *Acanthopagrus butcheri*, which were abundant for the first time following the environmental flow release, although their eggs were present throughout the sampling period. This sequence of events underscores the importance of freshwater flows in regulated estuarine systems and their interaction with nutrient dynamics as drivers of estuarine productivity.

2.2 Introduction

Estuaries are critical ecosystems linking riverine, marine and subsurface environments. They are subjected to salt and freshwater mixing, which can result in a salt-wedge estuary characterised by the presence of a halocline. The halocline is a highly productive zone where nutrients, phytoplankton and zooplankton accumulate (Viličić et al. 1989, Cauwet 1991, Lougee et al. 2002). These plankton bloom when temperatures increase in spring and are an important food source for newly spawned fish larvae (Newton 1996, Williams et al. 2013, Black et al. 2016). Therefore, the level of predator-prey interaction and the transfer of energy through the estuarine food web can be strongly influenced by the presence of the halocline, which is dependent on hydrological processes such as increased or reduced riverine flows (Newton 1994, Kimmerer 2002a, Jenkins et al. 2010, 2015).

The estuarine food web is also strongly influenced by biogeochemical processes, such as remineralisation and assimilation by phytoplankton, which control the cycling and fate of nutrients (Lomas & Glibert 1999a, Ueda et al. 2005, Dugdale et al. 2007). Nutrients form the basis of the estuarine food web and are increasing in coastal environments due to anthropogenic activities, particularly in the form of nitrogen (N) (Vitousek et al. 1997). Waste water is a major source of nitrate (NO_3^-) to estuarine waters, as are agricultural inputs, which are increasingly a source of ammonium (NH_4^+), due to a global shift of N fertiliser from oxidised to reduced forms of N (Valiela & Bowen 2002, Glibert et al. 2006, 2014). These nutrients enter coastal and estuarine waters via runoff and also from submarine groundwater discharge (SGD). SGD is an often overlooked source that may be traced using the isotopes of radium and radon (Lapointe et al. 1990, Valiela et al. 1990, LaRoche et al. 1997, Paerl 1997, Dowling et al. 2004, Hu et al. 2006). As N loads

increase, eutrophication and algal blooms become more likely in coastal waters, particularly as a result of increased NO_3^- concentrations (Glibert et al. 2016).

The key drivers of the abundance and diversity of phytoplankton taxa are NH_4^+ and NO_3^- (Lomas & Glibert 1999a, Berg et al. 2003). Assimilation of NH_4^+ is generally preferred by phytoplankton as it requires less energy, whereas NO_3^- must first be reduced to NH_4^+ within the algal cell. Generally, systems more enriched in NH_4^+ have phytoplankton communities dominated by mixotrophic algae such as dinoflagellates and cyanobacteria (Glibert et al. 2001, Berg et al. 2003, Rothenberger et al. 2009). Conversely, diatoms preferentially assimilate NO_3^-, particularly in NO_3^- rich environments (Goldman 1993, Lomas & Glibert 1999a, b). However, in many cases the presence of NH_4^+ at concentrations as low as 1 μmol L^{-1} inhibits NO_3^- uptake by phytoplankton (Dortch 1990, Lomas & Glibert 1999a). Therefore, the relative availability of NH_4^+ and NO_3^- exerts an important control on phytoplankton community composition.

When phytoplankton abundances increase in spring, the zooplankton biomass responds quickly. Copepods generally form the majority of the zooplankton biomass in estuaries and congregate at the halocline where primary productivity and nutrient concentrations are high (Cloern et al. 1983, Kimmerer 2002a, Williams et al. 2013, Jenkins et al. 2015). They are capable of actively searching for, capturing and choosing which food particles to ingest based on the nutritional adequacy of the food (Kleppel 1993, Gulati & Demott 1997, Koski et al. 1998, Shin et al. 2003). Fleming (1939) identified diatoms as one of the principal components of copepod diets, and much of the early literature assumed that the structure of the food web was a simple diatom-copepod link. However, more recent studies have found that, although diatoms promote somatic growth of copepods, they can

be deleterious to copepod egg hatching success and naupliar survival (Gifford & Dagg 1988, Kleppel 1993, Chaudron et al. 1996, Ban et al. 1997, Turner et al. 2001, Ianora et al. 2003, Paffenhöfer et al. 2005, Lauritano et al. 2012). There has since been increasing evidence that post-diatom blooms of mixed phytoplankton are linked to the bulk annual recruitment of copepods (Kleppel 1993, Runge & Lafontaine 1996, Jones et al. 2002).

Copepods are an important link from primary producers to higher consumers (Perbiche-Neves et al. 2007). The diet of larval black bream, *Acanthopagrus butcheri* (Munro 1949), for example, is dominated by calanoid copepods, particularly nauplii and copepodites (Williams et al. 2013). *A. butcheri* is an estuarine fish endemic to the south coast of Australia, and is an important recreational and commercial fishery species. Spawning typically occurs from August to December when water salinity is >10, dissolved oxygen (DO) is >2 mg L^{-1} and is triggered by temperatures >15°C (Haddy & Pankhurst 1998, Sarre & Potter 1999, Walker & Neira 2001, Nicholson et al. 2008, Williams et al. 2012, 2013). *A. butcheri* spawning is most successful when freshwater flows are sufficient for the development of strong salinity stratification and high productivity at the halocline (Jenkins et al. 2010, Williams et al. 2012, 2013). In these conditions eggs occur throughout the saline layer, with the highest concentrations in the halocline (Williams et al. 2013). This creates a spatial and temporal overlap of eggs and larvae with prey (copepods), which has been suggested to maximise larval survival (Williams et al. 2013).

Freshwater flows are the most important control on estuarine salinity structure (Kurup et al. 1998, Kimmerer 2002b). In estuarine systems with highly regulated freshwater flows, high pulse flows such as an environmental flow release (EFR), help maintain suitable

salinity conditions (Richter et al. 2006). Environmental flows are implemented for a number of purposes: to provide a resource or habitat, to increase connectivity and exchange of materials, to act as a disturbance in the hopes of resetting ecological communities (Sponseller et al. 2013). In this study an EFR was aimed at increasing stratification strength and therefore increasing the size of suitable habitat for *A. butcheri* larvae and their prey. This mechanism has been strongly linked to *A. butcheri* larvae survival by Jenkins et al. (2010), Williams et al. (2012), Williams et al. (2013) and Jenkins et al. (2015).

Therefore, we undertook a study of a highly regulated estuary over the periods before, during and after an EFR in order to follow the dynamics of water column structure, nutrients, phytoplankton, zooplankton and fish larvae, and elucidate their inter-relationships. This estuary, the Werribee River Estuary, Victoria, is known to have high NO_3^- concentrations as a result of groundwater inputs and therefore we hypothesised these inputs would be an important driver of productivity. The occurrence of increased freshwater flows via an EFR was hypothesised to increase stratification strength, which would drive estuarine productivity and have particular influence over the survival of *A. butcheri* eggs and larvae. The resultant data were used to infer the relative importance of biogeochemical processes and hydrodynamics as drivers of estuarine productivity.

2.3 Methods

2.3.1 Site description

The Werribee River Estuary, located 40 km southwest of the city of Melbourne, Victoria (Australia), is 8.25 km long and partially mixed, except in the upper estuary where it can become stratified (Fig. 2.1). The estuary forms the eastern boundary of the internationally listed Port Phillip Bay (Western Shoreline) & Bellarine Peninsula Ramsar site and is a key habitat for migratory water birds. To the west of the estuary is the Western Treatment Plant (WTP), which services 52% of Melbourne sewage (~ 450 ML day^{-1}), and to the east is an intensive agricultural precinct known as the Werribee Irrigation District (WID). The estuary is strongly influenced by tidal forces from Port Phillip Bay, into which it flows. Freshwater flows to the estuary are limited due to a number of upstream diversions including the Melton reservoir 45 km upstream and the Wyndham diversion weir 9 km upstream, and the river flow is typically ~4 ML Day^{-1}. Six sampling sites were chosen along the Werribee River Estuary approximately 1 km apart. Sites S4, S5 and S6 (the upper estuary) were the main focus of this study, chosen for their proximity to SGD hotspots. The average depth of the estuary is approximately 2 m but at low tide the depth between S5 and S6 decreases to less than 0.5 m.

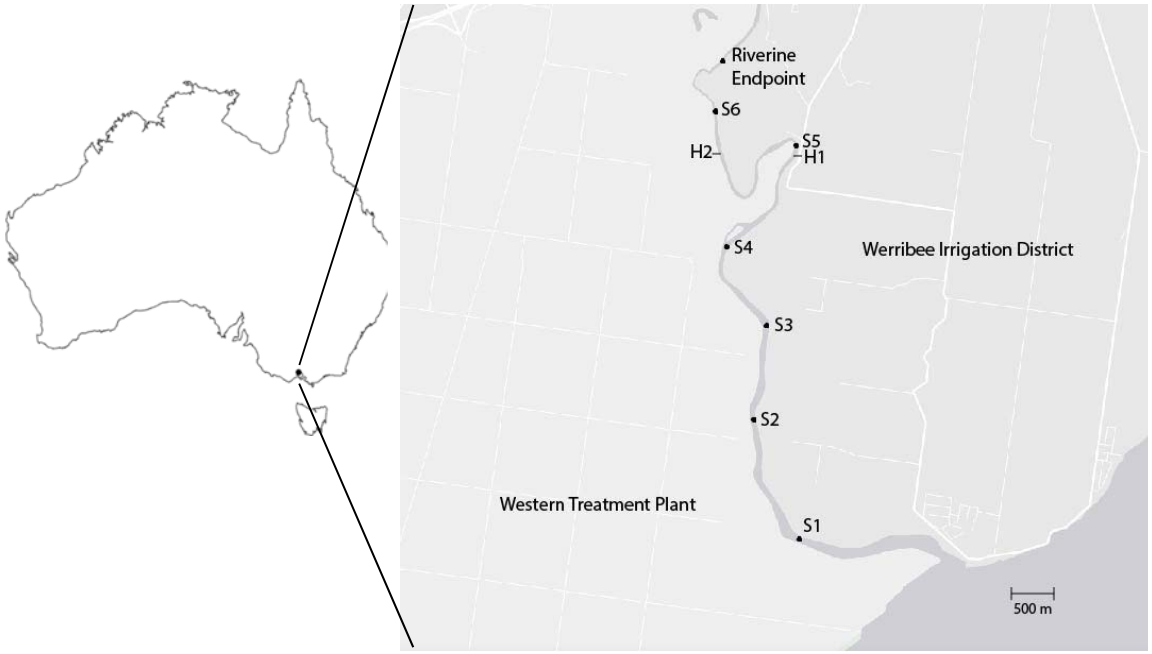


Figure 2.1: A map of the riverine endpoint and sampling sites along the Werribee River Estuary including the two SGD hotspots (H1 and H2).

2.3.2 Field sampling methods

Each site was sampled eight times between September and December 2014. Sampling was conducted approximately fortnightly, with the addition of weekly sampling in mid-November; shortly before and after a high rainfall event and EFR from the Melton reservoir (see Results section below). The EFR and rainfall event are hereafter referred to as the high flow event. A multi-parameter water quality sonde (Hydrolab DS5X) was used to measure depth (m), salinity (practical salinity unit scale; PSU), temperature (°C), pH, chlorophyll *a* (µg L^{-1}), turbidity (Nephelometric Turbidity Units; NTU) and DO (% saturation) profiles at each site. Water samples were taken from the surface and bottom water at each sampling site and the riverine endpoint and filtered through 0.22 µm filters. Particulate organic matter (POM) samples from each estuarine site were filtered onto 0.7 µm Whatman GF/Fs. Unfiltered water samples for ^{222}Rn, phytoplankton and zooplankton

samples were only collected in the upper estuary. Phytoplankton were collected in the filtrate that passed through an 80 µm sieve and preserved using a Lugol's Iodine solution. A water pump and later in the sampling period vertical net tows (80 µm mesh, 30 cm diameter conical net) were used to capture depth-integrated (bottom-to-surface) zooplankton samples which were preserved using buffered 96% ethanol. To collect fish eggs and larvae, three oblique tows were conducted at each site using a conical net with a 750-mm diameter opening and 333 µm mesh. Each tow was undertaken at c. 1.0–1.5 knots and lowered from the surface to within 1 m of the bottom at a rate of c. 0.5 m min^{-1}. The duration of the tows was about 3-5 minutes. Nets had flow meters fitted to the mouth of the net to measure the volume of water filtered. Fish eggs and larvae collected were preserved using 90% ethanol.

On 10/12/2014 a ninth sampling trip was undertaken to sample surface water and pore water in the upper estuary using a piezometer and peristaltic pump. A total of 3 surface water and 15 pore water samples were taken in close proximity to S5 and 500 m downstream of S6. Water samples extracted from the bed of the estuary using a piezometer were deemed groundwater when the salinity was <3. This was distinct from the saline bottom waters and therefore these samples were considered to represent the groundwater end-member entering estuary.

2.3.3 Laboratory methods

Filtered water samples were analysed for NH_4^+, NO_3^- and filterable reactive phosphorus (FRP). Their concentrations were determined spectrophotometrically using a Lachat QuikChem 8000 Flow Injection Analyser (FIA), following standard procedures (APHA

2005). The accuracy was within 2% relative error. Particulate organic matter (POM) collected on filters was extracted with acetone and analysed spectrophotometrically according to Strickland and Parsons (1972). The chlorophyll *a* concentrations (μg L^{-1}) were then determined using the equations of Jeffrey and Humphrey (1975). The activities of ^{222}Rn from unfiltered water were measured using a RAD7 Radon detector (Durridge Company), following the methods described by Burnett and Dulaiova (2003). 0.5 L of sample was collected by bottom-filling a glass flask and ^{222}Rn was subsequently degassed for 5 minutes into a closed air loop of known volume. Counting times were 120 minutes and the activities were expressed in Bq m^{-3}. Typical relative precision is <3% at 10,000 Bq m^{-3} and ~10% at 100 Bq m^{-3}. Initially, ^{222}Rn samples were taken on every second trip but as of 16/10/2014 were taken each time. Phytoplankton samples from five sampling dates (3/9/2014, 3/10/2014, 11/11/2014, 18/11/2014 and 3/12/2014) were sent to Microalgal Services, Victoria where taxa were identified and counted using a Zeiss Standard compound microscope equipped with Phase Contrast Optics with up to 400× magnification. A Sedgewick-Rafter counting chamber was used and data was reported in cells L^{-1} using reference material from Tomas (1997). Zooplankton from the same dates were counted in the laboratory under a light microscope with 100× magnification, using a Sedgewick-Rafter counting cell, identified with reference to Richardson et al. (2013). Eggs and larvae of *Acanthopagrus butcheri* were counted in the laboratory using a compound microscope with 20× magnification. Large samples were split into a manageable size using a Folsom plankton splitter (Williams et al. 2013). *A. butcheri* eggs and larvae were identified using published descriptions (Neira et al. 1998, Walker & Neira 2001).

2.3.4 Statistical analysis

Nonmetric Multidimensional Scaling (NMDS) was used to determine the community distribution of both phytoplankton and zooplankton in the upper estuary during the study period. This allowed the communities from the different sites and dates to be visualised and interpreted on a single plot using rank order dissimilarities. These analyses were done using the vegan package in R 3.3.2 (Oksanen et al. 2013, R Core Team 2016). To achieve the lowest stress level, phytoplankton abundance data were square-root transformed, while zooplankton were autotransformed using the metaMDS function. For both a Bray-Curtis dissimilarity matrix was used. Relevant water quality vectors were then fitted to these ordinations using the envfit function (constrained correspondence analysis) to determine the significant drivers of taxa distribution. These vectors included DO, salinity, temperature, turbidity, ^{222}Rn, NO_3^-, NH_4^+ and FRP. A Pearson correlation matrix was also performed using the Hmisc package in order to determine any significant linear relationships between abundance data and their drivers (Harrell Jr 2016). For all analyses, $p < 0.05$ was the level of significance set for the rejection of the null hypothesis. The sum of taxa abundances were used for analysis of a group referred to as the mixed algal assemblage (see Results section below), which included both diatoms and flagellates, excluding those that bloomed before the high flow event. This was deemed appropriate as the blooming taxa formed a distinct distribution on the NMDS plot (see Results section below), separate from the main community distribution. SigmaPlot was used to create the linear regression plots and filled contour plots of water quality parameters.

2.4 Results

2.4.1 Water quality parameters

The daily rainfall and average riverine flow entering the estuary are shown in Figure 2.2. Monthly rainfall in September, October and November 2014 was 12 to 23% below the 10 year average (http://www.bom.gov.au/climate/data/; station number 087031 Laverton). A small environmental flow was released on November 9 after seven days of no rainfall, which increased the maximum daily flow from 2 to 56 ML Day^{-1} (https://www.melbournewater.com.au/water/rainfall-and-river-levels#/; Cottrell St Ford). On November 13 a larger flow was released, which led to maximum daily flows of 100 to 200 ML Day^{-1} until November 16. On that day, the highest rainfall event of the year occurred (31.4 mm), which contributed to the maximum daily flow of 143 ML Day^{-1} (http://www.bom.gov.au/climate/data/; station number 087031).

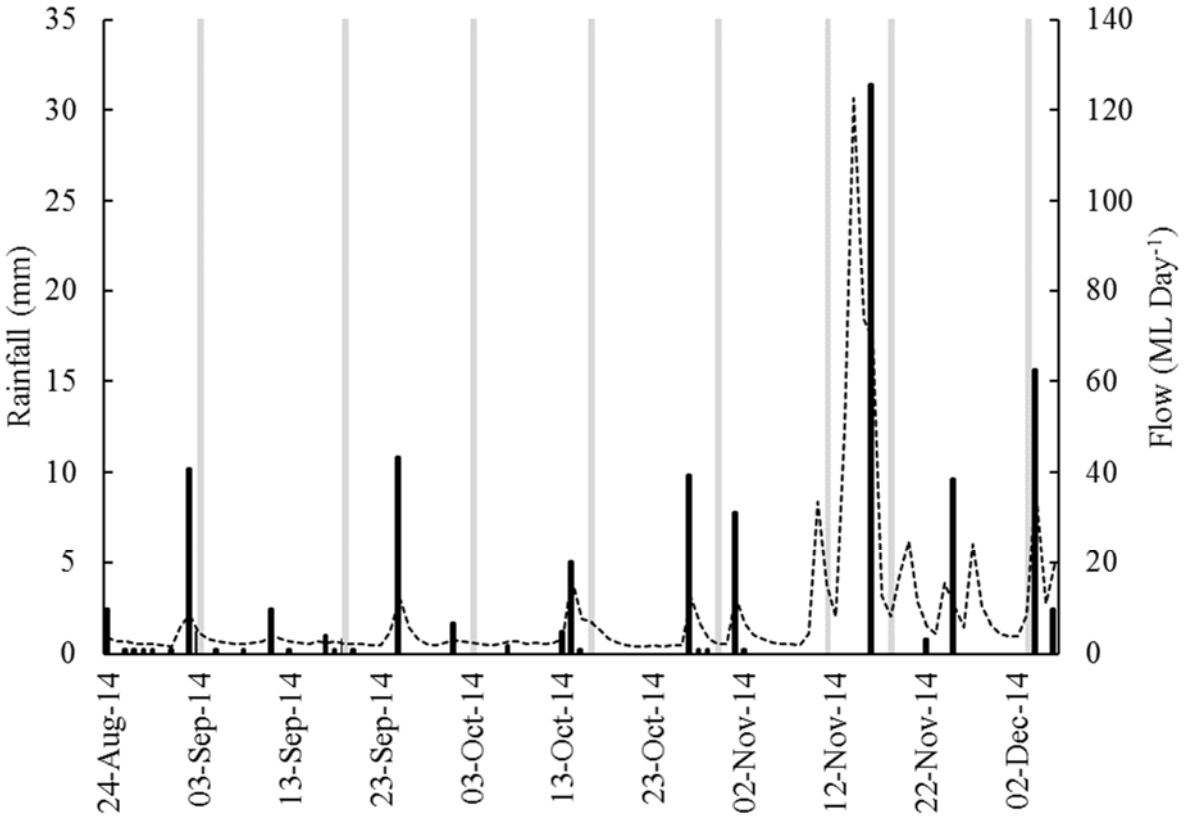


Figure 2.2: River flow (ML day^{-1}; dotted line) at Cottrell St Ford and rainfall (mm; black bars) at Laverton before and throughout the sampling period. Grey bars indicate sampling dates.

During the sampling period, salinity ranged from 1 to 37 PSU throughout the estuary (Fig. 2.3). Bottom water salinities were consistently higher than surface water salinities in the upper estuary. A halocline was considered to be present when salinity changed by more than 6 PSU over a depth of 0.5 m. On the first sampling date (3/9/2014) a halocline was present at between S4 and S6, but weakened downstream. Over the next two sampling dates (19/9/2014 and 3/10/2014) the upper estuary was partially mixed and the salinity did not decrease below 20 PSU even in the surface water at S6. A halocline developed again at S6 on 16/10/2014 and was also present at S5 on 30/10/2014 and 11/11/2014. After the high flow event a strong halocline was observed at S5 on

18/11/2014, whereas very low salinities occurred at S6 (1 to 7) and very high salinities at S4 (33 to 34 PSU). On 3/12/2014 the halocline was again present at S6 but the estuary was partially mixed downstream.

Chlorophyll *a* concentrations were highest at S6, particularly in the bottom waters, but fluctuated throughout the sampling period from 2 to 140 µg L^{-1} (Fig. 2.3). All other sites had low concentrations varying from 0 to 4 µg L^{-1} on most dates except S5 on 11/11/2014 when the chlorophyll *a* concentration increased to 10 µg L^{-1} in the bottom waters. DO ranged from 34 to 170 % throughout the sampling period (Fig. 2.3). It was highest on the days preceding the high flow event, particularly in the surface waters (120 to 160 %). DO then decreased following the high flow event, particularly in the bottom waters (45 to 80 %). Temperatures were 15 °C in the upper estuary at the beginning of the sampling period but were as low as 12.5 °C in the lower estuary. From 3/10/2014 onwards temperatures were consistently above 15 °C throughout the estuary.

The activities of ^{222}Rn within the estuary were highest in the surface water at S5 (2140 to 5890 Bq m^{-3}) on most dates (Fig. 2.3). However, on the 30/10/2014 and 11/11/2014 the ^{222}Rn activity was higher in the surface water at S4 (3140 and 4780 Bq m^{-3} respectively). In the bottom water ^{222}Rn activities were highest at S6 (2460 to 5100 Bq m^{-3}) on most dates excluding 11/11/2014, when bottom water ^{222}Rn activities were higher at S5 (4690 Bq m^{-3}) (Fig. 2.3). The highest ^{222}Rn activity (32844 Bq m^{-3}) was in water from the piezometer at S5, denoted as hotspot 1 (H1; Fig. 2.1). High ^{222}Rn activities of 15800 Bq m^{-3} also occur ~500 m downstream of S6, denoted as hotspot 2 (H2; Fig. 2.1).

The concentrations of FRP were high at S1 at the start of the sampling period (5 to 10 μmol L^{1}) and low in the upper estuary (0.3 to 4 μmol L^{-1}; Fig. 2.3). At the end of the sampling period after the high flow event FRP concentrations increased at S1 (11 to 17 μmol L^{-1}) and also up the estuary, where they reached a peak of 19 μmol L^{-1} at S3 and S4. At the riverine endpoint, the concentrations of FRP <1 μmol L^{-1} throughout the sampling period. Within the pore water at SGD H1 and H2, FRP concentrations ranged from 2 to 4 μmol L^{-1}. The highest concentrations of NH_4^+ were also detected near the mouth of the estuary on most dates (11 to 39 μmol L^{-1}; Fig. 2.3). In the upper estuary, NH_4^+ concentrations ranged from 4 to 10 μmol L^{-1} at the start of the sampling period. The NH_4^+ concentrations decreased to ≤1 μmol L^{-1} at S6 on 3/10/2014, 30/10/2014 and 11/11/2014, and decreased at S4 and S5 on 11/11/2014. After the high flow event, NH_4^+ concentrations increased to 7 to 32 μmol L^{-1} in the upper estuary. At the riverine endpoint, NH_4^+ concentrations ranged from 1 to 20 μmol L^{-1} and were higher than at S6 on all October sampling dates. At the SGD hotspots, NH_4^+ concentrations reached a maximum of 0.9 μmol L^{-1}. Much higher NO_3^- concentrations than both FRP and NH_4^+, were detected throughout the estuary, particularly at the upper three sites and at the riverine endpoint (Fig. 2.3). Very high NO_3^- concentrations (116 to 236 μmol L^{-1}) were observed at the riverine endpoint on all dates except 18/11/2014 (after the high flow event), when the NO_3^- concentration decreased to 33 μmol L^{-1}. At S6, NO_3^- concentrations were generally lower than at the riverine endpoint, ranging from 25 to 184 μmol L^{-1} in the surface water and 18 to 86 μmol L^{-1} in the bottom water. Only on 18/11/2014 were NO_3^- concentrations at S6 higher that at the riverine endpoint by ~5 μmol L^{-1}. Within the estuary, surface water NO_3^- concentrations were highest at S5 ranging from 76 to 164 μmol L^{-1} with two exceptions; at the start of the sampling period when NO_3^- was most concentrated at S4 (221 μmol L^{-1}) and before the high flow event

when NO_3^- was most concentrated at S6 (184 µmol L^{-1}). Bottom water NO_3^- concentrations at S5 were consistently lower than surface water concentrations ranging from 29 to 90 µmol L^{-1}. In the pore water, NO_3^- concentrations were highest at H1 ranging from 1863 to 2206 µmol L^{-1} and lower at H2 ranging from 878 to 914 µmol L^{-1}. Molar ratios of N and P within the water column indicated than N was most commonly the limiting nutrient, particularly in the lower estuary and bottom waters, while P became limited in the fresher surface waters of the upper estuary where NO^{-3} concentrations were highest.

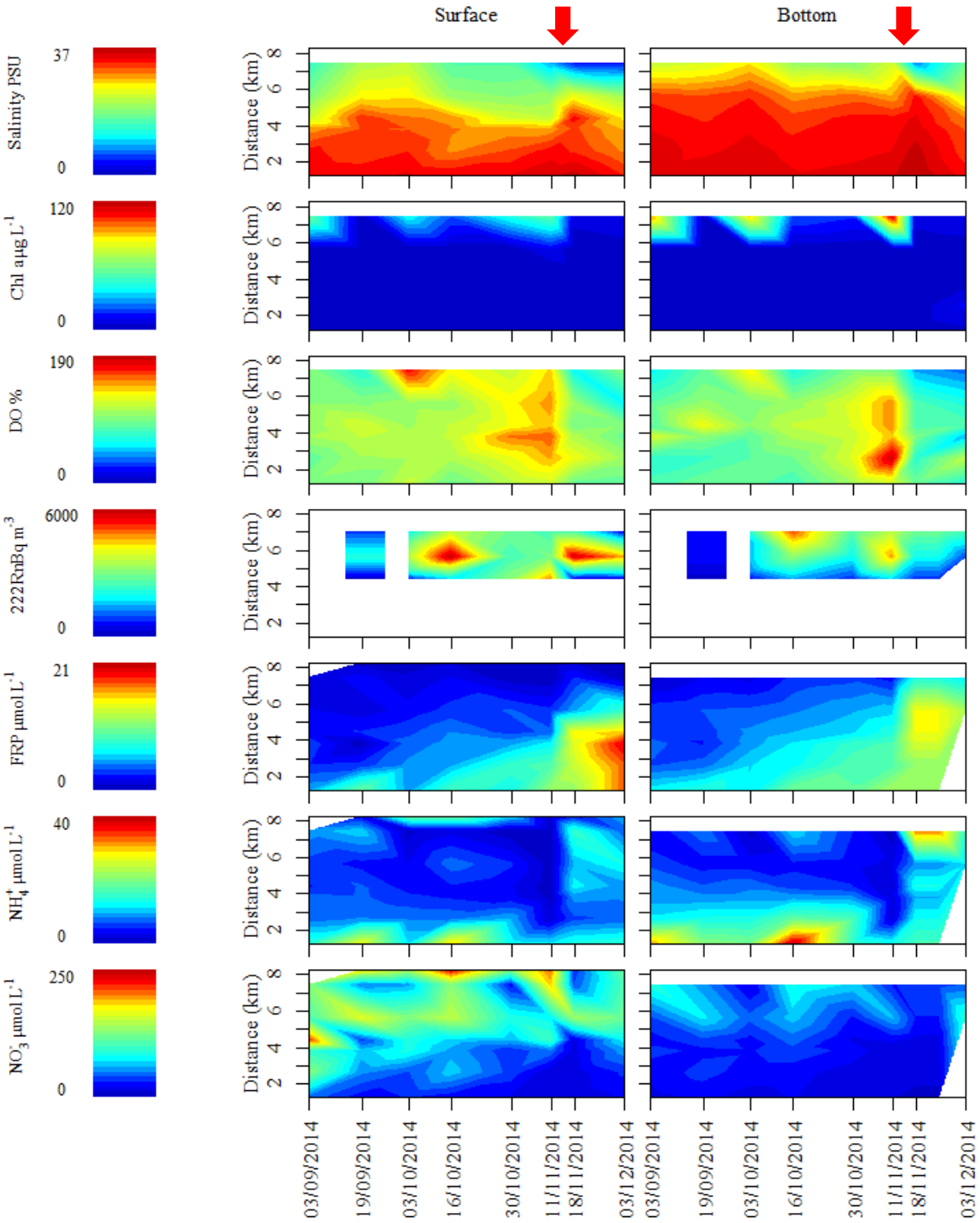


Figure 2.3: Contour plots of salinity (PSU scale), chlorophyll *a* (µg L^{-1}), DO (%), ^{222}Rn (Bq m^{-3}), FRP (µmol L^{-1}), NH_4^+ (µmol L^{-1}) and NO_3^- (µmol L^{-1}) in the surface and bottom water at each site and date. White spaces represent no data and red arrow represents the high flow event. Nutrient concentrations from the riverine endpoint (8.25 km) are included in the surface water plots.

2.4.2 Phytoplankton, zooplankton and Acanthopagrus butcheri

The distribution of phytoplankton on the NMDS plot shows that the phytoplankton community structure was significantly associated with three key physicochemical variables: FRP, salinity and NH_4^+ (Fig. 2.4). The majority of the algal taxa (circled in green) were positively associated with the NH_4^+ vector, with a wide spread along the salinity and FRP vectors. This group, referred to as the mixed algae assemblage, was dominated by flagellates (dinoflagellates, cryptophytes, chrysophytes, prymnesiophytes, euglenophytes and prasinophytes) and chlorophytes (some of which have flagellum). A smaller group of taxa negatively associated with NH_4^+ concentrations, referred to as the blooming taxa (circled in red), was dominated by the diatom *Cyclotella* sp. (dia.cyc). These blooming taxa were positively aligned with the significant DO vector, highlighting their association with high productivity. Within this distribution was the ^{222}Rn vector, which plots directly over *Cyclotella* sp. but is not significant. *Cyclotella* sp. was most abundant on 3/10/2014 at S6 and 11/11/2014 at S4, S5 and S6. After the high flow event, the abundance of *Cyclotella* sp. decreased to that of the start of the sampling period.

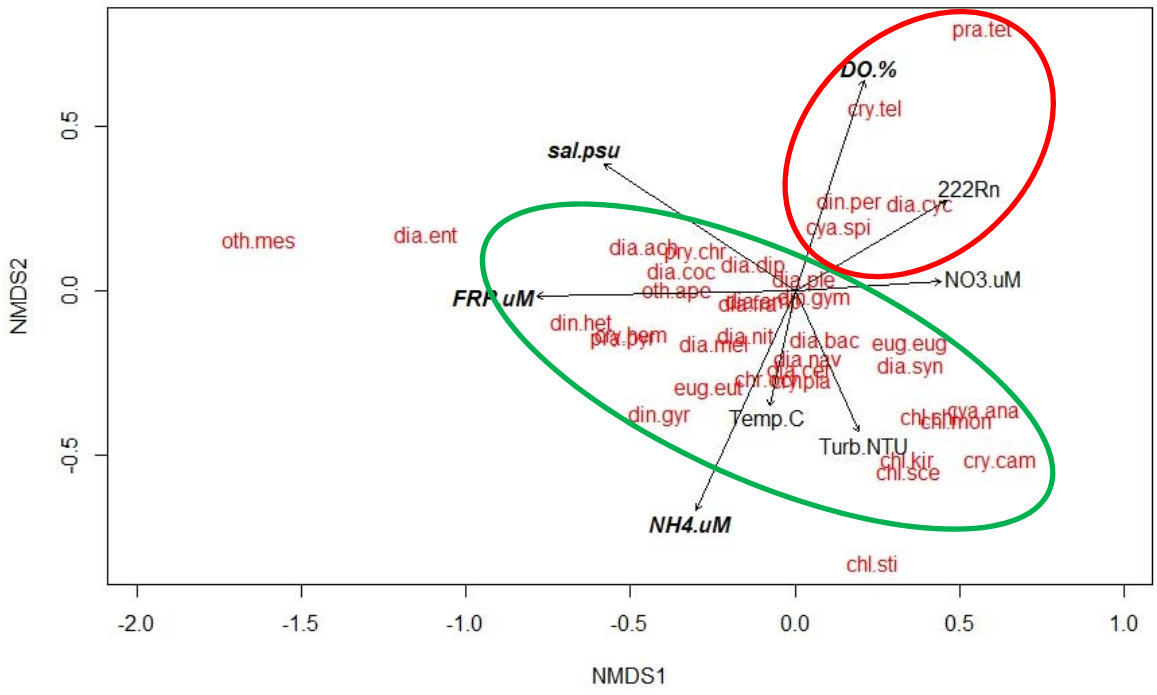


Figure 2.4: Plot of phytoplankton taxa rank abundance similarity (red; see Table 6.1 for abbreviations) overlayed with water quality vectors (black) using nonmetric multidimensional scaling ordination. Vectors italicised in bold are significant ($p < 0.05$) including DO, salinity, FRP and NH_4^+ (Table 6.2). Non-significant vectors remain because they provide further insights into the controls of taxa or groups (Table. 6.2). Dimensions = 3, transformation = square root, Bray-Curtis dissimilarity calculation, stress = 0.05. Rare taxa that were observed three times or fewer were excluded. The red circle represents the 3/10/2014-11/11/2014 bloom, the green circle represents the mixed algal assemblage.

The abundance of *Cyclotella* sp. and the other blooming taxa; the prasinophyte *Tetraselmis* spp. (pra.tet), the cryptophyte *Teleaulax acuta* (cry.tel), the dinoflagellate *Peridinium* sp. (din.per) and the cyanobacteria *Spirulina* sp. are depicted in Fig. 2.5a. The mixed algal assemblage is depicted in Fig. 2.5b and was dominated by *Euglena* spp. before the high flow event at S6 on 3/9/2014 when it contributed to half the total

phytoplankton abundance. The abundances of the mixed algal assemblage increased after the high flow event due to a number of taxa from the chlorophyte, chrysophyte and cryptophyte groups. In total, 86 phytoplankton taxa were identified from nine algal groups including; diatoms (22 taxa), chlorophytes (18), dinoflagellates (13), cryptophytes (9), cyanobacteria (7), chrysophytes (3), prymnesiophytes (3), euglenophytes (3) and prasinophytes (2), as well as 6 ungrouped taxa (other) including one ciliate; *Mesodinium rubrum*.

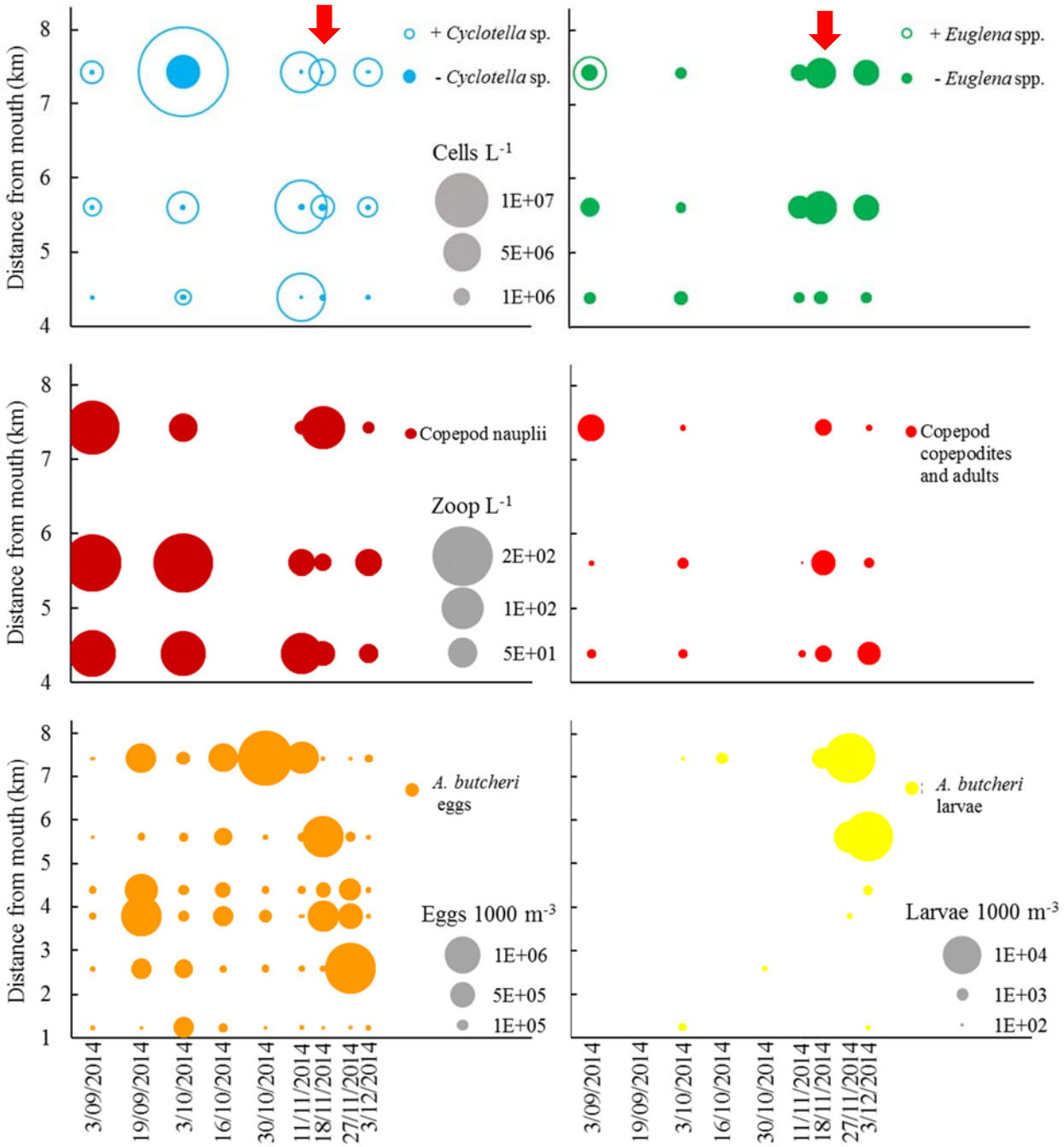


Figure 2.5: Abundance plots of a) blooming phytoplankton species including and excluding *Cyclotella* sp. (cells L^{-1}), b) the mixed algal assemblage including and excluding *Euglena* spp. (cells L^{-1}), c) calanoid nauplii (zoop L^{-1}), d) calanoid copepodites and adults (zoop L^{-1}), e) *A. butcheri* eggs (eggs 1000 m^{-3}) and f) *A. butcheri* larvae (larvae 1000 m^{-3}). The red arrows represents the high flow event.

The only significant physicochemical variable associated with the zooplankton community was NH_4^+ (Fig. 2.6). Increased NH_4^+ concentrations were found to be positively associated with calanoid copepods; *Gladioferens* (cal.glad), *Acartia* (cal.acar) and *Paracalanus* (cal.para), and cyclopoid copepods; *Oithona* (cyc.oith) and *Dioithona* (cyc.dio). Although marginally non-significant ($p < 0.1$), increased ^{222}Rn activities were observed to have a negative influence on those same taxa. Calanoid copepod abundances ranged from 0 and 47 zoop L^{-1} throughout the sampling period and were most abundant after the high flow event (and at S6 on 3/9/2014; Fig. 2.5d) when they contributed 5 to 35% of total zooplankton abundance. Cyclopoid and harpacticoid copepods were also identified but were low in abundances (0 to 2 zoop L^{-1}) except at S4 on 18/11/2014 when cyclopoid abundances were 6 zoop L^{-1}. Only calanoid copepods were observed with attached egg sacs. Copepod nauplii were more abundant than the adult copepods (7 to 196 zoop L^{-1}), but decreased in abundance throughout the sampling period (Fig. 2.5c). After the high flow event, their abundance initially increased at S6 but then continued to decrease. Total zooplankton abundance ranged from 45 to 480 zoop L^{-1}, of which 42% were copepod nauplii and adults on average. Other zooplankton identified included polychaetes, rotifers, mollusc larvae and crab zoea.

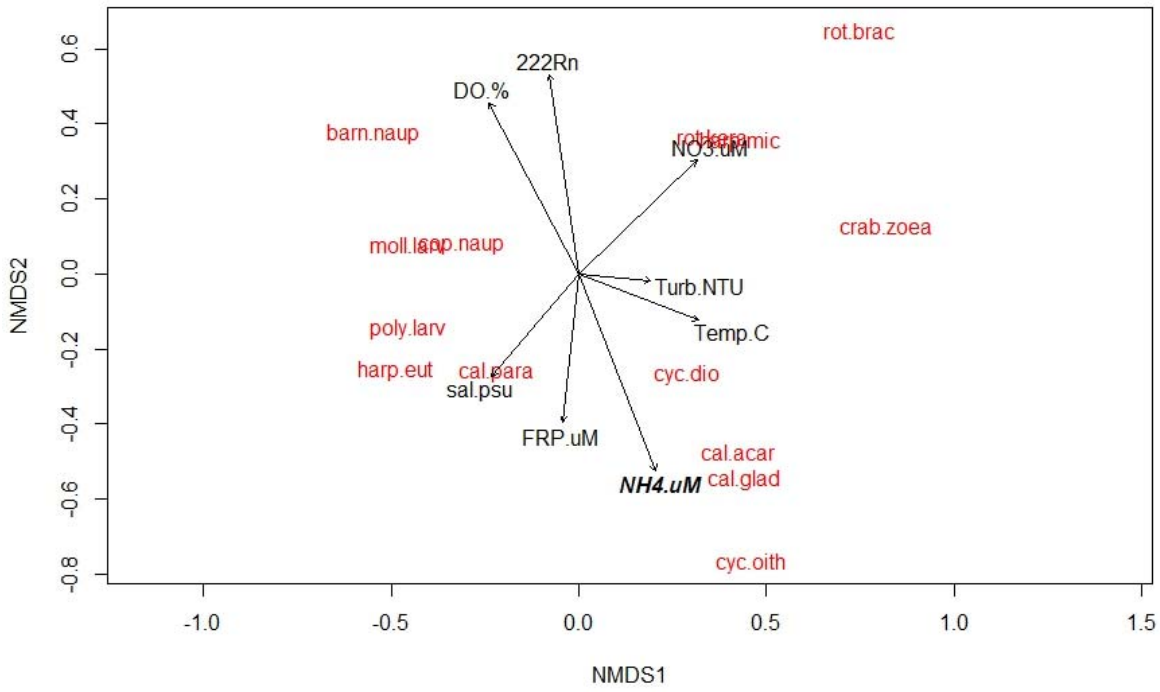


Figure 2.6: Plot of zooplankton taxa rank abundance similarity (red; see Table 6.3 for abbreviations) overlayed with WQ vectors (black) using nonmetric multidimensional scaling ordination. Vectors italicised in bold are significant ($p < 0.05$) including NH_4^+ (Table. 6.4). Non-significant vectors remain because they provide further insights into the controls of taxa or groups (Table. 6.4). Dimensions = 3, transformation = T, Bray-Curtis dissimilarity calculation, stress = 0.065.

In total, 2186817 eggs and 8531 larvae of *A. butcheri* were collected. Eggs and larvae were found at all six sites, with their abundances varying spatially and temporally. Most eggs (55%) and larvae (96%) were collected from sites S5 and S6. Eggs were found in abundance throughout the sampling period, with peak egg abundances collected from late October and throughout November (50%) (Fig. 2.5e). Larvae were first found on 3/10/2014, with peak larval abundances collected in late November to early December (Fig. 2.5f). *A. butcheri* larvae were only a small fraction (~5%) of the total fish larvae collected.

2.5 Discussion

2.5.1 Physicochemical structuring of the phytoplankton community

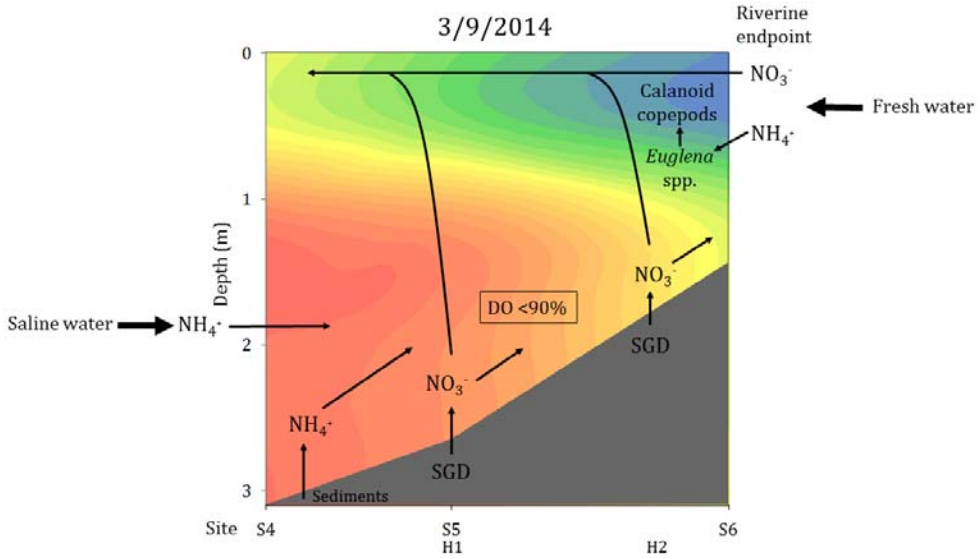
The results of this study reinforce the role of salinity and nutrients in structuring the phytoplankton community and highlighted the importance of freshwater inflows in driving estuarine productivity. Their interactions are outlined temporally using a conceptual diagram (Fig. 2.7). For the purposes of this discussion, we have split the phytoplankton community into the mixed algal assemblage and blooming taxa noted in the Results section and highlighted on the NMDS (Fig 2.3). When the role of FRP was explored it was found to stimulate growth in only a small number of less abundant taxa on one date when FRP was no longer limiting. Therefore, we can conclude that only some taxa were stimulated by increased FRP concentrations and it is not a main driver of the phytoplankton community as a whole. We expected that there would be a strong positive relationship between high NO_3^- inputs from groundwater and phytoplankton biomass. There was, however, no significant relationship between phytoplankton biomass and NO_3^-. In contrast, NH_4^+ played a significant role in structuring the phytoplankton community and the mixed algal assemblage had a positive relationship with NH_4^+ (Fig. 6.1a; $p < 0.04$). The maximum biomass of this group was typically observed during periods of lowest salinity when flagellates such as *Euglena* spp. and *Plagioselmis prolonga* (cryptophyte) and chlorophytes such as *Stichococcus* sp. and *Scenedesmus* sp. were dominant. Concentrations of NH_4^+ in the river water and groundwater were very low compared to the estuary during these periods, and the most likely source of NH_4^+ is therefore entrainment of NH_4^+ from the salt-wedge. This in turn appears to have two origins in this study – the sediment and Port Phillip Bay. On 3/9/14, the origin appears to

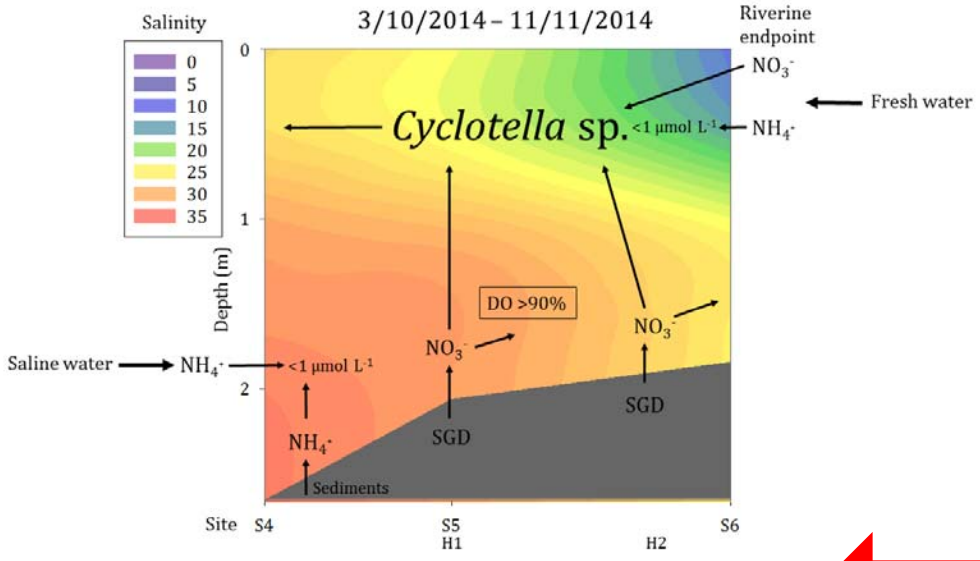
have been Port Phillip Bay as indicated by the highest observed concentrations being in the lower estuary which is influenced by the plume of the Western Treatment Plant. On 18/11/2014, after the high flow event, the highest NH_4^+ concentrations were observed at the tip of the salt-wedge in the upper estuary coinciding with low bottom water DO concentrations, and the most likely origin in this case was the recycling of organic matter within the sediment. This process would have been enhanced by the low DO in the upper estuary inhibiting nitrification and possibly also the higher input of organic matter from the river. This finding highlights the key importance of recycled nitrogen in driving estuarine productivity, and the way in which recycling was facilitated by increased stratification and decreased bottom water DO after the EFR.

The blooming taxa were negatively associated with NH_4^+ in the NMDS plot and increased in abundance on 3/10/2014 when the concentration of NH_4^+ had decreased to ≤1 µmol L^{-1} for the first time during the sampling period. *Cyclotella* sp. dominated the bloom and was the only blooming taxon to increase in abundance at S4 and S5 on 11/11/2014 when NH_4^+ concentrations also decreased to <1 µmol L^{-1} at those sites (Fig. 2.7, panel 2). *Cyclotella* sp. is a centric diatom, which commonly forms blooms as a result of its rapid assimilation of NO_3^- (Vanni 1987). It has previously been shown that *Cyclotella* sp. can dominate algal communities when NO_3^- concentrations are between 20 and 180 µmol L^{-1} in culture studies (Vanni 1987, Amano et al. 2012) and field studies (Muylaert & Sabbe 1996, El-Kassas & Gharib 2016). High NO_3^- concentrations were consistently found in the Werribee River Estuary throughout the study period, however, *Cyclotella* sp. was only found to dominate the phytoplankton community (i.e. reach abundances >2000 sqrt[cells L^{-1}]) when NH_4^+ concentrations were <1 µmol L^{-1}. The uptake of NH_4^+ requires less energy than NO_3^- uptake and therefore its presence likely

promoted the growth of the mixed algal assemblage and inhibited the growth of *Cyclotella* sp. (Dortch 1990, Lomas & Glibert 1999a, Glibert et al. 2016). Low NH_4^+ concentrations followed a bloom of *Euglena* spp. on 3/9/2014 (Fig. 2.7; panel 1), highlighting the role of nitrogen speciation controlling phyoplankton community succession (Fig. 2.7; panel 2) (Vilaclara et al. 1988, Pan & Rao 1997, Dugdale et al. 2007).

At first glance, the lack of a relationship between NO_3^- concentrations and *Cyclotella* sp. or the blooming taxa as a whole was surprising. This may be due to the differing time scales of phytoplankton growth and nutrient supply, as well as loss by advection when residence times decreased after high flows. However, residence times were often high in the upper estuary, allowing phytoplankton to bloom in high nutrient zones and therefore the lack of relationship was most likely due to active assimilation of NO_3^- by phytoplankton. There was, however, a significant relationship between bottom water ^{222}Rn activities and *Cyclotella* sp. (Fig. 6.1b; $p < 0.04$). Wong et al. (2013) concluded that correlation between NO_3^- concentrations and ^{222}Rn activities in the Werribee River Estuary implied that groundwater discharge was the dominant source of NO_3^-. Additionally, in our study, high concentrations of both ^{222}Rn and NO_3^- occurred in the groundwater water at both hotspots. These results suggested that ^{222}Rn activities in the estuary are a legacy tracer of NO_3^- input over a timescale of days; and that NO_3^- from SGD was the main source that caused the bloom of *Cyclotella* sp. (Fig. 2.7; Panel. 2). These findings are consistent with a small but growing body of evidence that link groundwater inputs to algal blooms in estuaries. At Little Lagoon, Alabama (USA) (a site of recurring harmful algal blooms), Su et al. (2014) concluded that a correlation between chlorophyll *a* concentration and Ra isotope activities ($^{223,224,226}Ra$) reflected assimilation





High flow event

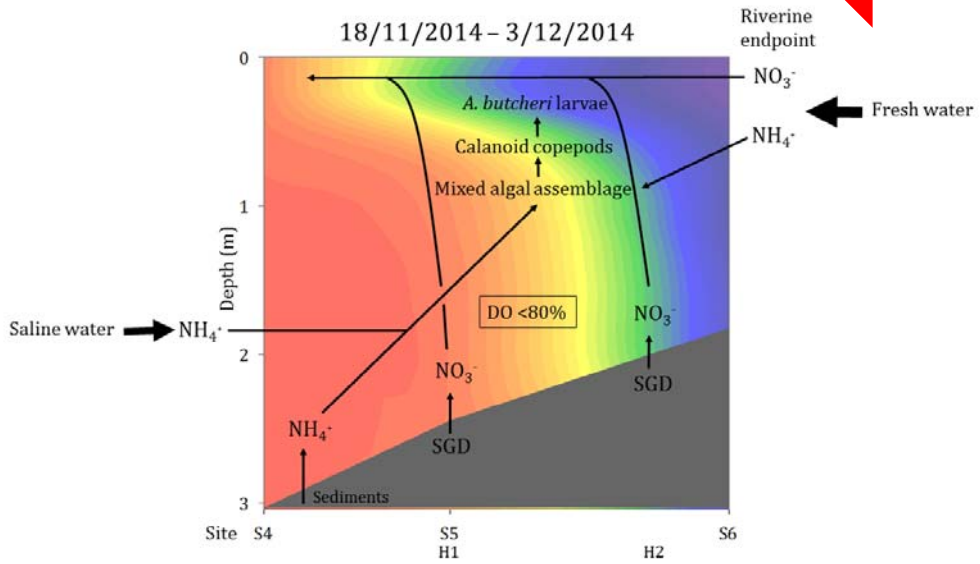


Figure 2.7: A conceptual diagram of phytoplankton, zooplankton and *A. butcheri* larvae communities and abundances in the upper Werribee River Estuary throughout the 2014 sampling period in response to the high flow event (red arrow), salinity stratification (contour plot), DO levels, NO_3^- additions (via SGD and riverine inputs) and NH_4^+ additions (via riverine and bay inputs and recycling from the sediments).

Panel 1: At the start of the sampling period a *Euglena* spp. bloom was driven by NH_4^+ inputs at S6, calanoid copepods were present but *A. butcheri* larvae were not.

Panel 2: before the high flow event NH_4^+ concentrations were depleted allowing for increased NO_3^- uptake and a bloom of *Cyclotella* sp., calanoid copepods disappeared, *A. butcheri* larvae remained absent.

Panel 3: after the high flow event stratification strength increased, NH_4^+ concentrations increased via remineralisation, a mixed algal assemblage became dominant, calanoid copepod abundances increased, and *A. butcheri* larvae were present in high abundances.

of N and P. Similarly, Basterretxea et al. (2010) and Lee et al. (2010) discovered that ^{224}Ra activities correlated with chlorophyll *a* concentrations and concluded that SGD drove phytoplankton biomass in Majorca, Spain and red tides off the southern coast of the Korean peninsula respectively.

2.5.2 Drivers of zooplankton and larval Acanthopagrus butcheri *dynamics*

Copepods form an important link in the estuarine food web between phytoplankton and *A. butcheri* larvae (Williams et al. 2013). Calanoid copepods were the dominant copepod group within the Werribee River Estuary. The majority of the copepod nauplii present

were likely calanoid copepod nauplii, based on the observation of egg bearing calanoid copepods. A marginally non-significant negative relationship existed between calanoid copepod abundance and *Cyclotella* sp. abundance (Fig. 6.2a; $p = 0.06$), as indicated by the marginal influence of ^{222}Rn on the zooplankton community (Fig. 2.6; $p < 0.09$). This relationship alluded to a possible deleterious effect of blooming *Cyclotella* sp. on calanoid copepods. There is no evidence within the literature to support this contention and according to Nival and Nival (1976), *Cyclotella* sp. are too small to be the main food source of copepods. However, at such high abundances it is possible they were consumed incidentally. Diatoms are known to promote somatic growth in copepods but more recently have been found to be deleterious to copepod egg hatching success and naupliar survival (Gifford & Dagg 1988, Kleppel 1993, Chaudron et al. 1996, Ban et al. 1997, Turner et al. 2001, Ianora et al. 2003, Paffenhöfer et al. 2005, Lauritano et al. 2012). Miralto et al. (1999) demonstrated that while a diatom bloom triggered increased egg production rate in two copepod taxa, it led to low egg viability and hatching rates (<25%). This was due to diatoms containing antiproliferative compounds from three aldehydes, which caused detrimental effects to copepod embryos (Miralto et al. 1999). Similarly, Turner et al. (2001) concluded that antimitotic compounds in some diatoms arrest normal embryonic division and cause reproductive failure. This question remains unresolved however, and a number of critics suggest that deleterious effects on copepod eggs is more related to nutritional inadequacy of the algal cell (Jónasdóttir et al. 1998 and references therein). Furthermore, if *Cyclotella* sp. had deleterious effects on calanoid copepod egg viability and hatching success within the Werribee River Estuary, a negative relationship should exist between *Cyclotella* sp. abundance and copepod nauplii abundance. No such relationship was observed, however, the time scale of this study may not have captured the generation time of copepods.

In contrast, the dominance of the mixed algal community was linked to increased calanoid copepod biomass. As indicated by the NMDS, calanoid copepod abundances increased significantly in response to NH_4^+ concentrations (Fig. 6.2b; $p < 0.01$) and also to increased abundances of the mixed algal assemblage at S5 and S6 (Fig. 6.2c; $p < 0.03$). Therefore the contention that the post diatom bloom of mixed phytoplankton is linked to the bulk annual recruitment of copepods (Kleppel et al. 1991, Runge & Lafontaine 1996, Jones et al. 2002) was well supported by this study. However, this relationship did not hold for S4 where mixed algal abundances were low and varied little throughout the sampling period. This may be due to the lack of stratification at that site compared to S5 and S6, where mixing at the freshwater/seawater interface would cause nutrient accumulation at the halocline (Viličić et al. 1989, Cloern 1991). The presence of this high nutrient zone would lead to increased phytoplankton abundances. Copepods are also known to congregate at the halocline and therefore, increased predator-prey interaction at that zone will result in the stronger relationship observed (Harder 1968, Lougee et al. 2002).

The presence of calanoid copepods is important for the survival of *A. butcheri* larvae, which are very selective consumers of calanoid nauplii and copepodites (Willis et al. 1999). Although *A. butcheri* egg abundances were often high throughout the study period, larval abundances were initially low or absent, possibly due to low calanoid copepod abundances. Following the high flow event and increased calanoid copepod biomass, *A. butcheri* larvae were observed in high abundance in the upper estuary (Fig. 2.7; panel 3). Freshwater flows resulted in increased stratification in this region, which have been linked with year class strength of *A. butcheri* (Jenkins et al. 2010, 2015). Greater *A. butcheri* egg and larval abundances are found at the halocline due to the neutral buoyancy

of *A. butcheri* eggs at intermediate salinities (Jenkins et al. 1999, Nicholson et al. 2008, Williams et al. 2012, 2013). Therefore, *A. butcheri* larvae that hatched after the flow event may have had a greater chance of encountering the more abundant calanoid copepods at the halocline. Once again, productivity was greatest at S5 and S6 where the halocline was strongest, further emphasising the importance of environmental flows in regulated river systems. In fact, this study is a rare example of how environmental flows can be used to stimulate aquatic production. Most environmental flows objectives are aimed at promoting movement of biota in a systems or the creation of a habitat (Sponseller et al. 2013). The idea of using an environmental flow to stimulate desirable production to support higher level objectives is not new (Rolls et al. 2012, Rolls et al. 2017), yet there are relatively few examples of this being tested and achieved in practice.

2.6 Conclusion

The sequence of events that led to the successful hatching and survival of *Acanthopagrus butcheri* larvae in the Werribee River Estuary were traced back to both biogeochemical processes and hydrodynamics. The release of an environmental flow during this study was found to be the mechanism that drove estuarine productivity and facilitated *A. butcheri* larval survival. Increased freshwater flows strengthened stratification within the upper estuary, which caused a decline in bottom water DO and the release, accumulation and entrainment of NH_4^+. This promoted the growth of a diverse mixed algal assemblage, which was linked to the increased calanoid copepod population. As a result, *A. butcheri* larvae were observed in abundance in the upper estuary, where the increased strength of the halocline led to increased predator-prey interaction.

Before the high flow event, high NO_3^- loads via SGD were the most likely cause of a bloom of the diatom *Cyclotella* sp. The bloom was facilitated by high residence times in the upper estuary and the depletion of the NH_4^+ pool, limiting competition from phytoplankton that preferentially assimilate NH_4^+. These results confirm that SGD is an important nutrient source to the Werribee River Estuary driving primary productivity. However, the resultant shift to a diatom dominated phytoplankton assemblage caused a decrease in calanoid copepod biomass, and therefore a lack of food for *A. butcheri* spawned at this time. These conditions were alleviated by the EFR and high rainfall event and as such, there is good evidence to support the provision of environmental flows as not only a remediation technique to improve habitat and system connectivity, but a way to stimulate the estuarine food web and aid in the survival of *A. butcheri* larvae. Further

work is required to fully understand how to best deliver environmental flows (i.e. the timing and magnitude) to maximise success of *A. butcheri* larvae.

2.7 Acknowledgements

The authors wish to thank Melbourne Water and the Department of Environment, Land, Water and Planning (DELWP) and in particular Bill Moulden, David Dawson, Fiona Warry & others who contributed to work in the field. We also thank Microalgal Services for their work on phytoplankton identification and counts. This work has been funded by the Australian Research Council Linkage Project Scheme (LP140100087), The University of Melbourne, Department of Environment, Land, Water and Planning (VIC), Gippsland Lakes Ministerial Advisory Committee, Water Technology and Melbourne Water. Caitlyn McNaughton was supported by a Monash University scholarship.

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Chapter 3: Salt wedge intrusion as the driver of spring productivity in the upper estuary of an unregulated river system.

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The Mitchell River estuary near site 3. Credit: Howard Stephenson https://www.flickr.com/photos/stara/7098020469/

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3.1 Abstract

Freshwater flows exert a strong control on stratification strength in salt wedge estuaries, which affects mixing forces, nutrient cycling and other important processes. In this study, salt wedge intrusion and its effects on the estuarine food web was investigated in the upper estuary of the Mitchell River, Australia. Strong salinity stratification depleted bottom water oxygen facilitated the release of recycled benthic nutrients. In the bottom water, high NH_4^+ concentrations supported phytoplankton biomass (as chlorophyll *a*), dominated by flagellate phytoplankton during high flow. Intermediate and low flows (<730 ML day^{-1}) and slightly increased surface water NO_3^- concentrations coincided with high phytoplankton biomass at the halocline, dominated by diatoms. A high flow event, and subsequent re-intrusion of the salt wedge in the middle of the sampling period, increased salinity and decreased DO in the bottom water. In response, distinct surface and bottom water phytoplankton assemblages occurred dominated by chlorophytes in the surface water (positively correlated to NO_3^-; $p < 0.05$) and the marine diatom S*keletonema costatum* in the bottom water. At this time flagellates provided less competition for available nutrients, possibly due to increased predation pressure by cyclopoid copepods, which appeared in high abundances after salt wedge intrusion. Freshwater fish larvae transported to the estuary during high flow periods were present in the upper sites. Small estuarine fish larvae were present at lower sites but the larvae of larger estuarine species were not observed. These results highlight the way in which freshwater flows, salt wedge intrusion and maintained stratification control nutrient cycling and the estuarine habitat, both of which determined estuarine productivity.

3.2 Introduction

Estuaries are highly productive coastal waterways where complex physico-chemical habitats are created by the mixing of freshwater and seawater, often leading to intense stratification. The interface between freshwater and seawater (the halocline) entrains nutrients and phytoplankton (Correll 1978, Holligan et al. 1984, Viličić et al. 1989, Cauwet 1991, Geyer 1993), and therefore primary productivity is strongly coupled with stratification (Malone et al. 1988, Cloern 1991, Lucas et al. 1998, Kimmerer 2002, Thompson 1998).

The degree of water column stratification is controlled predominantly by freshwater flows (Kurup et al. 1998, Kimmerer 2002). High freshwater inflows during winter and after episodic rainfall events, can entirely remove the saline layer and shorten estuarine residence times, which has been linked to decreased primary productivity (Alpine & Cloern 1992, Borsuk et al. 2004, Saeck et al. 2013). As inflows decrease in spring and summer, the salt wedge intrudes, increasing residence times and stimulating phytoplankton growth (Alpine & Cloern 1992, Holmes et al. 2000, Thompson 2001, Valiela & Bowen 2002, Ferguson et al. 2004).

The cycling of new and regenerated nutrients within the salt wedge plays an important role in the timing of spring and summer phytoplankton biomass peaks. Watershed nutrients have been found to promote spring phytoplankton peaks (Malone et al. 1988, Holmes et al. 2000, Watanabe et al. 2014), which are followed by summer peaks promoted by regenerated nutrients from the salt wedge (Malone et al. 1988, Alpine & Cloern 1992, Valiela et al. 1992, Holmes et al. 2000, Watanabe et al. 2014). The timing

of these events is also important for larval fishes, many of which are spawned and hatched in estuaries during spring and summer, when zooplankton biomass increases in response to phytoplankton biomass. However, anthropogenic activities, such as water abstraction, decrease freshwater flows and promote salt water intrusion, which may alter nutrient dynamics.

Benthic nutrient recycling is strongly controlled by salt wedge intrusion and estuarine stratification. High residence times in the tip of the salt wedge, promote low dissolved oxygen (DO) conditions, which has been linked with greater release of nitrogen (N) and phosphorus (P) from the sediments (Margalef 1978, Cloern 1984, Davis & Koop 2006, Wilson 2008). Studies have also linked increased porewater salinity due to salt wedge intrusion, to the release of ammonium (NH_4^+) (Haedrich 1983, Ketchum 1983, Milton & Arthington 1985, Paerl et al. 2001, Weston et al. 2010, Parker et al. 2012), which accumulates in low DO conditions as the potential for nitrification decreases. Lack of mixing into the upper freshwater layer also causes nutrient accumulation in the salt wedge (Cloern et al. 1983, Cauwet 1991, Ueda et al. 2005, Cook et al. 2010). These nutrient dynamics may play an important role in the food web in the upper estuary, where salt wedge intrusion fluctuates in response to episodic inflows.

Salt wedge intrusion and its physico-chemical affects on nutrient availability and chlorophyll *a* dynamics have previously been studied over multiple seasons by Kasai et al. (2010) in the Yura Estuary, Japan, which is highly modified due to a number of dams designed for flood control, water supply and hydropower. Watanabe et al. expanded on that study in 2014, and were the first to also examine the affects of salt wedge intrusion on phytoplankton and zooplankton community dynamics. In both studies, high riverine

derived nutrient concentrations facilitated the majority of marine and freshwater phytoplankton growth under the salt wedge regime (Kasai et al. 2010, Watanabe et al. 2014). However, there have been no studies of these dynamics in relation to biological production and fish larvae in the upper reaches of a nutrient poor estuary with unmodified flow. Such information is needed as a baseline to inform environmental flow requirements, which are commonly aimed at the maintenance of target species populations, but often overlook the nutrient and food web dynamics that determine trophic level responses (Rolls et al. 2017).

The Mitchell River, Victoria, was chosen as a study site because it is one of the largest unregulated rivers in SE Australia with a relatively intact forested catchment, low nutrient concentrations and high water clarity, and is known as an important spawning site of an important endemic fishery species black bream *Acanthopagrus butcheri* Munro, 1949. The aim of this study was to investigate the dynamics of the physical structure of the estuary and how this related to nutrient concentrations, primary production and phytoplankton, zooplankton and fish community composition and abundance. We found that the salt wedge was an important site for phytoplankton productivity driven by the recycling of nitrogen in the form of NH_4^+ and low riverin inputs of NO_3^-.

3.3 Methods

3.3.1 Site description

The Mitchell River estuary passes through Bairnsdale, 280 km east of Melbourne, Australia. The river begins in the Victorian Alps and drains into The Gippsland Lakes, a large series of interconnected coastal lagoons that flow into Bass Straight via a permanently open artificial sea-entrance constructed in 1889. Saline water from the Gippsland Lakes intrudes into the Mitchell River estuary as far as a rock sill, 25 km upstream, creating a salt wedge estuary below this point (Fig. 3.1). The average river flow varies throughout the year, typically 1450 to 3350 ML day^{-1} in winter and 350 to 1600 ML day^{-1} in summer during the last 10 years (http://www.bom.gov.au/waterdata/; station number 224217B). These flows are un-regulated and the Mitchell River is the largest un-regulated river system in Victoria, however it does provide agricultural irrigation water to the Lindenow valley and supports urban withdrawals for regional cities (Southern Rural Water 2014). Three sampling sites were chosen in the upper reaches of the Mitchell River estuary approximately 3 km apart; S1 (16.2 km upstream), S2 (13.5 km upstream) and S3 (10.3 km upstream; Fig. 3.1). These sites were chosen as they are at the tip of the salt wedge and this region has previously been shown to have high productivity (Williams et al. 2012, 2013). The depths at these sites, and throughout most of the estuary, range from 4 to 6 m.

Figure 3.1: A map of the Mitchell River Estuary including the three main sampling sites (S1 to S3), the extra fish larvae sampling sites (S4 to S6), the rock sill and the flow and rainfall monitoring site at Rosehill.

3.3.2 Field sampling methods

Each site was sampled five times, approximately fortnightly, on the dates 6/10/2015, 19/10/2015, 9/11/2015, 24/11/2015 and 7/12/2015. A multi-parameter water quality sonde (Hydrolab DS5X) was used to measure depth (m), salinity (Practical Salinity Units; PSU), temperature (°C), chlorophyll *a* (µg L^{-1}), dissolved oxygen (%) and turbidity (Nephelometric Turbidity Unit; NTU) profiles at 25 cm intervals throughout the water column at each site. Filtered water samples were taken from the surface, halocline and bottom water at each sampling site using 0.22-µm filters. A known volume of water was also passed through 0.7 µm Whatman GF/F filters to capture particulate organic matter

(POM). Phytoplankton samples were collected in the filtrate that passed through an 80 μm sieve and preserved using a Lugol's Iodine solution. Vertical net tows (30 cm diameter conical net, 80 μm mesh,) were used to capture zooplankton samples which were preserved using 96% ethanol. Fish larvae were collected via oblique net tow (75 cm dia. conical net, 333 μm mesh) using a flow meter to determine volume sampled and preserved using 96% ethanol. On 19/10/2015, 9/11/2015 and 7/12/2015, larval tows were also undertaken at three downstream sites; S4, S5 and S6 (Fig. 3.1).

3.3.3 Laboratory methods

Filtered water samples were analysed for ammonium (NH_4^+), nitrate (NO_3^-) and filterable reactive phosphorus (FRP). Their concentrations were determined spectrophotometrically using a Lachat QuikChem 8000 Flow Injection Analyser (FIA), following standard procedures (APHA 2005). POM collected on filters was extracted with acetone and analysed spectrophotometrically according to Strickland and Parsons (1972). The chlorophyll *a* concentrations (μg POM L^{-1}) were then determined using the equations of Jeffrey and Humphrey (1975). Phytoplankton samples from the surface, halocline and bottom water at S2 were sent to Microalgal Services, Victoria, where species were identified and counted using a Zeiss Standard compound microscope equipped with Phase Contrast Optics with up to 400× magnification. A Sedgewick-Rafter counting chamber was used and data was reported in cells L^{-1} using reference material from Tomas (1997). Zooplankton from all dates and sites were counted in the Water Studies Centre (WSC), Monash University, Clayton, under a light microscope at 100× magnification using a Sedgewick-Rafter counting cell, with reference to Richardson et al. (2013). Fish larvae samples were separated using a Folsom plankton splitter (Williams et al. 2013), and

counted in the laboratory using a compound microscope with 20× magnification. Larvae were identified using published descriptions (Neira et al. 1998).

3.3.4 Climate data

Average daily flow (ML day^{-1}) and daily rainfall (mm) data from the water monitoring station at Rosehill, ~10 km upstream of S1 (Fig. 3.1), was accessed from http://www.bom.gov.au/waterdata/; station number 224217B and http://www.bom.gov.au/climate/data/; station numbers 084147 respectively.

3.3.5 Statistical analysis

Nonmetric Multidimensional Scaling (NMDS) was used to determine the community structure of phytoplankton at each depth at S2 and zooplankton across all sites during the study period. This allowed patterns in community composition across depths/sites and dates to be visualised and interpreted on a single plot using rank-order dissimilarities. Analyses were done using the vegan package in R 3.3.2 (R Core Team 2016). A Bray-Curtis dissimilarity matrix was calculated from auto-transformed abundances using the metaMDS function. Relevant water quality vectors were then fitted to these ordinations using the envfit function (constrained correspondence analysis) to assess potential drivers of community composition. These vectors included surface, bottom and halocline DO, salinity, temperature, NO_3^-, NH_4^+, FRP and flow, as well as chlorophyll *a* on the zooplankton NMDS. Significant linear relationships were determined using a Pearson correlation matrix with the Hmisc package in R (Harrell Jr 2016). For all analyses $p < 0.05$ was the level of significance set for the rejection of the null hypothesis.

3.4 Results

3.4.1 Rainfall and flow

The daily rainfall and average riverine flow entering the estuary are shown in Figure 3.2. High flows of 5011 ML day^{-1} occurred in late August before the sampling period and decreased to 420 ML day^{-1} by 7/12/2015. The largest peak in average flow during the sampling period was 2592 ML day^{-1}, which occurred on 23/10/2015 after high rainfall fell throughout the catchment in the preceding days.

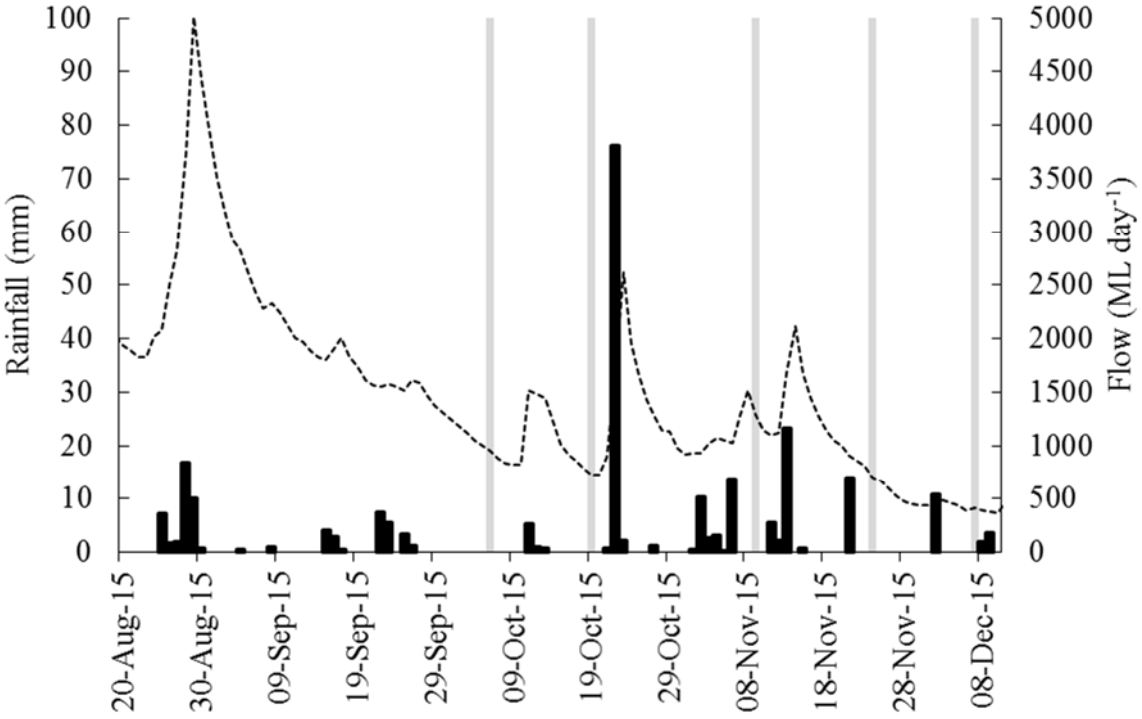

Figure 3.2: River flow (ML day^{-1}; dotted line) and rainfall (mm; black bars) at Rosehill before and throughout the sampling period. Grey bars indicate sampling dates.

3.4.2 Water quality parameters

Salinity stratification was observed throughout the sampling period with salinities typically ranging from 0 to 2 in the surface water and 14 to 21 in the bottom water (Fig. 3.3). The halocline occurred between 2 and 3 m over a depth of 30 cm. Only on one date (9/11/2015) was salinity stratification absent at S1, when the water column was entirely fresh. Dissolved oxygen (DO) was generally high in the surface water (80% to 120%) and low in the bottom water (0% to 30%), showing a similar pattern to salinity stratification (Fig. 3.3). The only date when DO was distinctly different from salinity stratification was 6/10/2015. On that date DO was high (75% to 100%) in the bottom water at S1 and S2, and only decreased to 25% below 4 m of depth at S3. On 9/11/2015, DO remained at 100% from surface to bottom. Temperature stratification was also present and surface waters were consistently 3 to 4 °C warmer than bottom waters, except on 9/11/2015 (Fig. 3.3). Temperatures increased throughout the sampling period from 19 to 23 °C in the surface water and 15 to 20 °C in the bottom water. Turbidity measurements peaked at 31.3 NTU on 7/12/2018 but the average turbidity ranged between 5.8 and 25 NTU throughout the sampling period.

Chlorophyll *a* concentrations were highest on 6/10/2015 when they peaked in the bottom water (Fig. 3.3). The highest concentration occurred at S1 (25 μg L^{-1}), followed by S2 (18 μg L^{-1}) and S3 (13 μg L^{-1}). However, when these values were compared against extracted chlorophyll *a* from POM they were found to be more than double the extracted concentrations, but did exhibit a significant positive correlation (Fig. 6.3; $p < 0.05$). All other chlorophyll *a* measures were found to correlate significantly at a ratio of ~ 1:1 (Fig. 6.3; $p < 0.05$). On 19/10/2015, 24/11/2015 and 7/12/2015 chlorophyll *a* concentrations

peaked at the halocline and slightly into the bottom water (ranging from 2.5 to 8 μg L^{-1}; Fig. 3.3). Chlorophyll *a* concentrations were lowest and most uniform on 9/11/2015 when they ranged from 0.5 to 1.6 μg L^{-1} throughout the water column at all sites, peaking in the bottom water at S3.

On 6/10/2015 NH_4^+ concentrations were <2.5 μmol L^{-1} in the bottom water at S1 and S2 but increased at S3 to 35 μmol L^{-1} (Fig. 3.3). Over the following dates NH_4^+ concentrations peaked at 15-35 μmol L^{-1} in the bottom water when salinity stratification was present and decreased to <3 μmol L^{-1} above the halocline. Lower concentrations were observed on 7/12/2015 when NH_4^+ was 6.5, 2 and 9 μmol L^{-1} in the bottom water of S1, S2 and S3 respectively. FRP concentrations were <2.2 μmol L^{-1} throughout the sampling period but were most concentrated in the bottom water and increased in concentration towards the end of the sampling period (Fig. 3.3). NO_3^- concentrations were <4.3 μmol L^{-1} throughout the sampling period and peaked in the surface water on 19/10/2015, 24/11/2015 and 7/12/2015 (Fig. 3.3). On 6/10/2015 and 9/11/2015, NO_3^- concentrations were low in the surface water and instead peaked in the bottom water at S3. Mixing of these nutrients along the salinity gradient showed an overall consumption of NH_4^+ and FRP within the estuary, while NO_3^- addition was found to occur in the surface and halocline on the dates when NO_3^- peaked above the halocline.

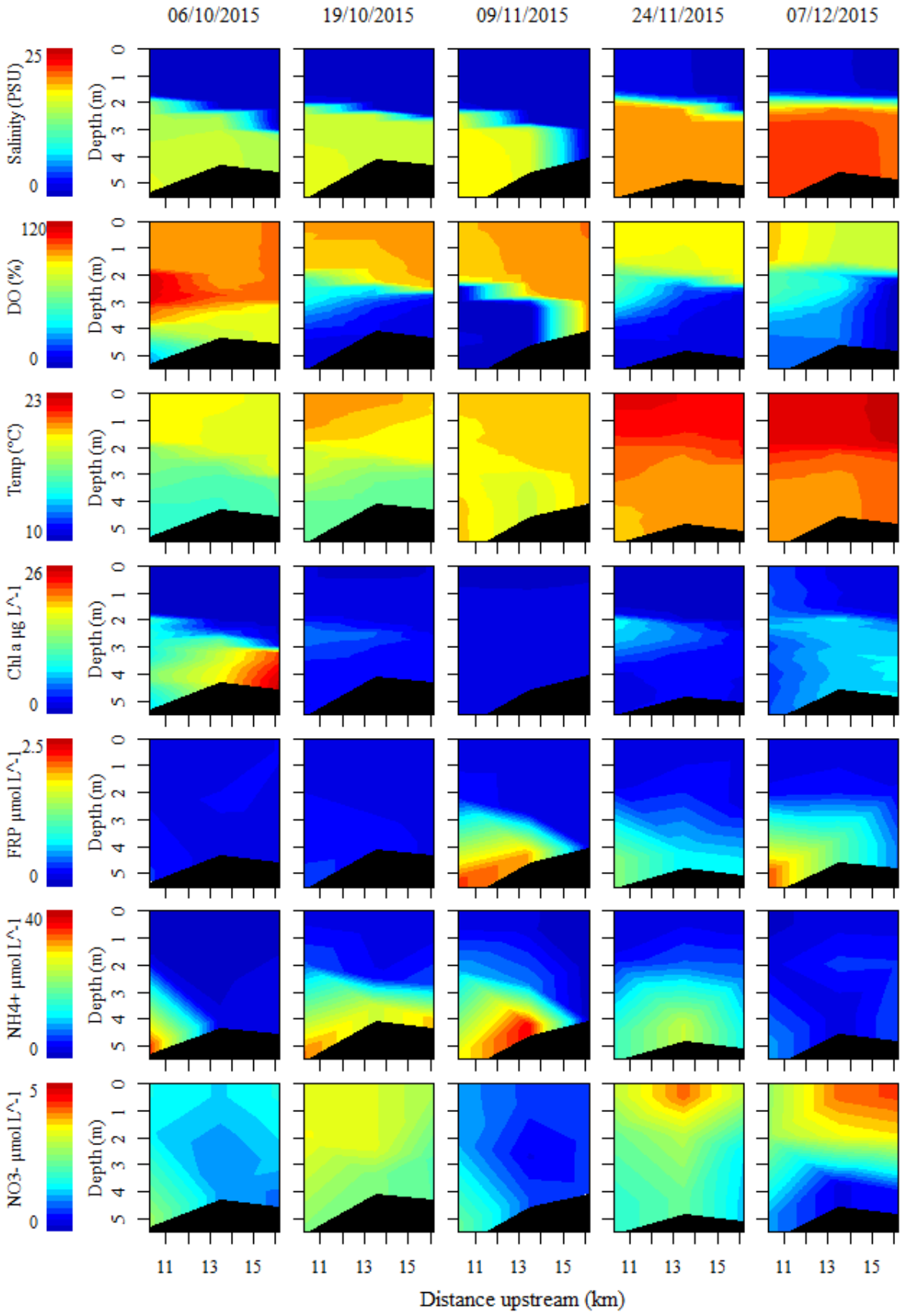
06/10/2015
19/10/2015
09/11/2015
24/11/2015
07/12/2015
Salinity (PSU)
25
0
DO (%)
120
0
Temp (°C)
23
10
Chl a µg L^-1
26
0
FRP µmol L^-1
2.5
0
NH4+ µmol L^-1
40
0
NO3- µmol L^-1
5
0
Depth (m)
0 1 2 3 4 5
11 13 15
Distance upstream (km)

Figure 3.3: Contour plots depicting the change in salinity (PSU scale), DO (%), Temperature (°C), Chlorophyll *a* (µg L^{-1}), NH_4^+ (µmol L^{-1}), FRP (µmol L^{-1}) and NO_3^- (µmol L^{-1}) throughout the water column in the upper estuary (S1 to S3) on each sampling date.

3.4.3 Phytoplankton, zooplankton and larval fish communities

Fifty-eight phytoplankton genera were identified from nine groups including diatoms (26), chlorophytes (6), cyanoprokaryota (1), and the flagellates; dinoflagellates (8), cryptophytes (7), chrysophytes (3), prasinophytes (3), prymnesiophytes (3) and euglenophytes (1) (Table. 6.5). Throughout the sampling period, 39 genera occurred in the surface water, 51 at the halocline and 45 in the bottom water. Although phytoplankton were consistently more diverse at the halocline, total abundance was only greatest there on 19/10/2015 and 24/11/2015 (Fig. 3.4). On 6/10/2015 and 9/11/2015 total phytoplankton abundances were greatest in the surface water, while on 7/12/2015 abundances were greatest in the bottom water. Flagellate genera, dominated by the cryptophyte *Hemiselmis* sp., dominated the algal assemblage on 6/10/2015 and 9/11/2015, contributing to 68-75% of total abundance (Fig. 3.4). Cryptophytes were also abundant in the halocline and bottom water on 24/11/2015, but diatoms became the most dominant group on all other sites and dates (Fig. 3.4). As a group, diatoms were most dominant on 19/10/2015 and in the bottom water on 7/12/2015, making up 63 to 74% of the total phytoplankton abundance (Fig. 3.4). The dominant diatom genera included a group of Naviculoid spp., *Nitzschia* spp., *Skeletonema costatum* and *Cyclotella* sp., the latter two of which was particularly dominant in the bottom water on 7/12/2015. Chlorophytes increased in abundance in the surface and halocline on 24/11/2015 and

7/12/2015, contributing to 19 to 26% of total phytoplankton abundance (Fig. 3.4). They were dominated by *Scenedesmus* sp. and *Monoraphidium* sp. Overall, the phytoplankton community changed little with depth until flow decreased below 710 ML day^{-1} and chlorophytes dominated the surface water, while diatoms dominated the bottom water.

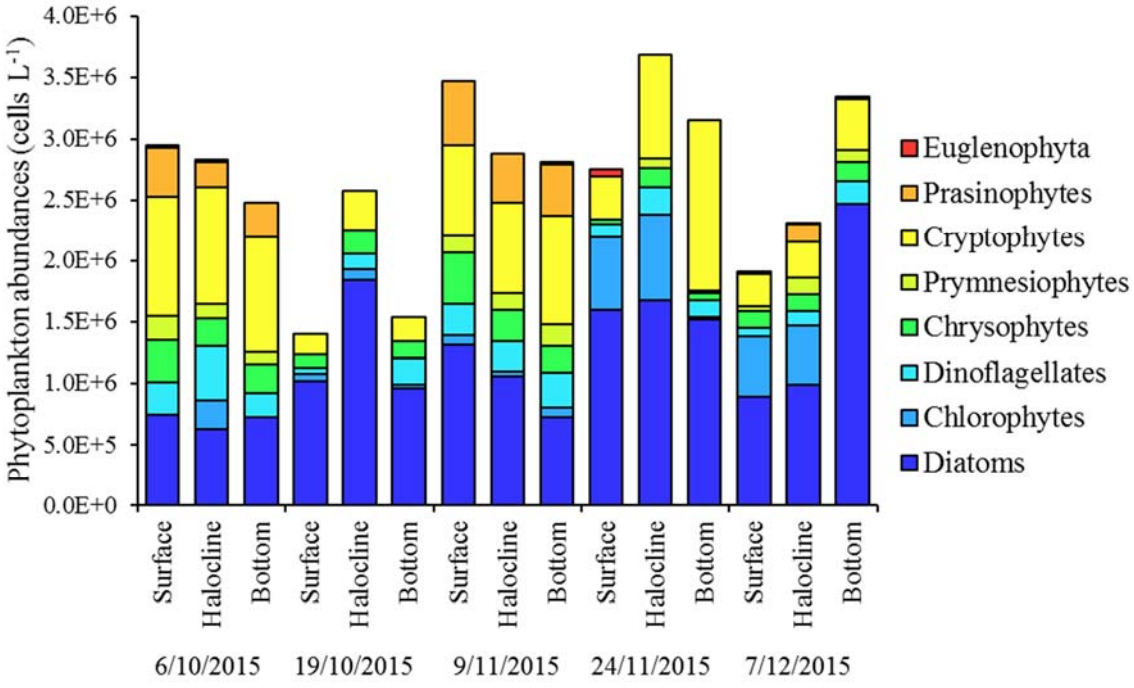

Figure 3.4: Bar plot of total phytoplankton group abundances (cells L^{-1}) at S2 at the surface, halocline and bottom on each sampling date.

The phytoplankton NMDS plot shows the water quality parameters that most strongly influenced the distribution of each phytoplankton group within the water column and throughout the sampling period (Fig. 3.5). Although not significant, the distribution of phytoplankton along the flow axis ($p = 0.12$) is indicative of how each group responded to high (left) and low (right) flow conditions. Prasinophytes and prymnesiophytes were abundant when flow was high, whereas chlorophytes dominated in low flow conditions.

This axis is closely mirrored by the significant NO_3^- axis ($p < 0.05$), which was a more significant driver of chlorophyte abundances, confirmed by the significant positive correlation between NO_3^- concentrations and chlorophyte abundances (Fig. 6.4, $p < 0.05$). On the perpendicular axis, turbidity had the most significant association with chrysophytes, dinoflagellates, cryptophytes and diatoms, which were most dominant when turbidity was low. The more vertical axes, somewhat controlled by salinity and temperature ($p < 0.1$), were indicative of how each phytoplankton group were distributed within the water column from surface to bottom.

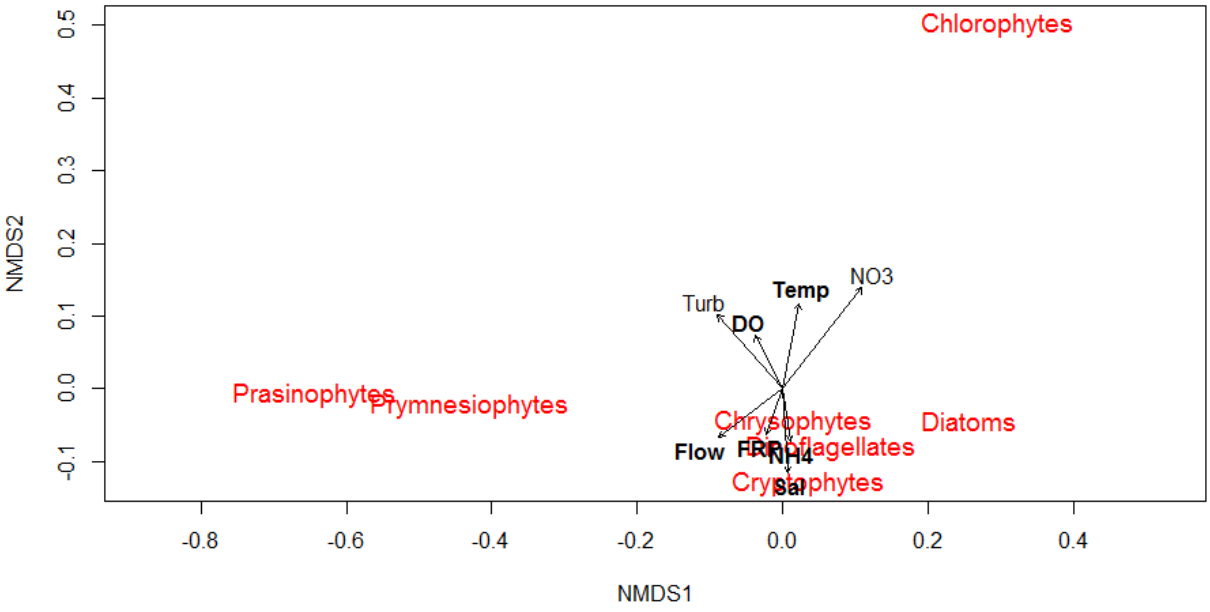

Figure 3.5: NMDS plot of the distribution of the dominant phytoplankton groups at S2 across the surface, halocline and bottom throughout the sampling period. Phytoplankton consistently present in abundances >60000 cells L^{-1} were included. Water quality vectors were overlayed, significant vectors include turbidity and NO_3^- ($p < 0.05$; Table. 6.6). Stress = 0.059.

The main zooplankton groups identified throughout the sampling period were copepod adults and nauplii, rotifers, polycheate larvae and mollusc larvae (Table. 6.7). A large abundance of copepod resting eggs and egg sacs were also observed. Total zooplankton abundances showed large variation between sites but greater variation across sampling dates (Fig. 3.6). The least variation occurred on 6/10/2015, 19/10/2015, 9/11/2015 (at S3) and 24/11/2015 when abundances ranged from 29 to 135 total individuals L^{-1}. Much lower abundances of <5 individuals L^{-1} were observed at S1 and S2 on 9/11/2015, while much higher abundances of 239 to 594 individuals L^{-1} occurred on 7/12/2015 (Fig. 3.6). Copepod adults and nauplii dominated the zooplankton biomass by over 80% on most dates and at most sites. On 6/10/2015 and 19/10/2015 calanoids dominated the copepod biomass, but on 24/11/2015 cyclopoid copepods of the genus *Oithona* appeared in similar abundances to calanoid copepods and then increased 8-fold on 7/12/2015 (Fig. 3.6). On this date, cyclopoid copepods contributed to 79 to 87% of adult copepod biomass and many were observed to be carrying egg sacs. Resting egg and nauplii abundances also increased after on 24/11/2015 and 7/12/2015.

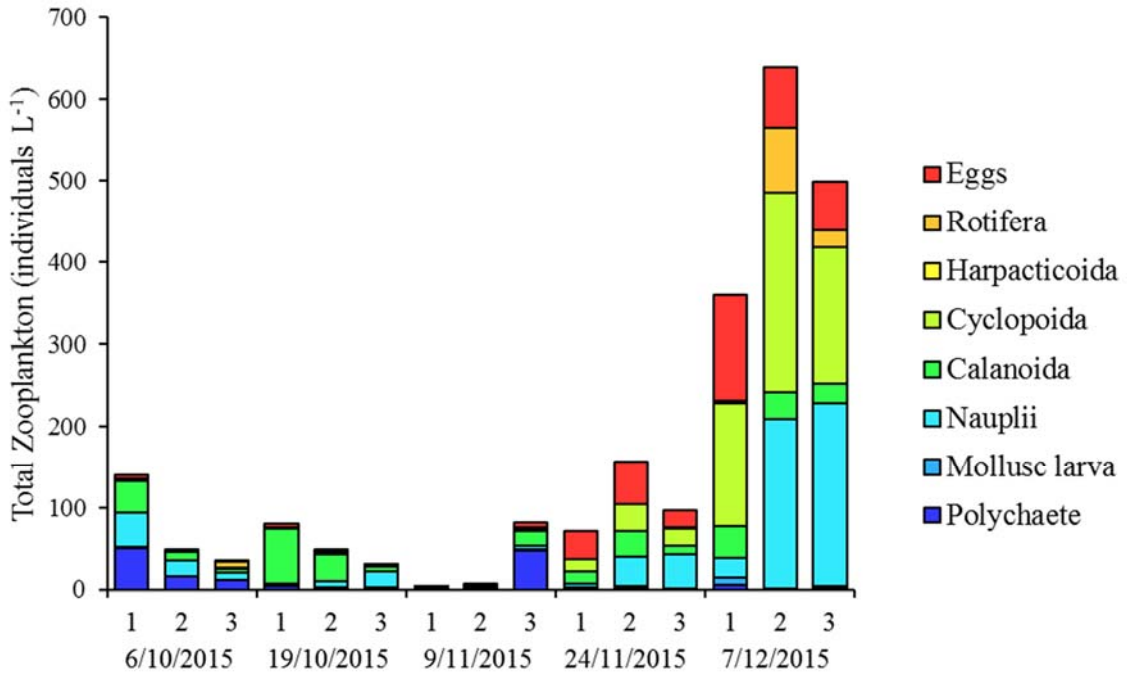

Figure 3.6: Bar plot of total zooplankton group abundances (individuals L^{-1}) at S1, S2 and S3 on each sampling date.

The dominant fish larvae identified were *Retropinna semoni* (smelt), *Philipnodon grandiceps* (flathead gudgeon), *Arenigobius bifrenatus* (bridled goby), *Redigobius macrostoma* (largemouth goby) and *Afurcagobius tamarensis* (Tamar goby). Larvae of *R. semoni*, the only pelagic fish species, were found in high abundances (375 to 865 larvae 1000 m^{-3}) at each site on 6/10/2015 and at S1 on both 19/10/2015 and 9/11/2015 (2490 and 1158 larvae 1000 m^{-3} respectively; Fig. 3.7). At all other times *R. semoni* larvae were found in low abundances (<50 larvae 1000 m^{-3}) or were absent. The remaining fish identified were benthic species that have pelagic larvae. *P. grandiceps* larvae were the most abundant and dominated this group by almost 100% at most sites. Larvae of benthic species were absent on 6/10/2015 but appeared in high abundances at S1 on 19/10/2015 (2490 larvae 100 m^{-3}; 80% *P. grandiceps* and 20% *A. bifrenatus*; Fig. 3.7). On 9/11/2015

and at S1 on 7/12/2015, 100% of the larvae of benthic species were *P. grandiceps* (247 to 1640 larvae 1000 m^{-3}). Very low abundances of <150 larvae 1000 m^{-3} occurred on 24/11/2015 but increased on 7/12/2015, with increased abundances of the *R. macrostoma* and *A. tamarensis* gobies at S3. When larval tows were conducted further downstream, no new larval species were identified and only *P. grandiceps* were found in high abundances; just downstream of S3 on 19/10/2015 and 9/11/2015 (Fig. 6.5).

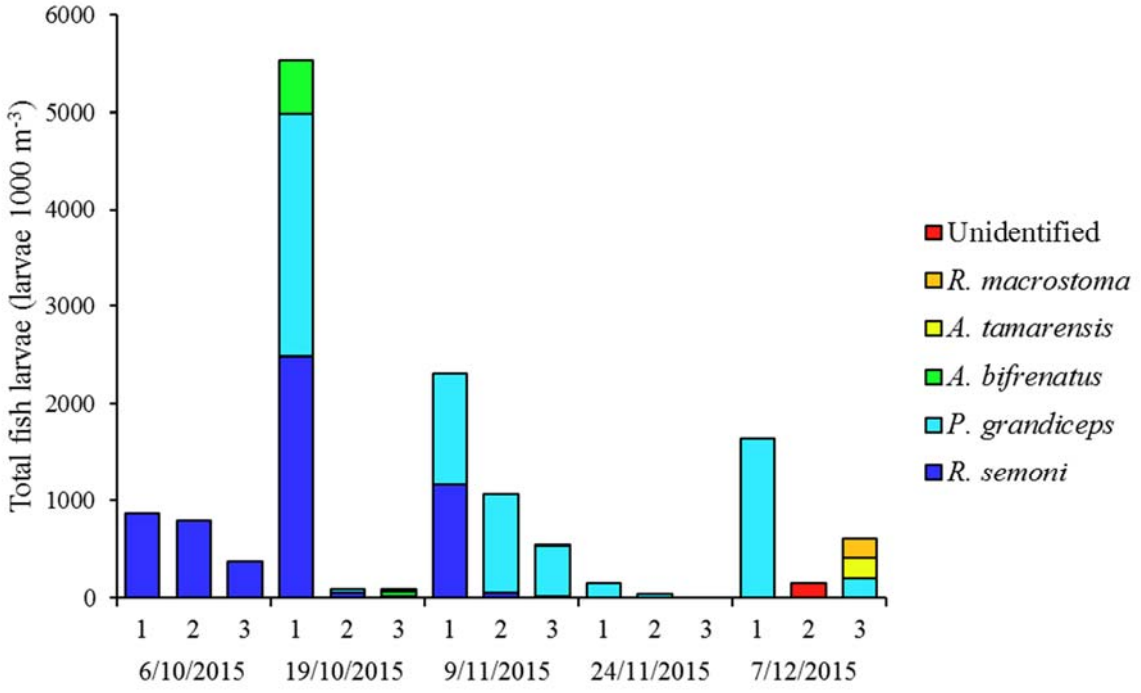

Figure 3.7: Bar plot of total fish larvae abundances (larvae 1000 m^{-3}) at S1, S2 and S3 on each sampling date.

Zooplankton and fish larvae communities from each site were presented on the same NMDS plot, overlayed with water quality parameters from the surface, bottom and halocline (Fig. 3.8). Only the significant vectors were plotted ($p < 0.05$), of which DO, temperature and flow were significant at every depth, salinity was significant in the

surface and bottom, NO_3^- concentrations were significant in the surface and halocline and chlorophyll *a* was only significant at the halocline. These distributions indicated that cyclopoid copepods were most strongly distributed in higher salinity water, while calanoid copepod distribution was more strongly controlled by chlorophyll *a* peaks at the halocline. *R. semoni* and *P. grandiceps* were more dominant under high flow conditions but *R. semoni* were confined to the less saline water.

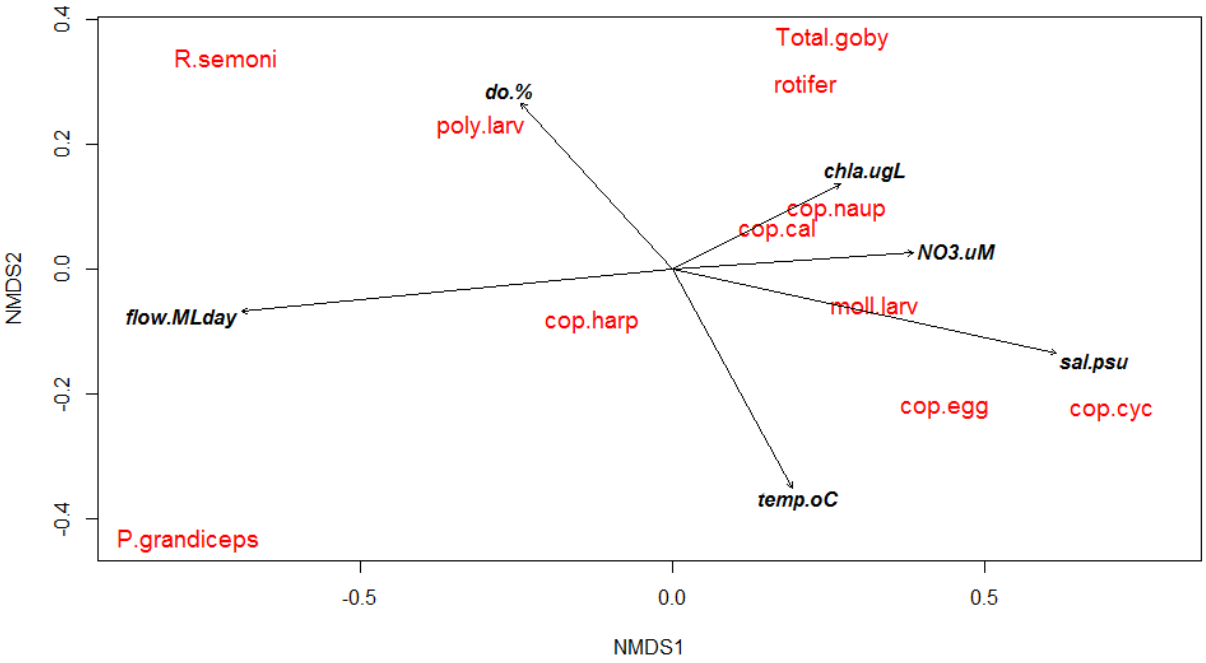

Figure 3.8: NMDS plot of the distribution of zooplankton and fish larvae at S1, S2 and S3 throughout the sampling period (see Table 6.7 for abbreviations). Significant water quality vectors from each depth are overlayed ($p < 0.05$), including flow (ML day^{-1}; surface, halocline and bottom), DO (%; surface, halocline and bottom), temperature (°C; surface, halocline and bottom), salinity (psu; surface and bottom), NO_3^- (µmol L^{-1}; surface and halocline) and chlorophyll *a* (µg L^{-1}; halocline; Table 6.8). Stress = 0.062.

3.5 Discussion

The upper reaches of estuaries are well known for their high productivity and importance for larval fish growth. In this study we investigated the dynamics of the physical structure, nutrients, phytoplankton, zooplankon and fish larvae within the Mitchell River estuary. To our knowledge this is the first detailed study of these intricately interlinked factors, which provides us with new insight into the factors driving the productivity of the upper reaches of an estuary with unregulated flows and very low incoming nutrient concentrations.

3.5.1 Salt wedge controls on nutrient dynamics and chlorophyll a

Strong stratification was observed in the estuary on all survey dates, but only at S2 and S3 on 9/11/2015, indicative of salt wedge intrusion after an episodic rainfall event. Stratification generally led to depleted oxygen concentrations in the bottom waters, and a concomitant accumulation of NH_4^+ and FRP most likely due to low dissolved oxygen and increased salinity stimulating release from the sediment consistent with previous studies of estuaries (Boatman & Murray 1982, Ketchum 1983, Cloern 1984, Milton & Arthington 1985, Paerl et al. 2001). In contrast dissolved inorganic nitrogen (DIN) concentrations in the surface water were typically very low compared to most previous studies of the upper reaches of estuaries (Malone et al. 1988, Holmes et al. 2000, Valiela & Bowen 2002, Fry et al. 2003, Kasai et al. 2010, Watanabe et al. 2014), reflecting the very low NO_3^- concentrations in the Mitchell River. Although we have no direct light measurements, turbidity and colour are relatively low in the Mitchell River and light is likely to have penetrated well into the halocline of this shallow estuary (Lloyd et al. 1987). In support of

this is the weak but significant ($p < 0.05$) negative relationship observed between chlorophyll *a* and NH_4^+ in the bottom water, which we interpret to reflect assimilation of NH_4^+ by phytoplankton in that zone. As a consequence of this, the highest phytoplankton biomass was typically observed at or below the halocline. Previous studies have also shown peaks in chlorophyll *a* at the halocline, which have been hypothesised by Kasai et al. (2010) to mean one of two things. First, chlorophyll *a* at the halocline may be due to the transportation of phytoplankton from the river mouth to upstream regions via circulation at the freshwater/seawater interface. Second, it may be due to marine phytoplankton in the bottom water utilising riverine nutrients at the interface. This second hypothesis was supported by Watanabe et al. (2014) and the results of both studies were consistent with the results of Thompson (1998), who also observed phytoplankton blooms at the freshwater/seawater interface. However, in the current study, the results suggest that productivity in the upper Mitchell River estuary is driven primarily by recycled nutrients rather than those derived from the river. Therefore, we present a third hypothesis, that chlorophyll *a* accumulates at the halocline where phytoplankton can access both bottom water recycled DIN and surface water riverine DIN.

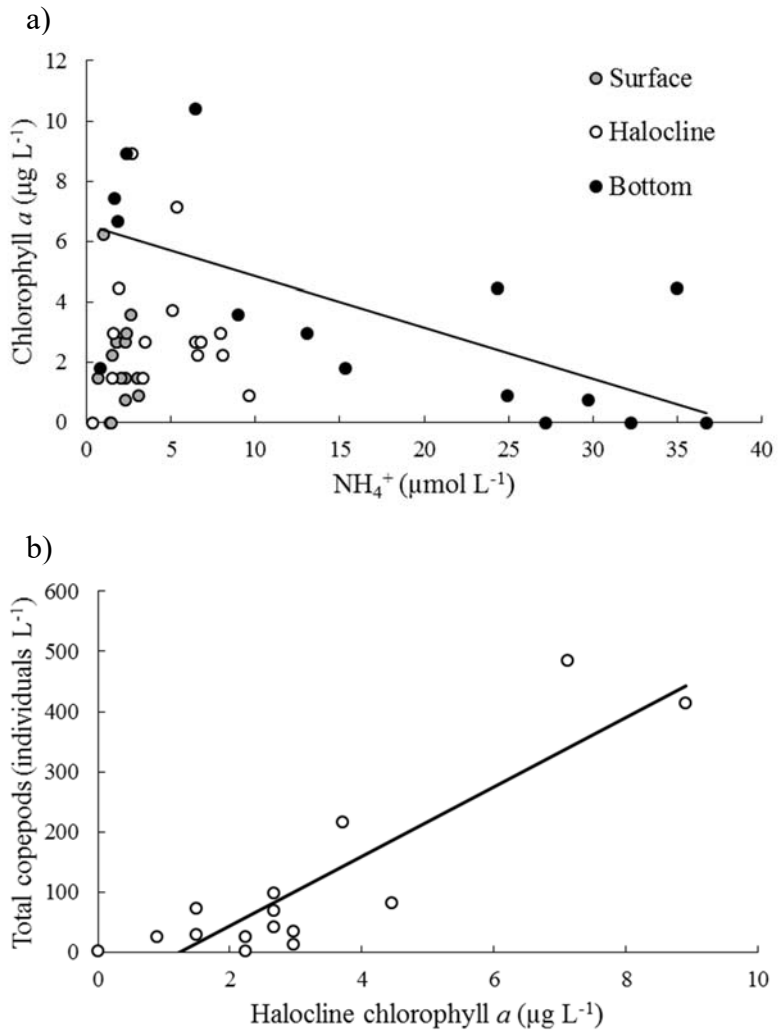

Figure 3.9: Plot of a) chlorophyll *a* (µg L^{-1}) vs NH_4^+ (µmol L^{-1}) at the surface, halocline and bottom ($r^2 = 0.44$, n = 15, $p < 0.008$), b) Plot of total copepod abundances including adults and nauplii (individuals L^{-1}) vs. chlorophyll *a* at the halocline (µg POM L^{-1}); n = 15, $r^2 = 0.79$, $p < 0.001$

3.5.2 Phytoplankton community dynamics

On examination of the phytoplankton community, the above hypothesis was further investigated along with the roles of flow strength and salinity stratification on phytoplankton productivity. It was observed that only in the presence of surface water

NO_3^- did phytoplankton accumulation occur at the halocline (on 19/10/2015, 24/11/2015 and 7/12/2014). These assemblages were dominated by diatoms, which have no control over their motility and therefore rely on turbulence and mixing forces to keep them suspended in the euphotic zone (Margalef 1978). Freshwater flows on these dates ranged from 730 to 410 ML day^{-1}, however, increased diatom abundances in the bottom water on 7/12/2015 (410 ML day^{-1}), indicated that mixing forces had decreased and sinking had begun. In contrast, flagellate phytoplankton have the ability to migrate vertically (3-5 m) to access high nutrient zones (Hamilton et al. 1997). Most consistent with this was the dominance of cryptophyte flagellates in the NH_4^+-enriched bottom water (Fig. 3.4). These phytoplankton dominated in high flow conditions (>950 ML day^{-1}).

After the flow event, when salt wedge intrusion caused bottom water salinities to increase, and freshwater flows began to decrease, distinct surface and bottom water phytoplankton communities emerged. High abundances of chlorophytes dominated the surface water, indicative of their adaptation to freshwater environments with low flow and high light conditions (Correll 1978, Capriulo et al. 2002, Fernandez & Galvan 2008, Winder & Jassby 2011). Chlorophytes were also significantly positively associated with NO_3^- concentrations (Fig. 6.4b; $p < 0.05$), which were highest in the surface waters under lower flow conditions. In the bottom water, diatoms dominated the phytoplankton assemblage, particularly on 7/12/2015 when the abundance of S*keletonema costatum* increased. S*keletonema costatum* is a marine diatom which survives best in salinities between 7 and 26 (Capriulo et al. 2002), and shows a preference for NH_4^+ assimilation (Das & Ray 2008, Mitrovic et al. 2008). Therefore, increased diatom abundances may not have been solely due to sinking caused by weak mixing forces, but increased availability

of bottom water NH_4^+ concentrations when salinity increased. As a result, bottom water NH_4^+ decreased in concurrence with increased *S. costatum* abundances on 7/12/2015.

The different surface and bottom water phytoplankton assemblages in the strongly stratified water column were similar to observations by Watanabe et al. (2014) and Ueda et al. (2005). Most similar were the results of Watanabe et al. (2014), who found that regenerated nutrients in the salt wedge of the Yura estuary, Japan, contributed to bottom water marine phytoplankton blooms, dominated by *Skeletonema* spp. Chlorophytes were also found to accumulate in the surface water of the estuary, but nearer the river mouth (not in the upper estuarine region) when periods of low flow increased residence times (Watanabe et al. 2014). In contrast, Ueda et al. (2005), who studied the shallow brackish Lake Obuchi, Japan, found a different community assemblage pattern under stratified conditions. Stratification in Lake Obuchi occurred during one month in summer, 2002, and resulted in surface waters of 10 psu, 14.2 μmol-NH_4^+ L^{-1} and 3.6 μmol-NO_3^- L^{-1}, and bottom waters of >20 psu, 14.2 to 70.4 μmol-NH_4^+ L^{-1} and 3.6 μmol-NO_3^- L^{-1}. These salinities and the availability of NH_4^+ were optimal for *S. costatum*, which was common in both layers but dominated in the surface water. Chlorophytes were the dominant phytoplankton in the bottom water, which was attributed to decreased turbulence and more eutrophic conditions (Ueda et al. 2005). In this case, high NH_4^+ and lower NO_3^- concentrations were observed in the bottom water. This is in contrast to both the Mitchell and Yura estuaries, where NO_3^- and NH_4^+ concentrations were relatively depleted in the bottom and surface waters respectively (Watanabe et al. 2014). Additionally, salinity was much lower in the surface water of both estuarine river systems and therefore phytoplankton were restricted by nutrient availability and salinity. However, the common

theme suggests that NO_3^-, even in low concentrations, plays an important role in chlorophyte productivity.

3.5.3 Zooplankton and fish larvae

Previous studies have shown that copepods generally dominate the zooplankton community in spring estuarine food webs, and their nauplii form an important part of the diet of fish larvae (Longhurst 1985, Newton 1996, Williams et al. 2013). Among pelagic zooplankton, the physical habitat of the water column plays an important role in copepod community composition, as do bottom-up and top-down forces of predator/prey interaction. The accumulation of chlorophyll *a* at the halocline in particular had a significant positive relationship with copepod abundances (Fig. 3.9b; $p < 0.05$). This suggests that copepod zooplankton accumulated near the halocline in part due to the availability of a food source. The same relationship was observed most strongly for calanoid copepods on the NMDS (Fig. 3.8), whereas cyclopoid copepods were shown to be strongly influenced by salinity and DO. *Oithona* are known to be abundant in marine environments, but also in estuaries where the salinity is >5 (Bayly 1965, Johnson & Allen 2012). Cyclopoid copepods are also able to better exploit the low oxygen environments due to their small size and low metabolic rate (Duggan et al. 2008). These factors explain the community shift from a calanoid dominated assemblage to a cyclopoid dominated assemblage after high rainfall and increased residence times caused zooplankton to be advected from the upper estuary. More specifically, the subsequent salt wedge intrusion favoured cyclopoid copepod recolonisation due to increased salinity and decreased DO in the bottom water.

Increased cyclopoid copepod and nauplii abundances resulted in an increase in total zooplankton abundances and therefore grazing pressure. This may have contributed to the shift in the bottom water phytoplankton assemblage from a flagellate dominated assemblage to a diatom dominated one. The small size of the cyclopoid *Oithona* (<1 mm), means they are better adapted to capturing smaller (2 to 40 μm) and more motile prey such as cryptophytes (Eaton 1971, Lampitt & Gamble 1982, Drits & Semenova 1984, Fulton 1984, Jonsson & Tiselius 1990, Atkinson 1995, Kiørboe & Visser 1999). Consistent with this, cryptophyte abundances decreased when cyclopoid copepods increased in numbers on 24/11/2015 and 7/12/2015. This may have reduced competition for available nutrients and favoured the diatom *S. costatum*, which became more abundant on these dates.

Within the larval fish community, there was no evidence that prey availability or abundance exerted any control over the presence/absence of either dominant fish larvae; *Retropinna semoni* or *Philypnodon grandiceps*. In fact, both *R. semoni* and *P. grandiceps* were abundant at S1 on 9/11/2015 when very little zooplankton prey was available due to high flows. *Retropinna semoni* is a freshwater fish that begins breeding in winter, a life history strategy aimed at avoiding the impacts of summer storms and fluctuating water levels, an adaptive value in seasonally unstable environments such as coastal streams (Milton & Arthington 1985). Therefore, *R. semoni* was likely spawned in the riverine environment and the larvae flushed into the estuary during high flows. This was consistent with its position on the NMDS near the flow vector and opposite the salinity vector (Fig. 3.8). *Philypnodon grandiceps* is also a freshwater species, and like *R. semoni* may have been advected downstream in high flows. In this case, the later appearance and increased persistence of *P. grandiceps* was likely due to a later spawning phenology.

However, *P. grandiceps* is also found in upper estuarine tidal habitats (Llewellyn 2007), and is known to spawn in estuaries like the benthic freshwater gobies identified (Newton 1996). Therefore, its presence further downstream (Fig. 6.5) may actually indicate that it was spawned and hatched in the estuarine environment or can survive in said environment. Estuarine goby larvae were the most likely to have been spawned and hatched within the estuary due to their presence at the end of the sampling period in the more downstream sites (Fig. 6.5). This may be an indication that environmental conditions were unsuitable for spawning or larvae survival at the start of the sampling period. In the upper estuary, DO was very low in the bottom water, particularly during salt wedge intrusion. DO concentrations < 2mg L^{-1} lead to reduced survival over long-term exposure and reduced feeding rates (Breitburg 1994). Concentration < 1 mg L^{-1} can lead to mortality after 24 hour exposure (Breitburg 1994). According to Miller et al. (2002), the larvae of pelagic fish are more sensitive to low DO than the larvae of benthic fish, which may explain the absence of the larvae of pelagic estuarine fish such as *Acanthopagrus butcheri*.

3.6 Conclusion

In this study of the Mitchell River estuary, unmodified freshwater flows exerted a strong control on the salinity structure, nutrient dynamics and food web of the upper estuarine region. The results emphasise the short term effects of salt wedge intrusion and its importance in an estuary that receives low watershed nutrient loads. Salt wedge intrusion facilitated the release of recycled benthic nutrients as NH_4^+, which accumulated in the low DO bottom water. In contrast NO_3^- was mostly confined to the surface water in low concentrations, which decreased under high flows indicative of low riverine N inputs. The availability of these N forms, the degree of freshwater flows and mixing forces, and the salinity structure, all played important roles controlling phytoplankton biomass and productivity. This was due to the various N preferences, motility and salinity sensitivity of the phytoplankton taxa and groups present. Grazing pressure by zooplankton was also hypothesised to affect the algal assemblage by decreasing competition for available nutrients. This occurred after salt wedge intrusion, which created environmental conditions better suited to cyclopoid copepods that preferentially grazed flagellate phytoplankton. In contrast, it was the physical forcing of the high flows that resulted in the appearance of the freshwater larvae of *Retropinna semoni* and *Philypnodon grandiceps*. However, *Philypnodon grandiceps* larvae were likely better adapted to the estuarine environment, like the goby larvae that were spawned further downstream.

The results from this study emphasise the complexities of the physico-chemical environment within a salt wedge estuary and its sensitivity to freshwater flows. Not only were advection and salt wedge intrusion important processes that influenced productivity in the Mitchell River estuary, but the maintenance of a stratified water column through

sufficient freshwater flows was also essential. Like other studies, mixing forces caused phytoplankton biomass to accumulate at the halocline, which in this study allowed access to both surface and bottom water DIN. As a result, it was clear that NO_3^-, even in low concentrations, exerted a strong control on phytoplankton productivity. This is a future concern if human activities in the Mitchell River catchment lead to increased watershed N loads to the Mitchell River estuary.

3.7 Acknowledgements

The authors wish to thank David Brehm and Oliver Thomas who contributed to work in the field and fish larvae counts, and Tony Miskiewicz who identified the fish larvae. We also thank Microalgal Services for their work on phytoplankton identification and counts. This work has been funded by the Australian Research Council Linkage Project Scheme (LP140100087), The University of Melbourne, Department of Environment, Land, Water and Planning (VIC), Gippsland Lakes Ministerial Advisory Committee, Water Technology and Melbourne Water. Caitlyn McNaughton was supported by a Monash University scholarship.

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Chapter 4: The response of natural phytoplankton populations from two estuarine systems to nutrient enrichment using bioassays

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The Mitchell River silt jetties near the lower sampling site. Photo supplied by East Gippsland Marketing Inc

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4.1 Abstract

Bioassays have long been used to determine factors potentially limiting algal growth rate, maximum yield and phytoplankton primary production. This used nutrient addition bioassays to test how NH_4^+ and NO_3^- additions affected the natural phytoplankton populations in both the Werribee and Mitchell River estuaries. It was found that high growth in unamended water from the Werribee Estuary (control) was similar to the nutrient addition bioassays due to high NO_3^- concentrations in these waters. In contrast, nutrient addition promoted significantly higher growth in the Mitchell Estuary compared to the control ($p < 0.05$). Therefore, there is potential for significant algal growth in response to *in situ* nutrient concentrations in the Werribee (observed *in situ* during 2014), and in response to nutrient additions in the Mitchell. Preferential assimilation of NH_4^+ was observed, and found to promote flagellate phytoplankton abundances and a more mixed algal assemblage in the Werribee bioassays. When NH_4^+ concentrations decreased to ~1 μmol L^{-1}, NO_3^- concentrations decreased, indicative of uptake inhibition in the presence of NH_4^+. High NO_3^- concentrations were found to cause increased diatom abundances in all treatments, and increased cyanobacteria abundances in treatments that received P-addition. Chlorophyte abundances increased significantly in the Mitchell bioassays, including the control ($p < 0.05$), which indicates high potential for chlorophyte blooms, but also their preference for low flow and high light conditions. These results helped confirm some aspects of how nutrient inputs control phytoplankton productivity in estuarine environments.

4.2 Introduction

Nitrogen (N) is generally considered to be one of the most important nutrients controlling phytoplankton productivity within estuarine systems as it often has the greatest potential to limit biomass accumulation (Twomey et al. 2005). The two dominant forms of N utilised by phytoplankton are ammonium (NH_4^+) and nitrate (NO_3^-), which are key drivers of phytoplankton abundance and diversity (Lomas & Glibert 1999a, Berg et al. 2003). The preferred source of N for phytoplankton is generally NH_4^+, as its assimilation requires the least energy, while NO_3^- must be reduced within the algal cell in a process requiring significant energy expenditure (Syrett 1956, 1981). These reduction reactions, catalysed by nitrate reductase and nitrite reductase, are often inhibited by high levels of NH_4^+, however the degree of inhibition and the effects on NO_3^- uptake and storage have been found to vary widely as outlined by Glibert et al. (2016). Systems more enriched in NH_4^+ generally have phytoplankton communities dominated by smaller flagellate taxa, while NO_3^--rich environments have been found to be dominated by diatoms (Goldman 1993, LaRoche et al. 1997, Lomas & Glibert 1999a, b, Glibert et al. 2001, Berg et al. 2003, Rothenberger et al. 2009).

Anthropogenic activities, such as fertiliser application and waste water management, have increased N loads to coastal environments mainly via runoff and groundwater inputs (Lapointe et al. 1990, Vitousek et al. 1997, Valiela & Bowen 2002, Glibert et al. 2006). These inputs add to NH_4^+ and NO_3^- pools that are already present as a result of processes such as remineralisation, denitrification and nitrification. Increased N loads not only stimulate phytoplankton assimilation in N limited environments, but also alter NH_4^+/NO_3^-

ratios, which may alter the composition of the natural phytoplankton community. As a result, higher trophic levels could be affected by bottom-up forces.

There are many other factors that exert control over phytoplankton community composition. One of these is predation pressure, particularly by copepods, which dominate the estuarine zooplankton community during spring. Pelagic copepods include calanoids and cyclopoids, whose diets differ due to differences in their size and motility. Larger calanoids tend to predate larger, less motile prey such as diatoms and larger flagellates (Berggreen et al. 1988, Nielsen & Sabatini 1996, Nakamura & Turner 1997). Smaller cyclopoids are more motile and therefore able to capture smaller, more motile prey (Eaton 1971, Drits & Semenova 1984). In general, copepods select prey based on their nutritional value, which varies widely between algal taxa (Kleppel 1993, Runge & Lafontaine 1996, Jones et al. 2002).

Another important factor that can exert multiple controls over estuarine phytoplankton community composition is hydrodynamics, which is also affected by anthropogenic activities. Salt-wedge estuaries, where fresh riverine water, flows over encroaching saline water, are particularly sensitive to altered hydrodynamics. This is because the presence or strength of the halocline in salt wedge estuaries can easily be affected by altered flow regimes. Freshwater flows can be altered naturally by winter rainfall and episodic events, or unnaturally by human regulation (such as dams and weirs). The strength of the halocline is important because friction and mixing forces at the freshwater/seawater interface can cause entrainment of nutrients and phytoplankton and result in a zone of high productivity. Mixing forces have a particularly strong control on diatoms and other non-motile phytoplankton, while motile phytoplankton such as flagellates can actively

move through the water column. Even so, the presence of a strong halocline should promote the growth of a mixed phytoplankton assemblage of both motile and non-motile groups.

The role of the halocline, hydrodynamics, and N inputs and recycling on estuarine productivity have previously been studied in two Australian estuaries; the Werribee River Estuary, 40 km southwest of Melbourne, Victoria (see Chapter 2), and the Mitchell River Estuary near Bairnsdale, Victoria, 281 km east of Melbourne (see Chapter 3). These estuaries were chosen for their contrasting hydrology, salinity stratification and nutrient loads. The Werribee River Estuary is in close proximity to the Western Treatment Plant and Werribee Irrigation District, which contribute to high NO_3^- concentrations (up to 180 μmol L^{-1}) via riverine and groundwater inputs (Wong et al. 2013). It is also highly regulated due to a number of upstream diversions, including the Melton reservoir 45 km upstream and the Wyndham diversion weir 9 km upstream. As a result, low flow conditions of ~4 ML day^{-1} were common during spring. Strong tidal forces from Port Phillip Bay (into which the Werribee River flows) cause the upper estuary to become partially mixed unless freshwater flows increase. Chapter 2 of this thesis describes in detail how the algal assemblage responded to varying concentrations of NH_4^+ and NO_3^-, altered, in part, due to an environmental flow event that triggered a food web response linked to the increased abundance of the larvae of the endemic fish species, *Acanthopagrus butcheri*. In contrast, the Mitchell River is the largest un-regulated river system in Victoria, with average freshwater flows in the last 10 years of 1450 to 3350 ML day^{-1} in winter and 350 to 1600 ML day^{-1} in summer (http://www.bom.gov.au/waterdata/; station number 224217B). The estuary begins at a man-made rock sill, 25 km from Lake King, into which it flows. Lake King forms part of the Gippsland Lakes, a series of

interconnected coastal lagoons that flow into Bass Straight via a permanently open artificial sea-entrance constructed in 1889. Saltwater intrusion creates a salt wedge estuary, which can become entirely fresh in times of high flow due to episodic rainfall events. Chapter 3 of this thesis describes in detail how salt wedge intrusion in the upper Mitchell River estuary facilitated the release of recycled benthic nutrients, and created a distinct water column separating bottom water NH_4^+ from surface water NO_3^-, which supported phytoplankton biomass at the halocline but also distinct surface and bottom water phytoplankton communities.

There were many interlinking hydrodynamic and biogeochemical processes controlling the phytoplankton communities throughout the sampling periods within both estuaries. These factors combined, make it hard to isolate the effects of N concentrations and NO_3^- /NH_4^+ ratios on phytoplankton. Therefore, this study aimed to use nutrient addition bioassays to test how NH_4^+ and NO_3^- affected the natural phytoplankton populations in both the Werribee and Mitchell River estuaries. Bioassays have long been used to determine factors potentially limiting algal growth rate, maximum yield and phytoplankton primary production (Dalsgaard & Krause-Jensen 2006). Phytoplankton growth in bioassays is usually determined by measuring chlorophyll *a* concentrations by spectrophotometric analysis or fluorescence, which gives information about the change in phytoplankton biomass. In this study, a PhytoPAM Phytoplankton Analyser (a pulse-amplitude-modulation (PAM) fluorometer) was used, which has the ability to measure fluorescence in four separate channels concurrently and thus determine effects of nutrient addition on major groups of algae (green algae, diatoms/dinoflagellates and cyanobacteria). Phytoplankton were also counted and identified by microscopy in order to better determine the response of specific groups and taxa. Bioassays are based on the

concept that addition of a limiting nutrient will increase growth rate compared to that in a control with *in situ* nutrient concentrations. In practice they more often reveal which nutrient might become limiting should phytoplankton populations increase above levels in the control (Beardall et al. 2001). Here however, the bioassay approach was used to test how changes in NH_4^+ and NO_3^- might influence growth and population composition of phytoplankton in the two systems. In general, it was hypothesised that addition of NO_3^- would increase diatom and chlorophyte abundances, while NH_4^+ would stimulate flagellate abundance as per the results observed in Chapters 2 and 3. However, high *in situ* concentrations of NO_3^- within the Werribee estuary were also hypothesised to allow high growth within the control population. Additionally, NH_4^+ was hypothesised to be used preferentially to NO_3^-, which would be traced by examining changes in nutrient concentrations throughout the bioassay growth period. These changes in phytoplankton community composition and abundance are important to examine due to their strong potential to influence copepods and other grazers.

4.3 Methods

4.3.1 Site description

The Werribee River estuary sampling site was located 5.6 km from the estuary mouth (Fig. 4.1) and was chosen within a commonly stratified region of the estuary in close proximity to a submarine groundwater discharge hotspot, known to be a source of NO_3^- (Wong et al. 2013). The second sampling site, located on the Mitchell River estuary, was also chosen for the presence of a strong salt-wedge. However, at the time of sampling, high rainfall and the resultant increase in freshwater flow removed the salt-wedge from the upper estuary where sampling took place in 2015 (see Chapter 3). Therefore, a sampling site was chosen downstream where stratification was present (Fig. 4.1). Each site was sampled four times between November 2016 and January 2017 but the results presented here encapsulate one sampling date from each estuary; 22/11/2016 in the Mitchell estuary and 28/11/2016 in the Werribee estuary; when phytoplankton were identified and counted.

Both estuaries were strongly stratified at the time and location of sampling. In the Werribee estuary, samples were obtained at 0.5 m where salinity was 6.85 psu, DO was 37.3% and temperature was 17.8 °C. Above this point salinity was 2.5 psu and DO was 62.8%, while below this point these parameters changed to 30.4 psu and <10% respectively and temperature increased to ~19 °C. In the Mitchell estuary, samples were obtained at 1 m where salinity was 2.3 psu, DO was 95% and temperature was 22.3 °C. Above this point salinity, DO and temperature were very similar, while below this point

salinity changed rapidly to 15.8 psu, while DO ranged between 71.2 and 85.3% and temperature was between 20.3 to 21.3 °C.

Figure 4.1: A map of the sampling sites on the Werribee River estuary (S5; left) and the Mitchell River estuary (right).

4.3.2 Field sampling methods

Phytoplankton samples were taken from a depth of 1 m in the Mitchell River estuary and 0.5 m in the Werribee River estuary and were collected in the filtrate that passed through an 80 µm sieve to remove larger grazers. These depths targeted the halocline where the change in salinity, temperature, and DO occurred and where chlorophyll *a* concentrations (µg L^{-1}) were highest. A multi-parameter water quality sonde (Hydrolab DS5X) was used

to measure these parameters with depth. Water samples for nutrient concentrations were taken from the surface and bottom water at each sampling site and filtered through 0.22 µm filters.

4.3.3 Laboratory methods

Filtered water samples were analysed for NH_4^+, NO_3^- and filterable reactive phosphorus (FRP). Their concentrations were determined spectrophotometrically using a Lachat QuikChem 8000 Flow Injection Analyser (FIA), following standard procedures (APHA 2005). The accuracy was within 2% relative error. Phytoplankton samples were collected in acid-washed 2L Nalgene bottles and stored at approximately ambient temperature in the dark overnight. Sub-samples were then transferred into 125 mL Nalgene bottles. Triplicate samples for each treatment were incubated in temperature-controlled water baths at 18°C. Light was supplied by Viva-lite natural daylight rendering tubes giving a photon flux of 65 to 80 mol quanta m^{-2} s^{-1} at the bottle surface, supplied on a 13/11 hr day/night rotation. The lids were left ajar to allow oxygen exchange but closed when the bottles were periodically shaken. Addition of nutrients for the bioassays were as follows:

- Ammonium (NH_4^+) treatment (50 µmol L^{-1} NH_4^+ as NH_4Cl and 85 µmol L^{-1} PO_4^- as K_2HPO_4)
- Nitrate (NO_3^-) treatment (50 µmol L^{-1} NO_3^- as $NaNO_3$ and 85 µmol L^{-1} PO_4^-)
- Control (+H_2O)

Nitrogen nutrient addition concentrations were based on the average NO_3^- concentration observed in the Werribee estuary during the 2014 sampling period. High concentrations of PO_4^- were added to ensure P limitation did not occur throughout the growth period

even though N:P ratios in the ambient water made this unlikely. The change in algal biomass in each treatment was measured as chlorophyll *a* fluorescence, using a PhytoPAM (Heinz Walz). Both chlorophyll *a* and nutrient concentrations were measured daily, or every second day, until maximum yield of chlorophyll *a* was reached. At this time, the bioassays were terminated using Lugol's iodine. Triplicate samples were also taken at the start as initial or *in situ* phytoplankton abundances. They were measured for chlorophyll *a* and nutrient concentrations before being fixed with Lugol's iodine.

Initial and final (after growth) phytoplankton samples were sent to Microalgal Services, Victoria, where taxa were identified and counted using a Zeiss Standard compound microscope equipped with Phase Contrast Optics with up to 400× magnification. A Sedgewick-Rafter counting chamber was used and data were reported in cells L^{-1} using reference material from Tomas (1997).

4.3.4 Statistical analyses

Algal growth rates were determined from the slope of a Log(biomass) vs time plot during exponential phase and represented as μ. One-way ANOVA was used to analyse the different growth responses between the treatments. Tukey's least significant difference procedure was then performed as a post hoc multiple comparison of treatment means. Untransformed data was used in all cases. Statistical analysis was undertaken using R 3.3.2 (R Core Team 2016) and for all tests the level of significance used was $p < 0.05$, however some marginally significant results ($p < 0.1$) are reported.

Data were analysed both with the inclusion and exclusion of the diatom *Cocconeis* spp. within the Werribee estuary samples. This was due to the extreme abundance this diatom reached in only the Werribee NH_4^+ treatment, which was not reflected in the change in nutrient concentration or the chlorophyll *a* concentrations. *Cocconeis* spp. is a benthic alga, known to attach to substrates and other taxa such as the filamentous green algae *Cladophora*, via a mucilagenous substance which it secretes (Holmes et al. 2000). Filamentous green algae was observed to grow on the sides of many bioassay bottles and therefore its presence may have been a factor affecting the dominance of *Cocconeis* spp. Chlorophyll *a* sampling by cuvette would likely exclude any attached phytoplankton, whereas transfer to bottles for identification may have caused attached phytoplankton to become dislodged. This may account for the absence of high chlorophyll *a* concentrations compared to other treatments, however does not explain why NH_4^+ uptake remained the same as other treatments without high *Cocconeis* spp. abundances. This factor calls into question the presence of such high abundances of *Cocconeis* spp. in the Werribee NH_4^+ treatment. Additionally, *Cocconeis* spp. was not a taxa of interest in either of the previous studies due to its low abundance (Chapters 2 and 3).

4.4 Results

4.4.1 Bioassay nutrient and chlorophyll a changes

The *in situ* concentrations of NO_3^-, NH_4^+ and FRP on the two sampling dates were greater within the Werribee estuary than the Mitchell (Table. 4.1). This produced higher maximum biomass yields (as chlorophyll *a*; µg L^{-1}) in all Werribee treatments compared to Mitchell treatments, even though the initial chlorophyll *a* concentrations were lower (Table. 4.1). The growth rates in each Werribee treatment were very similar but the growth rate in the NH_4^+ treatment was highest (Table. 4.1). Growth was slow until day three when the exponential growth phase began and peak growth rate occurred until day six, but high growth was sustained in the NO_3^- treatment until day seven (Fig. 4.2a and b). Maximum yield occurred on day nine, after which the yield in all treatments decreased and the bioassays were terminated. Although the growth rate was highest in the NH_4^+ treatment, it was highly variable and it was the NO_3^- treatment that reached the greatest maximum yield on average. However, the differences in maximum yield were not statistically significant between any of the treatments. In all treatments NH_4^+ was used most rapidly until it was ~1 µmol L^{-1}, at which point NO_3^- uptake increased (Fig. 4.3). In the control and NH_4^+ treatments, both N forms were depleted by day six, while in the NO_3^- treatment they were depleted by day seven. When both NH_4^+ and NO_3^- were at or near depletion, FRP uptake increased but it was still available when algal growth stopped in the addition treatments. Within the control treatments the relative decline in DIN compared to FRP was much greater.

Table 4.1: Nutrient concentrations (µmol L^{-1}) in the surface (S) and bottom (B) at the time of sampling (*in situ*), and in the control, NO_3^- and NH_4^+ treatments at the start (initial) and end (final) of the bioassay growth period; the *in situ* chlorophyll *a* concentrations (µg L^{-1}); maximum yield (µg chl L^{-1}); growth rate (µ) and R^2 for the growth rate determinations for each treatment. Errors are reported as standard error (n = 3).

	Werribee				Mitchell			
Treatment	***In situ***	**Control**	**NO_3^-**	**NH_4^+**	***In situ***	**Control**	**NO_3^-**	**NH_4^+**
Nutrient concentrations								
FRP (µmol L^{-1})								
Initial	2.3	1.93±0.04	88.3±0.4	86.5±0.9	0.2	0.19±0.05	85.9±0.5	85.5±0.1
Final		0.06±0.00	28±3	20±7		0.06±0.00	63±1	64±1
NO_3^- (µmol L^{-1})								
Initial	60	59.6±0.3	109.2±0.4	59.3±0.2	5.5	5.11±0.05	59.1±0.04	5.0±0.2
Final		-	-	-		0.5±0.4	-	-
NH_4^+ (µmol L^{-1})								
Initial	11	9.6±0.2	9.81±0.08	60.1±0.2	1.5	1.54±0.07	1.9±0.4	56.0±0.4
Final		0.14±0.07	0.17±0.02	0.07±0.04		0.17±0.02	0.10±0.02	-
Phytoplankton biomass								
Maximum yield (µg chl L^{-1})	1.15	23±5	41±1	38±11	2.45	6.5±0.3	31±3	35±6
Growth rate (µ)		0.24±0.01	0.26±0.02	0.30±0.02		0.061±0.006	0.267±0.009	0.276±0.008
Growth rate R^2 value		0.97±0.01	0.993±0.001	0.94±0.06		0.968±0.002	0.996±0.001	0.9987±0.0006

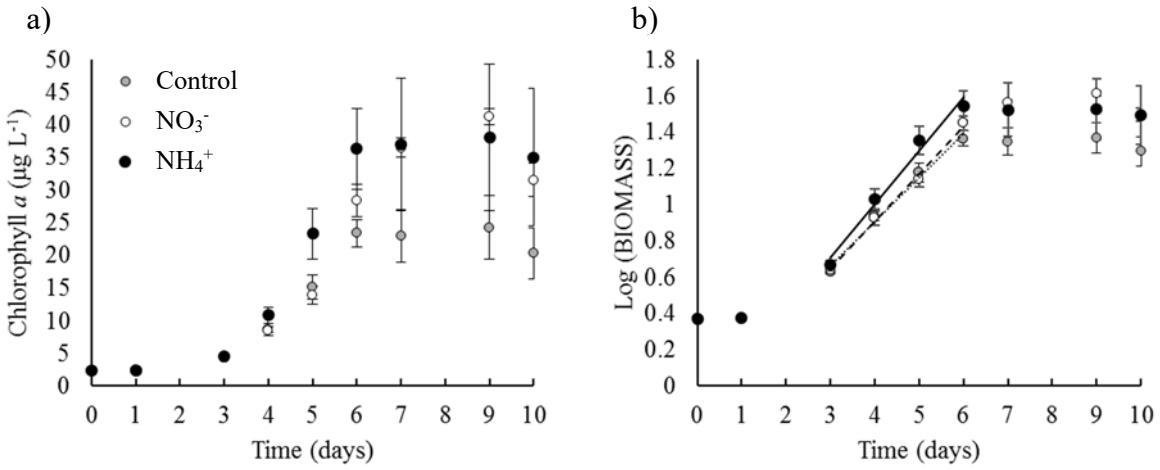


Figure 4.2: Change in a) total chlorophyll *a* concentration (µg L^{-1}) over the growth period in the Werribee River estuary samples and b) the log transformed values of chlorophyll *a* from a) and the region of exponential growth used to calculate the growth rates given in Table 4.1. Error bars represent standard error (n = 3).

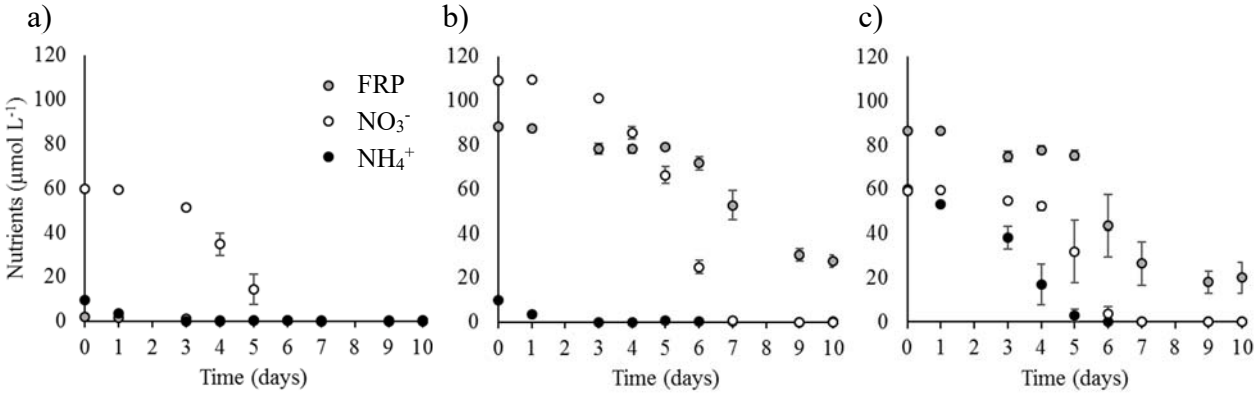


Figure 4.3: Change in nutrient concentrations (µmol L^{-1}) in a) the control, b) the NO_3^- and c) the NH_4^+ treatments over the bioassay growth period in the Werribee River estuary. Error bars represent standard error (n = 3).

The growth rates of the Mitchell populations in the NH_4^+ and NO_3^- treatments were very similar and were significantly higher than the growth rate of the control (Table. 4.1; Fig. 4.4; $p < 0.05$). Peak growth in the two addition treatments occurred between days three and five. Maximum yield occurred in the nutrient addition treatments on day eight, after which they were terminated along with the control. The NH_4^+ treatment had the highest average maximum yield compared to the NO_3^- treatment but high variability meant this was not significant. In all treatments NH_4^+ was used most rapidly until it was almost depleted, at which point NO_3^- uptake increased (Fig. 4.5). Algal growth within the control treatment was sustained throughout the growth period with no sign of plateau, however as a control it was terminated at the same time as the treatments.

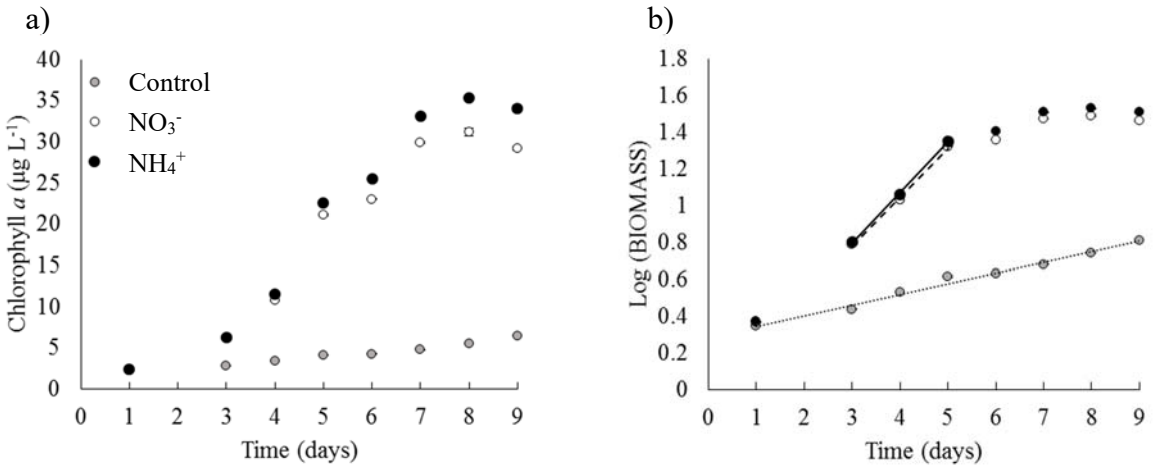


Figure 4.4: Change in a) total chlorophyll *a* concentration (µg L^{-1}) over the growth period in the Mitchell River estuary samples and b) the log transformed values of chlorophyll *a* from a) and the region of exponential growth used to calculate the growth rates given in Table 4.1. Error bars in a) are standard error (n = 3).

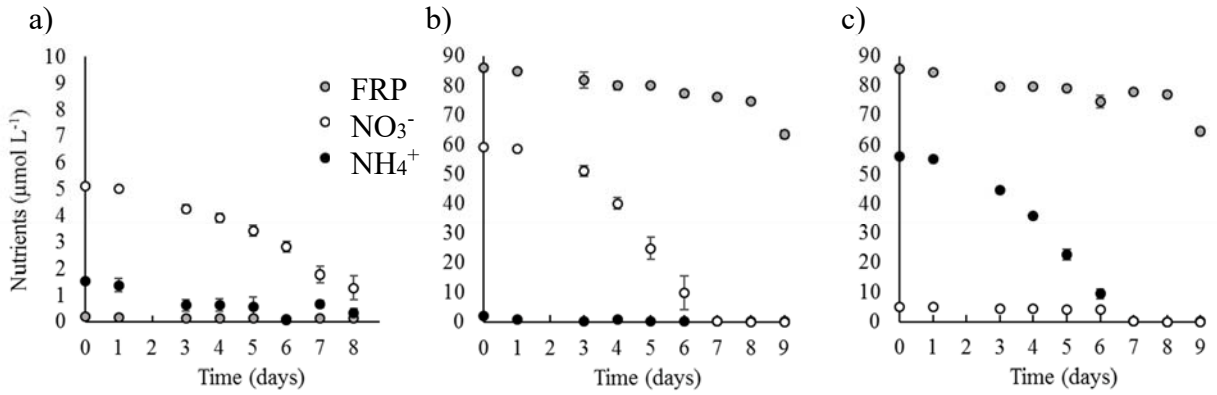


Figure 4.5: Change in nutrient concentrations (µmol L^{-1}) in a) the control, b) NO_3^- and c) NH_4^+ treatments over the bioassay growth period in the Mitchell River estuary. Note the different y-axis for the control treatment. Error bars represent standard error (n = 3).

4.4.2 The effect of nutrient addition on phytoplankton abundance and community structure

Total *in situ* (initial) phytoplankton populations were dominated by flagellates, diatoms and chlorophytes at both sites (Fig. 4.6). The flagellate phytoplankton groups included dinoflagellates, chrysophytes, prymnesiophytes, cryptophytes, prasinophytes and euglenophytes. Cyanobacteria were also observed in the Mitchell estuary but not in the Werribee estuary *in situ* samples. The dominance of each algal group changed after the growth period in not only the addition treatments but also the controls. In the Werribee, diatoms increased in dominance in the control and NH_4^+ treatments, chlorophytes increased in the control and NO_3^- treatments and cyanobacteria were observed in all treatments but most dominant in the NO_3^- treatment (Fig. 4.6a). Conversely, flagellate dominance decreased in all treatments as they did in the Mitchell bioassays (Fig. 4.6a and c). Diatom dominance also decreased in the Mitchell bioassays where chlorophytes became extremely dominant in each treatment and cyanobacteria increased somewhat in

all but the NH_4^+ treatment (Fig. 4.6c). However, for reasons outlined in the data analysis and discussion sections, the results from the Werribee estuary are also presented with the exclusion of *Cocconeis* spp. In that case, cyanobacteria dominated the Werribee NH_4^+ treatment, while diatoms, flagellates and chlorophytes were very similarly abundant (Fig. 4.6b)

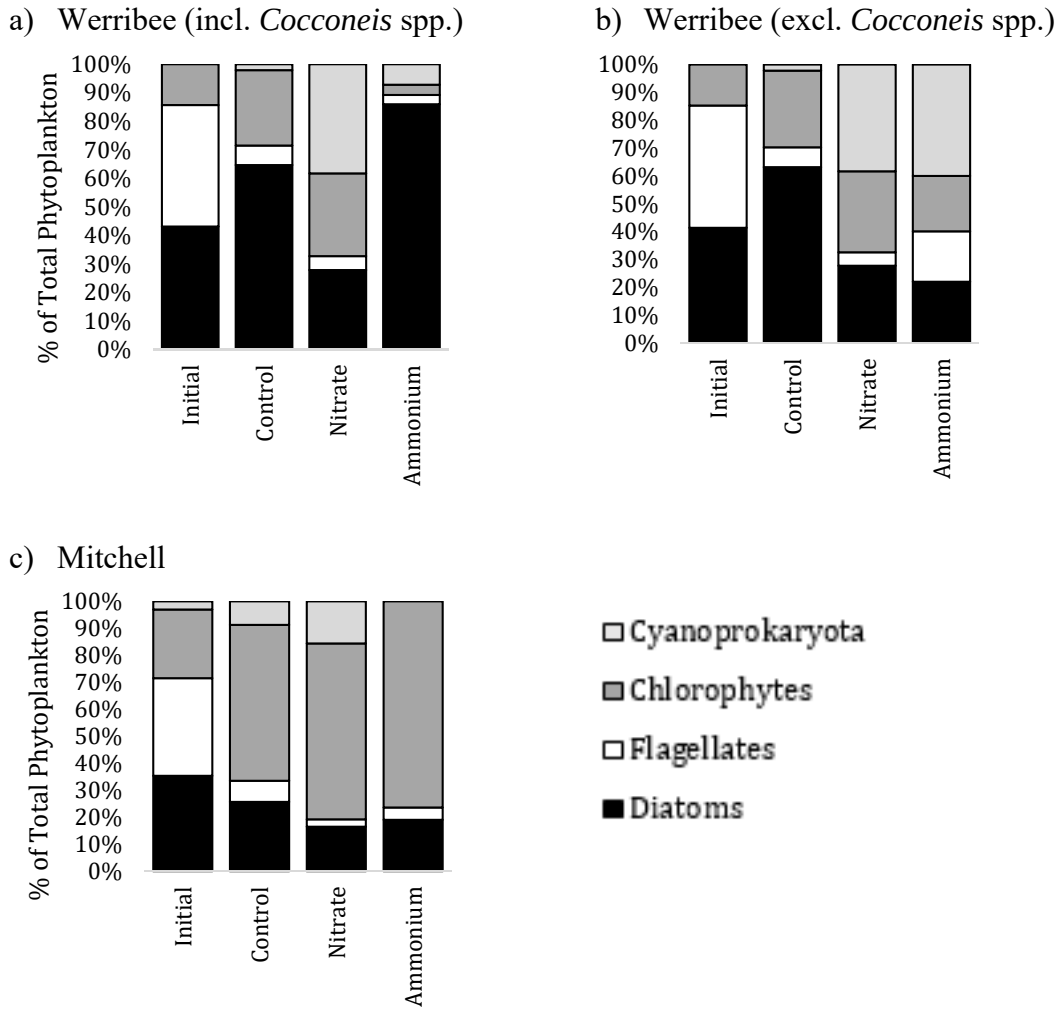


Figure 4.6: The percentage dominance of the main phytoplankton groups in the initial sample and each bioassay treatment after growth from a) the Werribee estuary including *Cocconeis* spp., b) the Werribee estuary excluding *Cocconeis* spp. and c) the Mitchell estuary.

The dominant and most abundant phytoplankton taxa that will be further discussed are the diatoms *Cocconeis* spp., *Nitzschia* spp. and *Cyclotella* sp., the dinoflagellates grouped as gymnodinioid spp., the chrysophyte *Ochromonas* spp., the cryptophyte *Hemiselmis* sp., the chlorophytes *Desmodesmus* sp. and *Klebsormidium* sp. and cyanobacteria. These taxa and groups were not only dominant within the estuaries during this sampling period but also during the 2014 (Werribee; see Chapter 2) and 2015 (Mitchell; see Chapter 3) sampling periods. The *in situ* abundances of these algal groups and taxa were not significantly different between the two sites during this 2016 bioassay study. However, in response to one of more treatments, the abundance of each of these taxa and groups increased. Significant differences in algal abundances between the two sites and within the treatments at each site are outlined in Table 4.2.

Total phytoplankton abundances increased most in response to the addition of NH_4^+ in the Werribee estuary (Fig. 4.7a). This was due to the diatom *Cocconeis* spp., which on average made up 82% of total phytoplankton and 95% of total diatoms in the NH_4^+ treatment. Therefore, the abundances of total phytoplankton, diatoms and *Cocconeis* spp. were significantly greater in the Werribee NH_4^+ treatment than the initial, control and NO_3^- treatments (Fig. 4.7; $p < 0.05$). These groups were also significantly more abundant in the Werribee NH_4^+ treatment than the Mitchell NH_4^+ treatment ($p < 0.05$). However, when *Cocconeis* spp. was excluded, the addition of NO_3^- resulted in the greatest total phytoplankton abundance at both estuaries (Fig. 4.8a). These abundances were significantly greater than the initial and control abundances at both sites ($p < 0.05$) and also significantly greater than abundances in the NH_4^+ treatment in the Werribee ($p < 0.05$). The addition of NH_4^+ increased the abundance of total phytoplankton (excluding *Cocconeis* spp.) at both sites significantly compared to the initial and control treatments

($p < 0.05$) (though only marginally compared to the control in the Werribee; $p < 0.1$). Total abundance was greater in the Werribee control compared to the Mitchell control and was significantly greater than the initial abundance in the Werribee ($p < 0.05$).

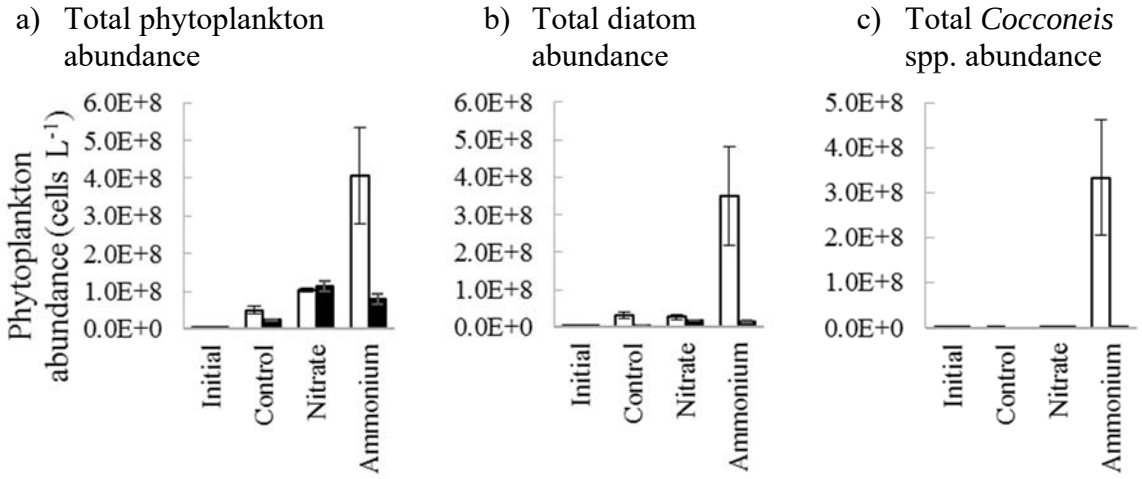


Figure 4.7: Bar charts of a) total phytoplankton abundance, b) total diatom abundance and c) *Cocconeis* spp. abundance in cells L^{-1} in the initial samples and after the growth period in each bioassay treatment. The Werribee samples (white bars), the Mitchell samples (black bars); significant differences are quoted in Table 4.2.

Chlorophytes contributed most to total phytoplankton abundance within the Mitchell estuary (Fig. 4.8b). The addition of NO_3^- and NH_4^+ both produced significantly greater abundances of chlorophytes compared to all other treatments, including the Werribee addition treatments ($p < 0.05$). In the Werribee, chlorophytes increased most in the NO_3^- treatment and the only non-significant result was between the control and NH_4^+ treatments (Fig. 4.8b; $p < 0.05$). The dominant taxa were *Desmodesmus* sp. and *Klebsormidium* sp. *Desmodesmus* sp. had the same pattern of significance as total chlorophytes in the Mitchell bioassays, but the highest average abundance occurred in the NH_4^+ treatment (Fig. 4.8c). In the Werribee bioassay treatments, significant (and

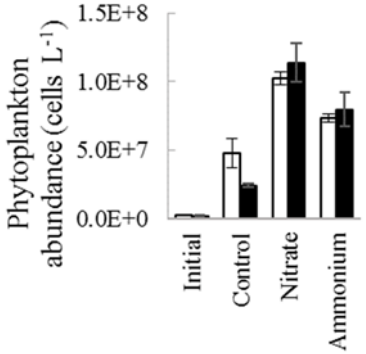
marginally significant) *Desmodesmus* sp. abundance increases only occurred compared to initial abundances ($p < 0.05$ and $p < 0.1$ respectively). *Klebsormidium* sp. was not observed *in situ* in the Werribee, however it was observed in each of the subsequent bioassay treatments. It had the highest average abundance in response to NO_3^- addition in both estuaries but significant results only occurred in the Mitchell where abundances in the NO_3^- treatment were significantly greater than the initial and control treatments (Fig. 4.8d; $p < 0.05$). The addition of NH_4^+ also produced increased abundances of *Klebsormidium* sp. in the Mitchell, but high variation between triplicate abundances resulted in no significant differences.

Cyanobacteria were the next most abundant phytoplankton group, dominated by *Pseudanabaena* sp. at both sites. In the Werribee bioassays, this genus increased significantly in the NO_3^- and NH_4^+ treatments compared to both the initial and control (Fig. 4.8e; $p < 0.05$). In the Mitchell, cyanobacteria increased most in the NO_3^- treatment but abundances were highly variable between triplicates and therefore no significant difference was observed. Cyanobacteria were not observed in the Mitchell NH_4^+ treatment.

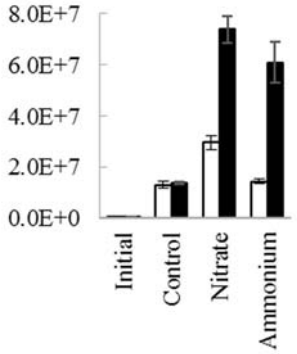
Total diatom abundance was dominated by the benthic taxa *Cocconeis* spp. in the Werribee NH_4^+ treatment as previously discussed. When *Cocconeis* spp. was excluded, the average diatom abundance was greatest in the control treatment, closely followed by the NO_3^- and then the NH_4^+ treatments with no significant differences between treatments (Fig. 4.8f). Diatom abundance in the Mitchell control was significantly lower than in the Werribee control ($p < 0.05$). It was also significantly lower than the abundances observed in the Mitchell NO_3^- and NH_4^+ treatments ($p < 0.05$). Excluding *Cocconeis* spp., the most

abundant diatoms were *Nitzschia* spp. and *Cyclotella* sp. The pattern of abundance change for *Nitzschia* spp. was similar to that of total diatoms (excluding *Cocconeis* spp.), but the significant increase of abundance in the Mitchell NH_4^+ treatment compared to the initial was marginal (Fig. 4.8g; $p < 0.1$). Conversely, abundances of *Cyclotella* sp. were greatest in the NO_3^-treatments for both estuaries and was also high in the Werribee control (Fig. 4.8h). However, only in the Mitchell estuary were any differences in abundance significant. *Cyclotella* sp. was significantly more abundant in all Mitchell treatments compared to the initial ($p < 0.05$), and the NO_3^- treatment was significantly more abundant than the control ($p < 0.05$) and marginally more abundant than the NH_4^+ treatment ($p < 0.1$).

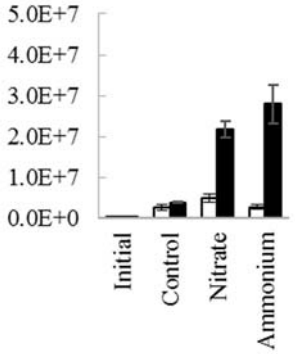
a) Total phytoplankton (excl. *Cocconeis* spp.)



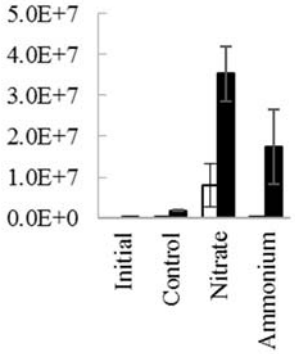
b) Total chlorophytes



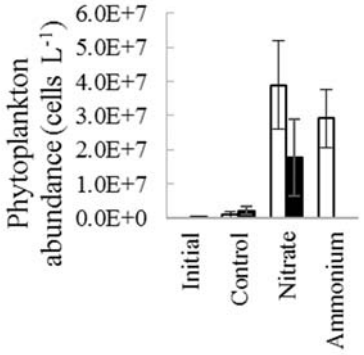
c) *Desmodesmus* sp.



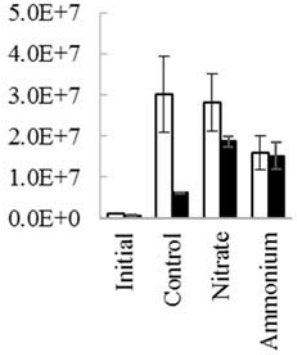
d) *Klebsormidium* sp.



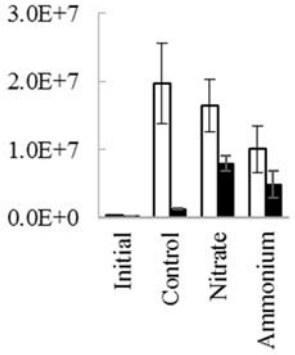
e) Total cyanobacteria



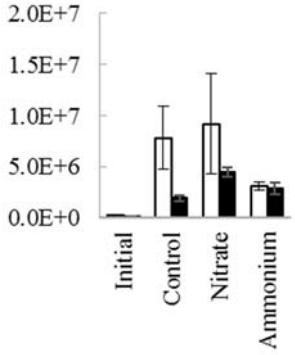
f) Total diatoms (excl. *Cocconeis* spp.)



g) *Nitzschia* spp.



h) *Cyclotella* sp.



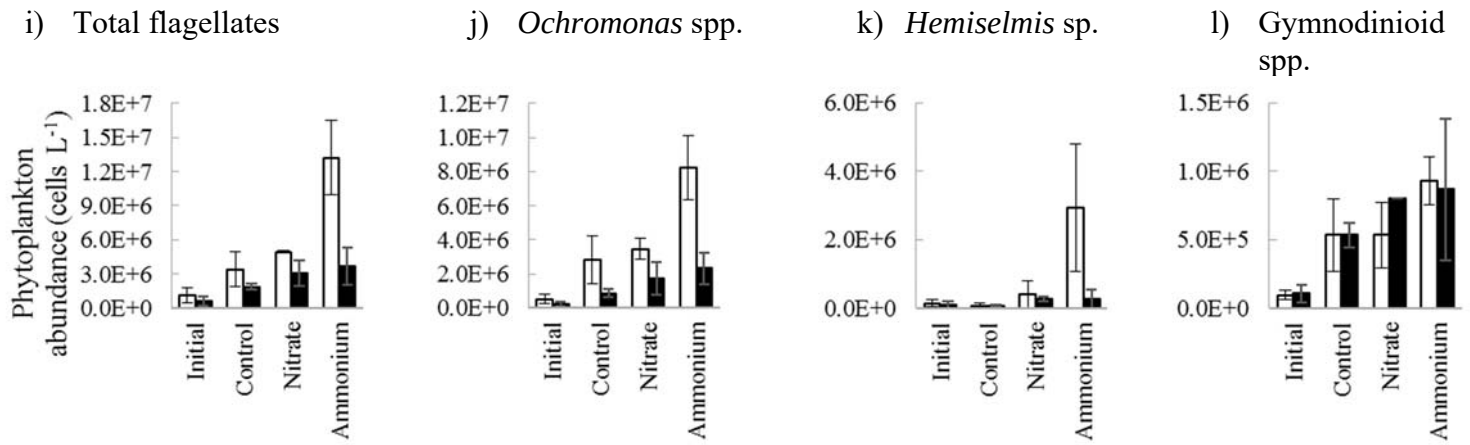


Figure 4.8: Bar charts of a) total phytoplankton excluding *Cocconeis* spp., b) total chlorophytes, c) *Desmodesmus* sp., d) *Klebsormidium* sp., e) total cyanobacteria, f) total diatoms excluding *Cocconeis* spp., g) *Nitzschia* spp., h) *Cyclotella* sp., i) total flagellates, j) *Ochromonas* spp., k) *Hemiselmis* sp. and l) Gymnodinioid spp. in cells L^{-1} in the initial samples, and at the end of the bioassay growth periods in the control, NO_3^- and NH_4^+ treatments. The Werribee samples (white bars); the Mitchell samples (black bars). Significant differences are provided in Table 4.2; note y-axis scales are different between graphs.

Total flagellate abundance increased most in response to NH_4^+ additions in both estuaries, but flagellates were significantly more abundant in the Werribee NH_4^+ treatment than the Mitchell NH_4^+ treatment (Fig. 4.8i; $p < 0.05$). Flagellate abundance in the Werribee NH_4^+ treatment was also significantly greater than abundances in the Werribee initial and control treatments ($p < 0.05$), but marginally compared to the NO_3^- treatment ($p < 0.1$). No significant differences in total flagellate abundances were observed across the Mitchell treatments. The chrysophyte *Ochromonas* spp. was the most abundant flagellate taxa, which showed the same pattern of significance as total flagellates, though the significant difference between the NH_4^+ and control treatment was only marginal (Fig. 4.8j; $p < 0.1$). There were no significant differences for the cryptophyte *Hemiselmis* sp., due to high variability between triplicates, however NH_4^+ addition was observed to have promoted the highest abundance on average in the Werribee (Fig. 4.8k). The dominant dinoflagellate group of gymnodinioid spp. showed marginally significant abundance increase in the Werribee NH_4^+ treatment compared to their initial abundance ($p < 0.1$), and also increased abundance within the Mitchell; however variation between replicates was large (Fig. 4.8l).

4.5 Discussion

The study presented here on the response of two natural phytoplankton assemblages to NO_3^- and NH_4^+ addition, helped us to understand and confirm some conclusions drawn from two previous case studies on the drivers of productivity in the Werribee and Mitchell River estuaries (Chapters 2 and 3 respectively). Firstly, it was determined that the natural phytoplankton populations from the two estuaries could be successfully grown in laboratory conditions, not just in response to nutrient additions but also under the *in situ* nutrient concentrations. This indicated that the nutrient concentrations present within both estuaries could support higher algal biomasses than were present at the time of sampling. In the Werribee estuary in 2014, this finding was observed *in situ*, when high nutrient concentrations, particularly NO_3^-, promoted algal growth to blooming proportions (see Chapter 2). This result, and the high growth rate in the Werribee control treatment, indicated that nutrient availability may not be a growth limiting factor within the Werribee estuary. Phytoplankton might simply be advected from the system faster than they can grow and utilise the high N load. Without constant nutrient addition however, algal growth halted in the control once NO_3^- was depleted (while NH_4^+ and FRP were still present). Within the Mitchell estuary, the addition of NO_3^- and NH_4^+ resulted in higher algal biomass (as chlorophyll *a*) than observed at any time during the 2015 sampling season (see Chapter 3). However, both NO_3^- and NH_4^+ addition produced similar algal growth rates that were not significantly different, and therefore it cannot be said with confidence which form was limiting or whether it was the addition of P that caused the most algal growth within the two treatments. On average however, NH_4^+ addition did promote a higher maximum yield than NO_3^- addition (as chlorophyll *a*), and

previous bioassay studies by Holland et al. (2012) strongly suggest that phytoplankton in that region were N limited most of the time, not P limited.

Chlorophyll *a* concentrations are generally a good proxy for algal biomass, but only by identifying the phytoplankton present in each bioassay treatment can we determine the effects of NO_3^- and NH_4^+ addition on specific algal groups and taxa. Additionally, total phytoplankton abundance results showed slightly different patterns of growth between treatments than chlorophyll *a* concentration results. This was because the PhytoPAM was not calibrated for all taxa present. However, many of the significant differences between total phytoplankton abundances (excluding *Cocconeis* spp.) were the same as the significant differences in maximum yield of chlorophyll *a*, particularly in the Mitchell bioassays (Table. 4.2). It is unknown what caused the bloom of *Cocconeis* spp. within the Werribee NH_4^+ treatment. Similar NH_4^+ concentrations were added to the Mitchell NH_4^+ treatment but no bloom occurred. It is possible that the presence of the bioassay bottle may have provided a substrate for *Cocconeis* spp. to attach via its mucus-producing raphe, however all treatments were subject to the same environment and did not produce such high abundances. Additionally, nutrient uptake was not increased in the Werribee NH_4^+ treatment compared to any other treatment, and when *Cocconeis* spp. was excluded from the analysis the remaining phytoplankton were similarly abundant to populations under other nutrient addition treatments. Therefore, the remainder of this section will discuss phytoplankton composition with the exclusion of *Cocconeis* spp.

Diatoms are an important algal group within estuarine environments as they often dominate the water column in NO_3^--rich systems during spring (Glibert et al. 2016). Of particular interest is the small centric diatom, *Cyclotella* sp., which is known to rapidly

assimilate NO_3^- (Vanni 1987, Amano et al. 2012), and as hypothesised, increased most in response to high NO_3^- concentrations in the bioassays of both estuaries. This result was most clearly observed in the Mitchell bioassays, where *in situ* NO_3^- concentrations were low, resulting in a significant increase in *Cyclotella* sp. abundance in response to the addition of NO_3^- ($p < 0.05$). In contrast, high *in situ* NO_3^- concentrations in the Werribee estuary resulted in high *Cyclotella* sp. abundances in both the control and NO_3^- treatments (Fig. 4.8h). However, lower abundances were observed within the Werribee NH_4^+ treatment, which were exposed to the same high NO_3^- concentrations as the control treatment. Therefore, it is proposed that high NH_4^+ concentrations inhibited NO_3^- uptake by *Cyclotella* sp., a well-known phenomenon (Dortch 1990, Lomas & Glibert 1999a, Glibert et al. 2016). This was also observed in the Werribee during 2014, where *in situ* NO_3^- concentrations were consistently >28 μmol L^{-1}, but *Cyclotella* sp. was only found to bloom when NH_4^+ concentrations were < 1 μmol L^{-1} (see Chapter 2). A similar finding was also observed in the Mitchell during 2015, where the abundance of *Cyclotella* sp. was highest when NH_4^+ concentrations decreased below 2 μmol L^{-1} (Fig. 6.4b). This only occurred on one date. On all other dates, *Cyclotella* sp. abundances were lower, but were found to increase significantly in response to increased NO_3^- concentrations (Fig. 6.4b; $p < 0.05$). This indicates that high NH_4^+ concentrations did not fully inhibit NO_3^- uptake by *Cyclotella* sp.

Table 4.2: Table of significant differences in phytoplankton abundance from Figs. 4.7 and 4.8

Treatment	Significant difference between rivers				Significant difference between treatments: Werribee						Significant difference between treatments: Mitchell					
	IN	**C**	**NO3**	**NH4**	**IN C**	**IN NO3**	**IN NH4**	**C NO3**	**C NH4**	**NO3 NH4**	**IN C**	**IN NO3**	**IN NH4**	**C NO3**	**C NH4**	**NO3 NH4**
Total Phytoplankton				***			***		***	***		***	***	***	***	
excl. *Cocconeis* spp.					***	***	***	***	*	***		***	***	***	***	
Total Diatoms				***			***		***	***		***	***	***	***	
excl. *Cocconeis* spp.		***			***	*				***		***	***	***	***	
Cocconeis spp.				***			***		***	***						
Nitzschia spp.		***			***	*						***	*	***		
Cyclotella sp.											***	***	***	***		*
Total Flagellates				***			***		***	*						
Total Dinoflagellates							***									
Gymnodinioid spp.							*									
Chrysophytes				***			***		*	*						
Ochromonas spp.				***			***		*	*						
Total Cryptophytes																
Hemiselmis sp.																
Total Chlorophytes			***	***	***	***	***	***		***		***	***	***	***	
Desmodesmus sp.			***	***	*	***	*					***	***	***	***	
Klebsormidium sp.	0		***									***		***		
Total Cyanobacteria	0					***		***								
Maximum yield (μg chl L^{-1})						***	***				***	***	***	***	***	
Growth rate (μ)	N/A				N/A	N/A	N/A				N/A	N/A	N/A	***	***	

Notes:
*** p < 0.05
* p = 0.05 to 0.1
0 No phytoplankton taxa present initially
IN Intial treatment
C Control treatment
NO3 NO_3^- treatment
NH4 NH_4^+ treatment

Although *Cyclotella* sp. was dominant within the Werribee estuary in 2014 (see Chapter 2), and was abundant in the Mitchell during 2015 (see Chapter 3), *Nitzschia* spp. was the dominant diatom within both estuaries during the 2016 sampling. Like *Cyclotella* sp., *Nitzschia* spp. is a bloom forming genus, and it responded well to both NO_3^- and NH_4^+ additions. However, on average it increased most in abundance in response to NO_3^-, and although these results were not statistically significant, it may indicate that *Nitzschia* spp. is a better assimilator of NO_3^- than NH_4^+. A similar result was found in a study by Mitrovic et al. (2008) where both *Cyclotella* sp. and *Nitzschia* spp. blooms caused a decrease in oxidised nitrogen concentrations. However, these blooms were found to be most strongly correlated to low flow (<400 ML day^{-1}) and water temperature (>23°C) (Mitrovic et al. 2008). Therefore, lack of turbulence within the bioassay bottles during this experiment may have been another factor that caused the high abundances of both *Cyclotella* sp. and *Nitzschia* spp. Additionally, there are many attached forms of *Nitzschia* spp. and therefore the presence of the bottle itself may have contributed to the increased abundances observed. However, this may also lead to an underestimation of the abundance of these attached forms, which may not have been transferred to the sample collected for identification. Nonetheless, these results may indicate the potential for *Nitzschia* spp. blooms to occur within the Werribee estuary in times of low flow, like the *Cyclotella* sp. blooms. Low flow is common in the Werribee due to riverine water regulations, and when *Cyclotella* sp. bloomed in 2014 the average flow was only ~4 ML day^{-1} (see Chapter 2). The environmental flow release that caused the end of the *Cyclotella* sp. bloom in 2014 peaked at ~200 ML day^{-1}.

Freshwater flows strongly control stratification strength in estuaries, which when increased is linked to higher water column NH_4^+ concentrations as a result of benthic

nutrient recycling. The only phytoplankton group to significantly increase in abundance in response to NH_4^+ addition were flagellate phytoplankton in the Werribee bioassay (Fig. 4.8i; $p < 0.05$). The flagellate taxa that increased most were among the most dominant during the 2014 and 2015 study periods including the dinoflagellates grouped as gymnodinioid spp., the chrysophyte *Ochromonas* spp. and the cryptophyte *Hemiselmis* sp. (see Chapters 2 and 3). In contrast, no significant increase in flagellate abundances were observed in the Mitchell NH_4^+ treatment. The main difference between the NH_4^+ treatments was the concentration of NO_3^-, which was much higher in the Werribee *in situ*. Therefore access to both NH_4^+ and NO_3^- was likely important for flagellate growth. We also hypothesise that competition by chlorophytes for available NH_4^+ may have contributed to this outcome. It is also possible that the significant and marginally significant results from the Werribee bioassays may have been influenced by other sources of nutrition, such as nanoplankton or bacteria. Many flagellate taxa are mixotrophic, meaning not only are they phototrophic but also phagotrophic and capable of engulfing food particles. This process is more likely in a light and/or nutrient poor habitat, and therefore may have occured in the bioassay treatments when nutrient levels were at or near depletion (Nygaard & Tobiesen 1993, Burkholder et al. 2008). Flagellates including crytophytes, chrysophytes, prymnesiophytes and dinoflagellates are the main groups capable of mixotrophy (Andersson et al. 1989, Bockstahler & Coats 1993, Sanders et al. 2001). Specifically, phagotrophy is common in *Ochromonas* sp. (Andersson et al. 1989), and gymnodinioid spp. (Stoecker 1999), which may have contributed to their increased abundances throughout the bioassay experiment. If occurring, this process would also result in a reduced abundance of the phytoplankton being consumed, which may affect community composition and final yield. This may have also been compouded by any microzooplankton not excluded from the bioassays due to their small size.

There has been increasing evidence that post-diatom blooms of mixed phytoplankton are linked to the bulk annual recruitment of copepods (Kleppel 1993, Runge & Lafontaine 1996, Jones et al. 2002). This provides copepods with a wide range of nutrition from which to select their prey (Gulati & Demott 1997, Koski et al. 1998, Shin et al. 2003). Flagellates, diatoms and chlorophytes were similarly dominant *in situ* in the Werribee and Mitchell estuaries, which is considered a mixed phytoplankton assemblage (Figs. 4.6b and c initial). After bioassay growth, the only treatment to retain similarly dominant flagellate, diatom and chlorophyte abundances was the Werribee NH_4^+ treatment, although cyanobacteria abundances did increase (Fig. 4.6b). This is similar to the algal assemblage observed within the Werribee estuary in 2014 when recycled NH_4^+ concentrations increased after an environmental flow release. The more mixed phytoplankton assemblage of diatoms, chlorophytes and flagellates that emerged, strongly correlated with increased NH_4^+ concentrations (Fig. 6.1a; $p < 0.04$). In response, copepod abundances were found to increase (Fig. 6.2c; $p < 0.03$). These results suggest that in the Werribee estuary where NO_3^- concentrations are high, increased NH_4^+ is necessary to promote flagellate abundances, a mixed phytoplankton assemblage and higher trophic levels.

In the Mitchell estuary, the algal assemblage present *in situ*, became dominated by chlorophytes in all treatments at the end of the bioassay. Chlorophytes are fast growing and a number of conditions within the bioassays may have contributed to their increased abundances including: low salinity, good light source, lack of turbulence and high nutrient availability (Correll 1978, Capriulo et al. 2002, Winder & Jassby 2011). In the 2015 Mitchell sampling period, chlorophytes were also observed to increase in the fresh surface water when flow decreased (see Chapter 3). In that study, chlorophyte abundances

increased significantly in response to increased NO_3^- concentrations (Fig. 6.4a; $p < 0.05$), a pattern which was also observed in both the Mitchell and Werribee bioassays. However, in the Mitchell, chlorophytes also increased in response to NH_4^+ additions. The two most abundant taxa, *Klebsormidium* sp., a filamentous long chain chlorophyte, and *Desmodesmus* sp., a colonial chlorophyte, responded best to NO_3^- and NH_4^+ addition respectively. Both taxa have been studied for their capacity to remove N and P from wastewater, and the results of those studied confirmed the respective N uptake results (Chícharo et al. 2006, Winder & Jassby 2011). These findings suggest that nutrient enrichment in the Mitchell estuary may result in chlorophyte blooms in the surface water under low flow conditions.

Cyanobacteria did not form a dominant part of the Mitchell algal assemblage *in situ* and were not observed *in situ* in the Werribee, however became very abundant in some bioassay treatments (Fig. 4.8e). Increased abundance of cyanobacteria is a sign of eutrophication in freshwater lakes, where cyanobacteria blooms are common (Murrell & Lores 2004). Blooms within estuarine and coastal environments are less common but have been found to occur within the Gippsland Lakes as blooms of *Nodularia spumigena* Mertens and *Synechococcus* sp. (Webster et al. 2001, Cook et al. 2008, Cook et al. 2010, Cook & Holland 2012, Holland et al. 2012). In those studies, abundances increased in response to regenerated P uptake and increased surface water dissolved inorganic N loads after catchment wide fires respectively. *Nodularia spumigena* and *Synechococcus* sp. were not observed in either estuary in this study however, and the dominant cyanobacterium was *Pseudanabaena* sp., a filamentous genus, which has been found to efficiently remove NO_3^- from wastewater with high N/P ratios. Within the bioassay treatments, *Pseudanabaena* sp. was most abundant in treatments with high NO_3^-

concentrations and P addition. Therefore, increased abundances of cyanobacteria within the Werribee may be P-limited, but as a P-addition-only treatment was not included, this cannot be conclusively stated.

4.6 Conclusion

This study investigated the response of two natural estuarine phytoplankton assemblages to nutrient addition using bioassays. The results obtained have helped us to understand some of the findings from two previous case studies on estuarine productivity presented in this thesis. Of particular interest was the diatom *Cyclotella* sp., which was confirmed to respond to both high *in situ* NO_3^- concentrations and addition of NO_3^-. This was best observed in the Mitchell bioassay experiment, where low *in situ* nutrient concentrations allowed for a clearer distinction between treatments after nutrient addition. In all bioassay treatments, NH_4^+ was observed to decrease before NO_3^-, which did not decrease until NH_4^+ concentration were ~1 μmol L^{-1}, which suggested a combination of phytoplankton preference for NH_4^+ and NH_4^+ inhibition of NO_3^- uptake. This inhibition was found to be a positive mechanisms within the Werribee estuary in 2014 as it prevented *Cyclotella* sp. from reaching high abundances on many sampling dates. Increased NH_4^+ concentrations were also found to be an important source of N to promote flagellates abundances, as well as a mixed algal assemblage. Chlorophyte and cyanobacteria responses to bioassay incubation may have been highly influenced by the high light and low flow conditions of the bioassay environment, however significant influence of nutrient addition were also observed. Very high chlorophyte abundances in the Mitchell to both N additions suggested the potential for chlorophyte blooms, particularly in low flow conditions if nutrient inputs increase. Cyanobacteria responded best to high NO_3^- concentrations in the presence of P addition.

Overall, high nutrient concentrations in the Werribee promoted high algal growth, which is a concern for the health of the estuary and the food web. In the Mitchell, only nutrient

additions produce high algal growth, which indicates the sensitivity of the system to increased human impacts. The presence/addition of NO_3^- produced the greatest and most negative changes in algal abundance and community assemblage. These results suggest that nutrients levels should be monitored in the future to ensure NO_3^- concentrations do not increase and NH_4^+ is present to inhibit the bulk of NO_3^- uptake in NO_3^--rich environments.

4.7 Acknowledgments

The authors wish to thank David Brehm who contributed to work in the field. We also thank Microalgal Services for their work on phytoplankton identification and counts. This work has been funded by the Australian Research Council Linkage Project Scheme (LP140100087), The University of Melbourne, Department of Environment, Land, Water and Planning (VIC), Gippsland Lakes Ministerial Advisory Committee, Water Technology and Melbourne Water. Caitlyn McNaughton was supported by a Monash University scholarship.

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Chapter 5: Discussion and concluding remarks

This thesis has investigated the controls and drivers of estuarine productivity in two Australian estuaries of contrasting flow regimes, nutrient loads and stratification strength. Figures 5.1 and 5.2 provide conceptual diagrams of the key hydrodynamic and biogeochemical processes controlling the estuarine food web in the Werribee and Mitchell River estuaries throughout the sampling periods.

In this chapter, the major findings of Chapters 2 – 4 are summarised, before a discussion in which the productivity in the two estuaries is compared and the importance of hydrodynamics and biogeochemical processes in estuarine environments are summarised in regards to human impacts and remediation. Finally, potential avenues for future work are proposed.

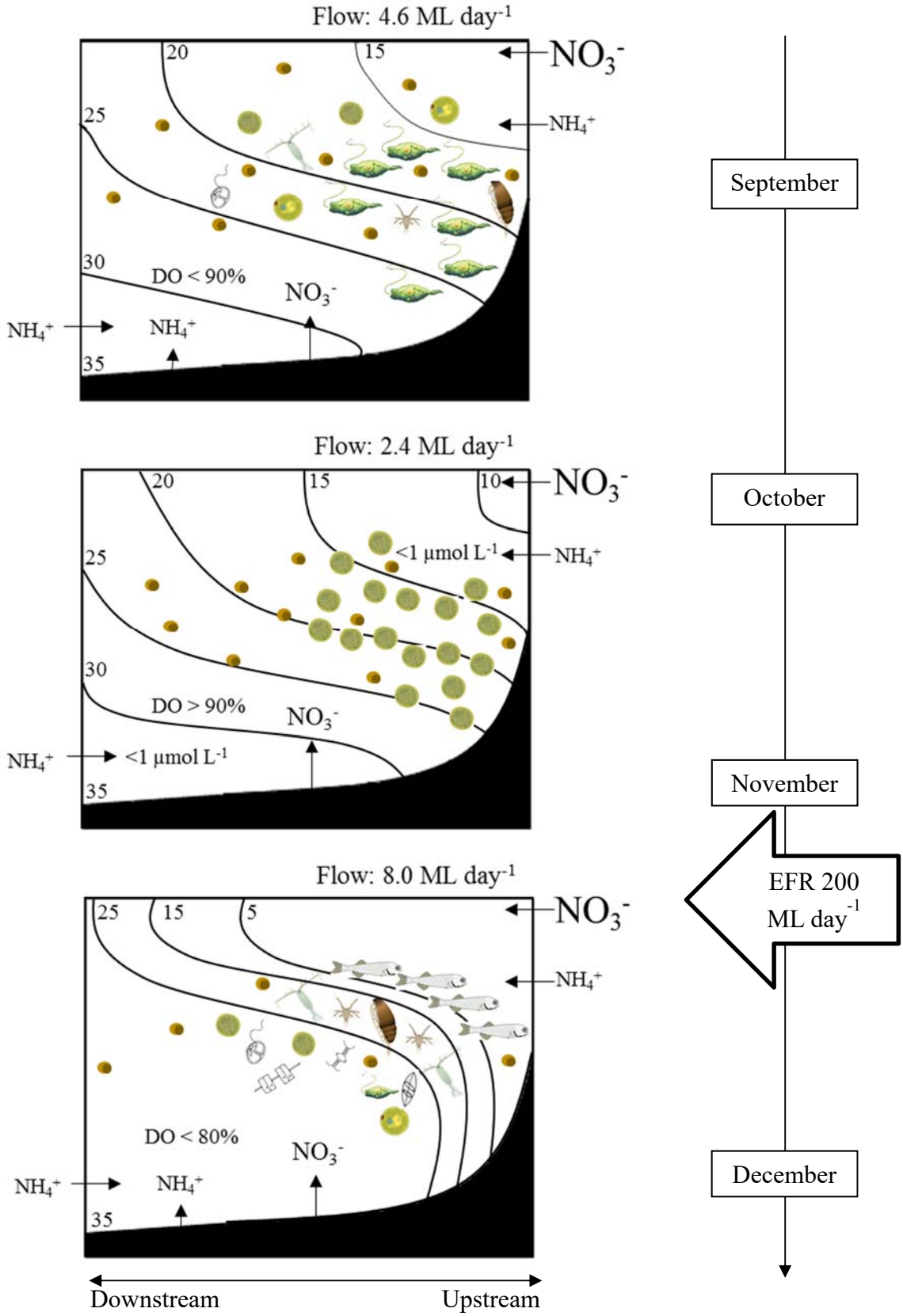
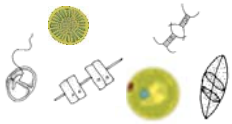


Figure 5.1: A conceptual diagram of salinity stratification (isohalines; PSU), nutrient sources and food web dynamics in the Werribee estuary under differing flow conditions.

Key:

Euglena spp.: Bloomed in early September and depleted NH_4^+ concentrations

Cyclotella sp.[1]: Bloomed when NH_4^+ concentrations were <1 µmol L^{-1}



Mixed phytoplankton[2]: Increased in abundance when NH_4^+ concentrations increased after the EFR

Copepod nauplii[1]: Abundant when a mixed phytoplankton assemblage was present

Calanoid copepods[3,4]: Abundant when a mixed phytoplankton assemblage was present

Cyclopoid copepod[4]: Present in low abundances

Acanthopagrus butcheri larvae[5]: Present after the EFR

Acanthopagrus butcheri egg: Present throughout the sampling period

(Saxby 2003[4], 2004[2], 2010[1], Thomas 2004[5], Kraeer & Van Essen-Fishman 2010[3])

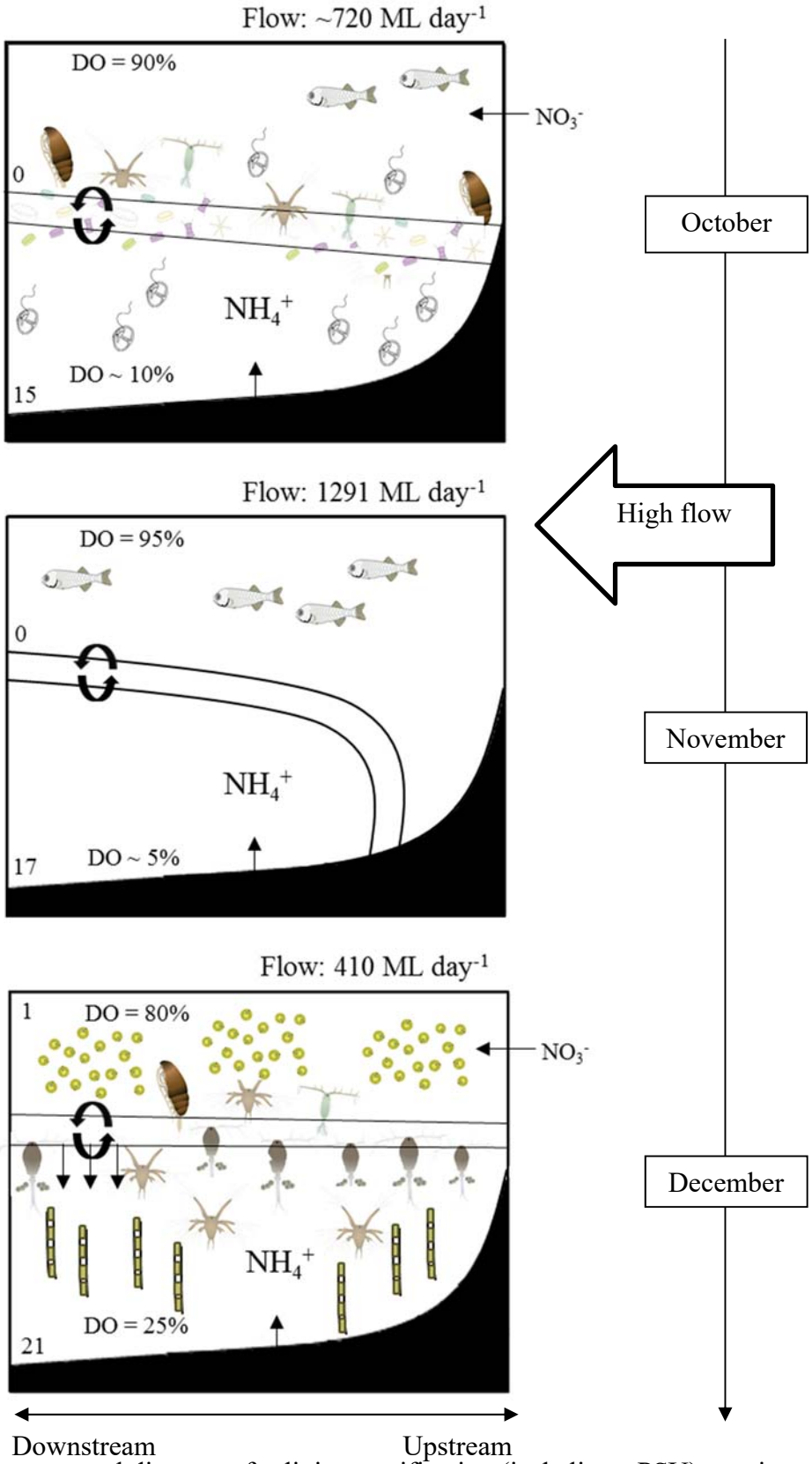


Figure 5.2: A conceptual diagram of salinity stratification (isohalines; PSU), nutrient sources and food web dynamics in the upper Mitchell estuary under differing flow conditions.

Key:

Mixed diatoms[6]: Accumulated at the halocline when mixing forces were sufficient

Skeletonema costatum: Accumulated in the bottom water as salinity increase, flows decreased and mixing forces declined

Chlorophyte phytoplankton[2]: Accumulated in the fresh surface water under lower flows

Flagellate phytoplankton: Dominated the bottom water until replaced by *Skeletonema costatum*

Copepod nauplii[1]: Abundant throughout except when flushed out of the estuary under high flows

Calanoid copepods[3,4]: Abundant throughout except when flushed out of the estuary under high flows

Cyclopoid copepod[3]: Dominated when salinity increased after salt wedge intrusion

Freshwater fish larvae[5]: Flushed into the estuary under high flows

Sufficient mixing forces

Declining mixing forces

(Kleine 2001[6], Saxby 2003[4], 2004[2], 2010[1], Thomas 2004[5], Kraeer & Van Essen-Fishman 2010[3])

5.1 Chapter Summaries

5.1.1 Chapter 2: The Werribee River Estuary

This chapter describes the field study that was undertaken to investigate how environmental flows stimulate estuarine productivity in the highly regulated, nutrient-rich Werribee River estuary. The Werribee estuary is a known spawning site of *Acanthopagrus butcheri*, but only a few year classes of *A. butcheri* are observed. *Acanthopagrus butcheri* are very selective feeders at early life stages and their spawning and successful recruitment is linked to freshwater flow and increased stratification strength. Therefore the salinity structure within the estuary and the food web supporting these fish larvae are of great importance for successful recruitment to the adult life stage. It was hypothesised that altered hydrodynamics and nutrient inputs impacted upon important biogeochemical processes in the estuary, negatively affecting *A. butcheri* recruitment. This chapter documents phytoplankton, zooplankton and fish larval dynamics before and after an environmental flow release which aimed to the hydrodynamics and promote optimal conditions for *A. butcheri* larvae.

High algal biomass (as chlorophyll *a*) and phytoplankton abundances occurred in the upper Werribee estuary when flow was low and residence times were high. High biomass was initially dominated by a bloom of the flagellate *Euglena* spp., which depleted NH_4^+. When NH_4^+ concentrations were <1 µmol L^{-1}, a bloom of the diatom *Cyclotella* sp. occurred, assimilating the high NO_3^- concentrations delivered via groundwater inputs. Groundwater was traced using radon (^{222}Rn), which correlated significantly with increased *Cyclotella* sp. abundances ($p < 0.04$). *Cyclotella* sp. is not considered harmful

but in blooming proportions was found to have a negative association with calanoid copepod abundances, likely due to ingestion impacting upon reproduction. The environmental flow event coupled with increased rainfall triggered the end of the *Cyclotella* sp. bloom and caused increased stratification within the upper estuary. As a result, bottom water DO decreased, which facilitated NH_4^+ accumulation in the bottom water. Increased NH_4^+ concentrations were associated with a significant increase in the abundance of flagellate species ($p < 0.04$) and of calanoid copepods ($p < 0.01$). Increased flagellate abundances were also significantly positively correlated with increased calanoid copepod abundances ($p < 0.03$). This provided optimal prey for larval *A. butcheri*, which were found in abundance for the first time following the EFR, though their eggs were present throughout the sampling period. The positive associations between a more mixed algal assemblage (including flagellates, diatoms and chlorophytes), calanoid copepods and *A. butcheri* larvae occurred when the estuary was most strongly stratified after the EFR. Therefore, the increased strength of the halocline resulted in a zone of accumulation where predator/prey interaction increased.

This sequence of events outlines the importance of environmental flows to the highly regulated Werribee estuary, where high residence times and nutrient loads resulted in eutrophication, an unsuitable habitat and insufficient diet for *A. butcheri* larvae. It also led to the hypothesis that NH_4^+ played an important role as an inhibitor of NO_3^- uptake by blooming taxa, and also as the preferred N form for flagellate phytoplankton, which formed an important portion of the mixed algal assemblage promoting productivity of higher trophic levels.

This study made some important findings that have only been observed in a few previous cases. Firstly the direct link between ^{222}Rn (Bq/m^3) and the diatom *Cyclotella* sp. (cells L^{-1}) was important as it indicates that ^{222}Rn could be used as a proxy for nutrient concentrations (in this case NO_3^-) when groundwater is nutrient enriched. Previous links between groundwater measurements and chlorophyll *a* concentrations have been found but not a direct link to a blooming phytoplankton taxa (Basterretxea et al. 2010, Lee et al. 2010, Su et al. 2014). Secondly this chapter is a rare example of how environmental flows can be used to stimulate aquatic production. Most environmental flows are aimed at promoting movement of biota in a systems or the creation of a habitat (Sponseller et al. 2013). The idea of using an environmental flow to stimulate desirable production to support higher level objectives is not new, yet there are relatively few examples of this being tested and achieved in practice (Rolls et al. 2012, Rolls et al. 2017).

5.1.2 Chapter 3: The Mitchell River Estuary

This chapter describes a field study that was undertaken in the upper Mitchell River estuary to investigate how salt wedge intrusion and stratification strength influences nutrient dynamics, the estuarine habitat and the estuarine food web. Many studies have focussed on one or two aspects of salt wedge intrusion such as how it facilitates nutrient release and/or impacts upon some aspect of the estuarine food web. However, to my knowledge, none have considered how salt wedge intrusion to the upper estuary drives phytoplankton, zooplankton and fish larvae productivity in an estuary with low watershed N inputs. This type of comprehensive study was considered necessary in the Mitchell River estuary because it is the main tributary to Lake King where HABs commonly occur. Therefore, when considering remediation practises for the lakes, it is important to

know how they may affect phytoplankton productivity and biomass in the region, particularly considering the Mitchell estuary is an important spawning ground of *A. butcheri*. This study of an unregulated river/estuarine system also serves as baseline, which will help to inform EFRs.

Strong salinity stratification was present in the upper Mitchell estuary on most sampling dates, except when freshwater flows were very high. Increased bottom water salinity as a result of salt wedge intrusion and low bottom water DO, facilitated benthic nutrient recycling and the accumulation of NH_4^+ in the bottom water. Phytoplankton biomass (as chlorophyll *a*) was significantly negatively correlated with NH_4^+ concentrations in the bottom water ($p < 0.05$), an indication of assimilation and that NH_4^+ strongly supported algal biomass in the system. Flagellate phytoplankton were often dominant in the bottom water and halocline, particularly under high flow conditions when their motility allowed them to exploit high nutrient zones. As flows decreased, NO_3^- concentrations increased in the surface water, facilitating increased phytoplankton biomass at the halocline, dominated by diatoms due to sufficient mixing forces. Chlorophyte phytoplankton and the diatom *Cyclotella* sp. were significantly positively correlated to NO_3^- concentrations ($p < 0.05$). The increased abundances of chlorophytes were confined to the high light environment of the fresh surface water at the end of the sampling period, when flows were lowest. In contrast, the bottom water was dominated by the diatom *Skeletonema costatum* at this time, which was best adapted to the NH_4^+-rich environment and increased salinity after salt wedge re-intrusion.

Increased salinity after salt wedge re-intrusion also exerted a strong control on higher trophic levels. In general, copepods were found to accumulate at the halocline as

indicated by the positive correlation between total copepod and phytoplankton biomass (as chlorophyll *a*; $p < 0.05$). Calanoid copepods were initially dominant in the zooplankton assemblage until a rainfall event flushed zooplankton from the upper estuary. As copepods returned, calanoids were dominated by cyclopoid copepods, which are better adapted to increased salinity, decreased DO and preying upon flagellate phytoplankton. Grazing pressure increased as cyclopoid abundances increased, a likely cause of decreased flagellate phytoplankton abundances at the end of the sampling period. Physical forcing of freshwater flows appeared to be the main control of fish larvae present. *Retropinna semoni* is a freshwater fish, which spawned in the river and was flushed into the upper estuary under high flows. The other dominant fish larvae, *Philipnodon grandiceps*, had a later spawning phenology but was also likely flushed into the estuary from upstream. However, its presence further downstream may have been due to its adaptation to estuarine environments. The only fish to spawn within the estuary were benthic gobies, whose larvae were observed in low abundances in the mid estuarine region at the end of the sampling period. This later spawning phenology and the absence of other estuarine fish larvae indicated that non-optimal conditions were present within the Mitchell estuary during most of the sampling period. Low DO as a result of persistent stratification and then salt wedge intrusion was considered the likely cause.

This sequence of events outlines the importance of salt wedge intrusion in facilitating nutrient release from the sediment and promoting the estuarine food web. Most interestingly, the peak spring phytoplankton biomass was supported by recycled NH_4^+, as opposed to watershed nutrients, which are commonly dominated by NO_3^-. Very low NO_3^- concentrations reflect the fact that the Mitchell River drains a relatively intact catchment. However, low NO_3^- concentrations were present in the surface water and were found to

stimulate diatom productivity at the halocline and chlorophyte productivity in the surface waters. This suggests that if NO_3^- concentrations were to increase in the future, chlorophyte blooms may develop. These benign blooms may be preferable to the HABs of the cyanobacterium *Nodularia spumigena,* but the conditions that promote chlorophyte algal blooms also promoted a more recent HAB of the cyanobacterium *Synechococcus* sp. in the Gippsland Lakes (Cook et al. 2010, Cook & Holland 2012).

This study also highlighted the importance of unmodified freshwater flows to a nutrient-poor estuary. The maintenance of a stratified water column meant that mixing forces facilitated accumulation at the halocline, which increased the interaction between nutrient sources, phytoplankton biomass, zooplankton biomass and larval fish populations. However, *A. butcheri* larvae were not observed throughout the sampling period, which suggests spawning or hatching conditions were not optimal. This will be further discussed below.

5.1.3 Chapter 4: Bioassay experiments

This chapter describes the nutrient addition bioassay experiments undertaken to assess how the natural phytoplankton assemblages in the Werribee and Mitchell estuaries responded to the additions of NH_4^+ and NO_3^- under laboratory conditions. In particular, there was a focus on how NH_4^+ and NO_3^- were utilised throughout the bioassay growth period, and whether addition of one N form would stimulate higher abundance in a phytoplankton group or taxa than the other N form. Due to findings in the previous chapters, it was hypothesised that NH_4^+ would stimulate flagellate phytoplankton productivity and be utilised in preferentially, while NO_3^- would stimulate diatoms and

chlorophytes productivity. Maximum biomass yield and growth rate were also determined using chlorophyll *a* concentrations, which gave an indication as to the potential for algal blooms.

High *in situ* nutrient concentrations in the Werribee control bioassay stimulated an algal growth rate and maximum yield similar to that of the nutrient addition treatments. In the Mitchell bioassays, significantly higher growth rates and maximum yields were stimulated as a result of both NH_4^+ and NO_3^- addition compared to the control treatment ($p < 0.05$). Therefore, increased nutrient loads to the Mitchell estuary could potentially stimulate algal blooms, while *in situ* nutrient concentrations in the Werribee estuary have been confirmed to stimulate algal blooms, however in these studies no harmful blooming taxa were identified. Nutrient uptake patterns were also observed throughout the bioassay growth periods. Assimilation of NH_4^+ was observed first, followed by the assimilation of NO_3^- when NH_4^+ concentrations decreased to ~1 µmol L^{-1}. This is further evidence of NH_4^+ inhibiting NO_3^- uptake and confirms algal preference for NH_4^+ uptake. Flagellate phytoplankton responded best to NH_4^+ addition and significantly increased in abundance in the Werribee NH_4^+ bioassay treatment where NO_3^- concentrations were also high ($p < 0.05$). This was the only treatment to retain the most mixed assemblage, similarly dominated by flagellates, diatoms and chlorophytes, which was found to be an important food source for copepods in Chapter 2. The addition of NO_3^- was confirmed to promote the increased abundance of *Cyclotella* sp., which was significant in the Mitchell NO_3^- treatment ($p < 0.05$). Chlorophyte abundances increased significantly in response to both N forms in the Mitchell bioassays, which were significantly more abundant than chlorophytes in the Werribee treatments ($p < 0.05$). Additionally, chlorophyte abundance in the nutrient-poor Mitchell control was as high as the abundance in the nutrient-rich

Werribee control. These results indicate a high potential for chlorophyte blooms in the Mitchell estuary, where increased abundances were observed *in situ* in 2015 when low flow and high light conditions prevailed. These conditions were more exaggerated throughout the bioassay growth period. Cyanobacteria abundances increased most in response to NO_3^- concentrations, but only in treatments with P addition. Separate P addition treatments were not conducted and therefore this result would need further examination within the Werribee.

The results of this experiment appear to confirm two broad statements that have been recurring throughout this thesis: diatoms dominate NO_3^--rich systems, while increased NH_4^+ concentrations generally promote phytoplankton communities dominated by mixotrophic algae such as flagellates. However, flagellates only increased significantly in response to high NH_4^+ conditions when *in situ* NO_3^- concentrations were also high. There are also many exceptions to this rule at a taxa and species level. Overall, this experiment initiated a comparison of the nutrient concentrations and algal communities of both study sites. The dominant phytoplankton taxa were found to be similar between sites and respond similarly to nutrient addition. Throughout the remainder of this discussion chapter, a more in depth comparison will be made between the two study sites, which will also focus on important factors that could not be compared with the use of bioassays experiments such as hydrodynamics and food web interactions.

5.2 A direct comparison of estuarine biomass

A comparison of estuarine productivity in the Werribee and Mitchell River estuaries is most easily achieved by comparing the phytoplankton and zooplankton biomass in both systems. Figure 5.3 depicts how phytoplankton and zooplankton biomasses changed throughout spring and early summer, as well as the growth achieved in the bioassay control treatments of both estuaries. Phytoplankton biomass from the halocline has been presented, or in the absence of stratification, biomass at ~1 m of depth was used. Both S5 and S6 are presented from the Werribee estuary because of the large difference in phytoplankton biomass between the two sites. S5 is a good representation of phytoplankton biomass in the lower estuarine region, where chlorophyll *a* concentrations were similarly low. In the Mitchell estuary, S2 was presented because biomass differed little between sites.

5.2.1 Estuarine biomass in the Werribee River estuary

Before a comparison between the two sites is undertaken, the difference between biomasses at S5 and S6 in the Werribee estuary will be discussed. Phytoplankton biomass was consistently higher at S6, sometimes hundredfold (Fig. 5.3a). In contrast, zooplankton biomass was often higher at S5 (Fig. 5.3b), and therefore increased grazing pressure may account for some difference in phytoplankton biomass. However, on some dates, zooplankton biomass was similar or higher at S6 (early September; Fig. 5.3) and therefore top-down controls were likely not the main factor driving phytoplankton biomass at S6. Nutrient availability exerts a strong bottom-up control on phytoplankton, however nutrient concentrations, particularly NO_3^-, were high at both S5 and S6 due to

riverine and groundwater inputs in close proximity. Therefore, physical forces were the most likely cause of contrasting biomass. Between S5 and S6 is a very shallow region of estuary, approximately 50 m in length, impassable by boat in during low tide. Combined with highly regulated flows upstream, this results in high residence times at S6, where the estuary deepens again. Therefore, phytoplankton accumulate at S6, until high flows occur and their abundances decrease (Fig. 5.3a; ML-Nov and E-Dec).

The effect of residence time was also observed when sampling took place for the bioassay experiment. *In situ* (initial) phytoplankton biomass was observed to be much lower at S6 during 2016/17 compared to 2014 (Fig. 5.3). This was due to peaks in flow just before sampling (Fig. 6.6), except mid-December when biomass peaked at S6 and also S5 after a longer period of low flow (Fig. 5.3c). Under laboratory conditions, residence times were controlled and therefore any difference in final biomass was due to nutrient concentrations. In the Werribee control bioassays, growth at S5 exceeded growth at S6 in all cases except late November (Fig. 5.3c). This was the only time that NO_3^- concentrations were the same at both S5 and S6 (56 μmol L^{-1}). Therefore, the same phytoplankton biomass was observed after the growth period (Fig. 5.3c). On all other dates, NO_3^- concentrations were greater at S5 and therefore biomass increased most in those bioassay treatments.

5.2.2 Estuarine biomass: a comparison between estuaries

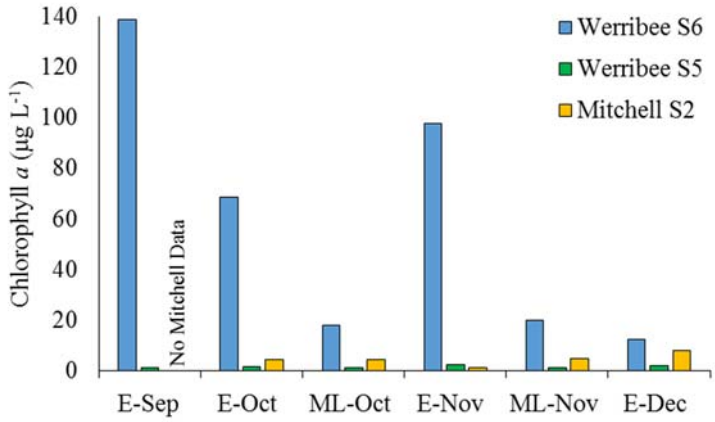
The highest phytoplankton biomass was observed at S6 in the Werribee estuary due to high nutrient concentrations and residence times as discussed previously. High nutrient concentrations were also present at S5, however lower phytoplankton biomasses were

observed in comparison to the nutrient poor Mitchell estuary, at all times except early November, when high flows flushed plankton from the Mitchell estuary (Fig. 5.3a). Zooplankton biomass was generally higher in the Werribee estuary compared to the Mitchell (Fig. 5.3b), which suggests a strong top-down control on phytoplankton biomass in the Werribee Estuary. Total zooplankton in the Werribee estuary included much higher abundances of mollusc and polychaete larvae due to having a greater marine influence. In contrast, salinities in the Mitchell estuary never reached those of marine systems, and the zooplankton biomass was dominated by copepods. However, the abundance of copepods in the Mitchell estuary was greater than copepod abundances observed in the Werribee estuary.

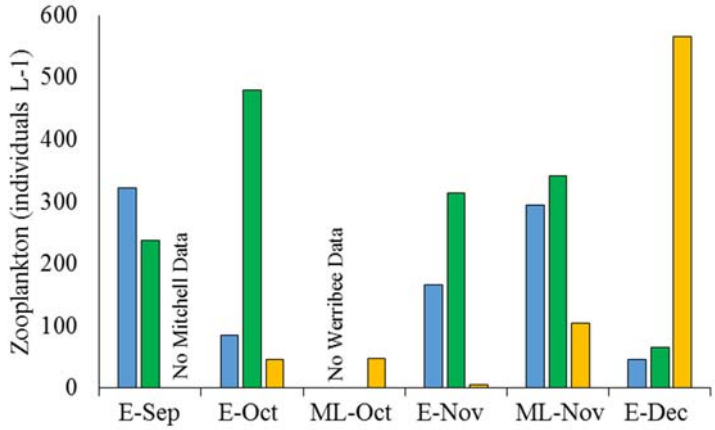
The hypothesis of top-down control was further supported by the bioassays due to the exclusion of zooplankton. An absence of grazing pressure resulted in a 14-fold increase in phytoplankton biomass at S5 in the Werribee control treatment (Fig. 5.3c). In comparison, phytoplankton biomass increased only marginally or not at all in the Mitchell control treatment. This reflects the low nutrient concentrations in the estuary, which were also lower in 2016/17 than 2015. It also suggests that grazing pressure may be minimal in the Mitchell estuary, where cyclopoid copepod abundances increased in early December 2015 coinciding with the highest phytoplankton biomass observed at the halocline (Fig. 5.3a and b). However, this emphasises the importance of phytoplankton and zooplankton identification in these types of studies. In this case, cyclopoid copepods likely predated their preferred prey of flagellate phytoplankton, which were replaced in the phytoplankton assemblage by increased diatom abundances. Therefore, grazing pressure did occur but was not reflected in total phytoplankton biomass.

Stratification strength also plays a role in phytoplankton biomass accumulation and may account for some of the difference observed in the Werribee and Mitchell estuaries. In the Mitchell estuary, phytoplankton biomass above and below the halocline decreased to similar concentrations observed at S5 in the Werribee estuary, which was predominantly partially mixed. This highlights the importance of freshwater flows in generating a stratified water column, where phytoplankton accumulate at the halocline. These processes facilitated phytoplankton productivity within the Mitchell estuary by increasing phytoplankton access to nutrients above and below the halocline. A further discussion of how these, and other hydrodynamic and biogeochemical processes drove estuarine productivity in both systems follows below.

a) Phytoplankton biomass



b) Zooplankton biomass



c) Bioassay phytoplankton biomass

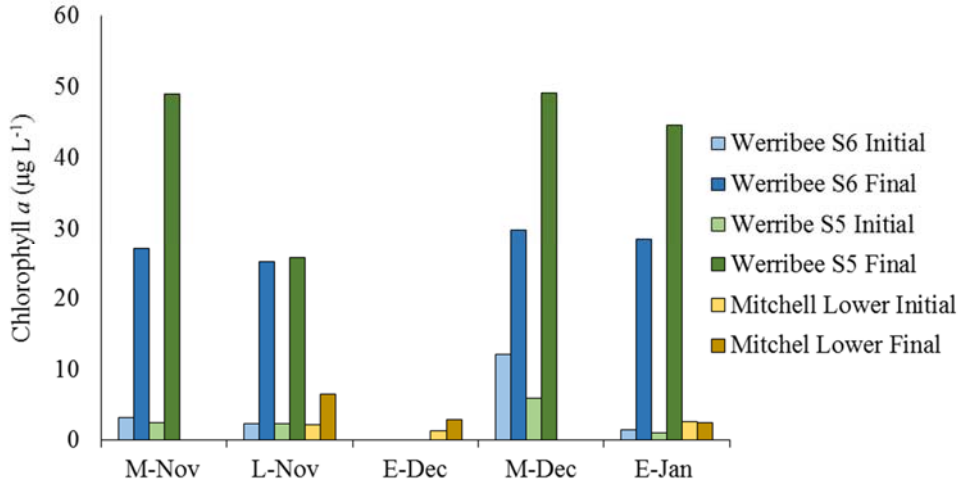


Figure 5.3: a) Bar plot of phytoplankton biomass (chlorophyll *a* µg L^{-1}) at the halocline or ~1 m depth, b) Bar plot of zooplankton biomass (individuals L^{-1}), c) Bar plot of initial and final phytoplankton biomass (chlorophyll *a* µg L^{-1}) in the control bioassay treatments. All At S5 and S6 in the Werribee estuary and S2 or the lower region of the Mitchell estuary.

E = early, M = mid, ML = mid to late, L = late

5.3 The role of hydrodynamics and biogeochemical processes on estuarine productivity: a comparison of two contrasting estuarine systems

Estuarine productivity is controlled by many interlinking hydrodynamic and biogeochemical processes that vary over spatial and temporal scales and are strongly impacted by human activities. The results obtained throughout this thesis provide a snap shot of estuarine productivity within the Werribee and Mitchell River estuaries during the spring in which they were studied. Although the exact sequence of events are unlikely to occur in the same way again, the processes controlling productivity within both estuaries are common to many estuarine systems and many of the findings can help inform future policy makers. Additionally, by studying two different estuarine systems, where the same endemic fish species is known to spawn, we can explore the relative importance of internal and external processes on estuarine productivity. But first, it must be noted that although sampling took place in different years, the location, geography and climate of both estuaries is sufficiently different that the timeframe of each study does not negate a comparison.

5.3.1 The role of hydrodynamics

There were two major differences between the hydrodynamics of the Mitchell and Werribee River estuaries; regulation of riverine flow and tidal forces. The Werribee estuary is much shorter than the Mitchell estuary and opens directly to Port Philip Bay and therefore tidal exchange results in high salinities, even in the upper estuary. In

contrast, the Mitchell River estuary is much longer and opens to a series of interconnected estuarine lakes, connected to Bass Straight via a permanently opened sea entrance. Therefore, tidal forces are weak and salinities never reach that of seawater in the Mitchell estuary. However, the strongest control on salinity structure in estuarine environments is the degree of freshwater flow (Kurup et al. 1998, Kimmerer 2002). As a regulated system, the Werribee estuary receives much lower freshwater flows than the unregulated Mitchell estuary. As a result the Werribee estuary was characterised as a partially mixed estuary in average flow conditions (~4 ML day^{-1}), but became more stratified in the upper estuary after an EFR from the Melton dam (200 ML day^{-1}). In contrast, the Mitchell estuary is a salt wedge estuary under medium and low flow conditions (≤950 ML day^{-1}), but high freshwater flows flushed the salt wedge from the estuary resulting in an entirely fresh water column in parts of the upper estuary (>1250 ML day^{-1}). These changes to the hydrodynamics of each system affect residence times, salinity regimes and stratification strength, which in turn affect phytoplankton biomass and productivity and higher trophic levels.

5.3.2 The effects of hydrodynamics on phytoplankton, zooplankton and fish larvae

The degree of freshwater flows strongly controls residence times within the water column, as previously discussed. In the Werribee estuary, low flows caused high residence times, particularly in the upper estuary where the abundance of *Euglena* spp. increased at the start of the sampling period. Residence times were still high when *Cyclotella* sp. and the other blooming taxa dominated the algal assemblage. However, as a result of the EFR and coupled rainfall event, residence times decreased and *Cyclotella* sp. was likely flushed from the estuary. This flow event was not strong enough to flush

motile organisms, such as copepods from the estuary, but high flows in the Mitchell estuary were. The high flows that removed the salt wedge from the Mitchell estuary decreased residence times to such an extent that phytoplankton and zooplankton biomass were also removed from the upper estuary. In times of lower flow, residence times remained high in the surface waters, but strong salinity stratification and decreased mixing between layers caused increased residence times in the salt wedge. This resulted in DO depletion in the salt wedge, which does not affect the algal community but can affect higher trophic levels that require oxygen. Low DO favoured cyclopoid copepods and was therefore one of the factors controlling their dominance over calanoid copepods in the Mitchell estuary. It also delayed spawning of small estuarine fish.

The volume of freshwater flows to estuarine environments also dictates salinity and therefore the extent of suitable habitat for salinity sensitive organisms. This was a more important factor in the Mitchell estuary, where stratification created two distinct salinity zones in the water column. As the difference between the surface water and bottom water salinities increased after salt wedge intrusion, two distinct phytoplankton assemblages were formed. These were dominated by chlorophytes in the fresh surface water and the diatom *Skeletonema costatum* in the saline bottom water. Salinity effects were also observed in the higher trophic levels. Cyclopoid copepods were first observed when bottom water salinities increased and the saline layer deepened. In contrast, these changes coincided with the disappearance of *Retropinna semoni* fish larvae, a freshwater fish likely flushed into the upper estuary in high flows but unable to adapt to increased salinity.

Stratification strength is arguably the most important factors controlled by flow in estuaries. This is because the presence of the halocline in strongly stratified estuaries is strongly linked to the accumulation of nutrients, phytoplankton, zooplankton and fish larvae (Harder 1968, Viličić et al. 1989, Cauwet 1991, Newton 1996, Jenkins et al. 2010). The importance of the halocline can be most easily observed by comparing phytoplankton biomass in both estuaries (section 5.3.2). Higher phytoplankton biomass (as chlorophyll *a*) was observed at the halocline in the Mitchell River, compared to any depth in the Werribee estuary (excluding algal blooms and S6). This highlights the fact that the halocline is an important zone of accumulation in a nutrient-poor estuary. However, as described in section 5.3.2, grazing pressure played an important role in the different phytoplankton biomasses observed.

Stratification strength also played an important role within the Werribee estuary after the EFR. The increased the strength of the halocline provided a zone of accumulation where predator/prey interactions increased. As a result, not only did the increased stratification allow *A. butcheri* eggs to float at the halocline, but there was a greater abundance of suitable prey available once the larvae hatched. These results highlight the importance of the physical forces and mixing dynamics created by freshwater flows and stratification strength, which also strongly control nutrient dynamics.

5.3.3 How hydrodynamics control nutrient dynamics

Many of the processes discussed above also affected the nutrient dynamics in both estuarine systems. Increased residence times resulted in decreased nutrient concentrations when high algal growth was stimulated. Most notably, NH_4^+ concentrations were

decreased due to assimilation by *Euglena* spp. and *S. costatum* in the Werribee and Mitchell estuaries respectively. However, increased residence times also resulted in the accumulation of NH_4^+, particularly in the Mitchell estuary where decreased DO and increased salinity in the salt wedge facilitated benthic nutrient recycling and then NH_4^+ accumulation. In the Werribee estuary, accumulation of NH_4^+ occurred under similar circumstances; decreased bottom water DO in stratified conditions. Inputs of NH_4^+ were also a result of riverine and tidal transport from the Werribee catchment and Port Philip Bay respectively. More concentrated in the riverine flows however, was NO_3^-, which was also delivered to the Werribee estuary via groundwater. As a result, NO_3^- concentrations were high throughout the estuary and the water column. In contrast, much lower NO_3^- concentrations were observed in the Mitchell estuary and were generally confined to the surface water due to stratification. However, in high flow conditions (>950 ML day^{-1}), NO_3^- concentrations in the surface water decreased, likely due to dilution by low NO_3^- rainwater. At times NO_3^- concentrations increased in the bottom water, likely due to nitrification when DO was sufficient.

5.3.4 The effects of nutrients dynamics on phytoplankton, and food web interactions

Increased nutrient loads and altered nutrient ratios can lead to eutrophication and harmful algal blooms (HABs), which are predominantly driven by increased NO_3^- inputs (Cloern 2001, Glibert et al. 2016). In the Werribee estuary, where NO_3^- inputs were high, chlorophyll *a* concentrations were indicative of eutrophication on some dates (>20 µg/L) (Håkanson et al. 2007), but no HABs were observed. Instead, a non-harmful algal bloom occurred, dominated by *Cyclotella* sp., which is known to assimilate NO_3^- (Vanni 1987, Muylaert & Sabbe 1996, Amano et al. 2012, El-Kassas & Gharib 2016). In the presence

of this bloom, calanoid copepod abundances decreased (Fig. 6.2a; $p = 0.06$) and *A. butcheri* larvae were absent. However, this bloom did not persist throughout the sampling period, even though NO_3^- concentrations remained high. This was hypothesised to be due to the presence of NH_4^+, which is known to inhibit NO_3^- uptake in phytoplankton (Dortch 1990, Domingues et al. 2011, Glibert et al. 2016). The results of each bioassay treatment supported this hypothesis, and showed preferential uptake of NH_4^+ until a concentration of ~1 μmol L^{-1}, when NO_3^- uptake increased rapidly. In the Werribee estuary during 2014 the same threshold was observed. *Cyclotella* sp. reached blooming abundances only when NH_4^+ concentrations were <1 μmol L^{-1}, however below this threshold *Cyclotella* sp. remained a dominant taxa within the algal assemblage. This reflects the suggestion by Glibert et al. (2016) and references therein, that the extent of NH_4^+ inhibition on NO_3^- uptake depends on the algal species present, the environmental conditions they have been exposed to and their physiological status.

Concentrations of NH_4^+ >1 μmol L^{-1}, were present at the start of the sampling period until depletion occurred due to *Euglena* spp. assimilation. *Cyclotella* sp. then bloomed until the EFR, which increased stratification strength and facilitated NH_4^+ accumulation. As a result a more mixed phytoplankton assemblage of flagellates, diatoms and chlorophytes emerged, significantly correlated with increased NH_4^+ concentrations (Fig. 6.1a; $p < 0.05$). This assemblage significantly correlated within increased calanoid copepod abundances (Fig. 6.2c; $p < 0.05$), and coincided with the appearance of *A. butcheri* larvae for the first time in the sampling season. The results of the bioassay experiment showed a similar response to NH_4^+ addition. The Werribee NH_4^+ treatment was the only treatment to retain a mixed phytoplankton assemblage of similarly abundant flagellates, diatoms and chlorophytes after the growth period. It was also the only treatment in which

flagellate abundances significantly increased ($p < 0.05$). This same result was not observed in the Mitchell bioassay treatments, where the main difference was the initial concentration of NO_3^-. Therefore both NH_4^+ and NO_3^- were key forms of N controlling phytoplankton productivity that formed the basis of the estuarine food web supporting *A. butcheri* larvae in the Werribee estuary.

In the Mitchell estuary chlorophyte abundances were significantly correlated to increased NO_3^- concentrations in the Mitchell estuary ($p < 0.05$), and dominated the surface water assemblage in low flow conditions. After NO_3^- addition in the bioassay experiment, chlorophyte abundances increased further, indicating the potential for blooms if NO_3^- loads to the Mitchell estuary increased. In addition, if freshwater flows to the Mitchell estuary were regulated in the future, low flow conditions would be more prevalent, and possibly promote increased chlorophyte growth and blooming conditions. Chlorophytes blooms may not be toxic but like all blooms cause low DO conditions when they die and rapidly decompose (Paerl et al. 2001). This may exacerbate and extend periods of low DO below the halocline, currently a result of stratification and salt wedge intrusion. These conditions may further inhibit fish from spawning, hatching and surviving in the Mitchell estuary as was hypothesised in Chapter 3.

5.3.5 Acanthopagrus butcheri

The Werribee and Mitchell River estuaries are both known spawning grounds for the endemic black bream; *Acanthopagrus butcheri*. Spawning and larval survival in the Werribee estuary during 2014 has been discussed thoroughly throughout this Chapter and Chapter 2. The timing of its appearance in the larval life stage strongly conforms with

previous studies of optimal water quality parameters, available prey and freshwater flow/stratification conditions (Jenkins et al. 2010, Williams et al. 2012, Williams et al. 2013). However, in terms of the Mitchell estuary study, *A. butcheri* larvae have only briefly been discussed in Chapter 3, due to their absence from the larval population during the 2015 sampling season. Three possible explanations for their absence are presented here. First, very low DO levels were observed below the halocline on most sampling dates. According to studies by (Hassell et al. 2008a, 2008b), hypoxia reduces embryo survival and hatching rates to zero at 30% saturation, while moderately hypoxic conditions (45-55% saturation) delay hatching, reduce hatching rates and increase the likelihood of larval deformities. At the upper sampling sites, DO saturation was commonly below these thresholds in the salt wedge, while increased DO levels occurred downstream, but *A. butcheri* larvae remained absent. Second, cyclopoid copepods dominated the zooplankton assemblage in very high abundances in late spring. These copepods and their nauplii are not the preferred diet of *A. butcheri* larvae, however, calanoid copepods were present and therefore this explanation is less likely. Third, spawning may not have occurred during the sampling season and instead occurred later in summer. It could not be confirmed whether *A. butcheri* eggs were present during the sampling period or not as genetic testing was not undertaken. Therefore it is unknown whether larvae were simply not surviving or whether their eggs were absent. However, new evidence from Jenkins et al. (in press) has found that successful *A. butcheri* spawning in the Gippsland Lakes estuarine tributaries occurred in January 2016 and again in late February-early March 2016. This was determined by back-calculated spawning dates of juveniles sampled in the Gippsland Lakes using daily increments in otoliths (Jenkins et al. in press). It cannot be definitively stated that the Mitchell estuary was one of the tributaries in which successful *A. butcheri* spawning occurred, however the

spawning of benthic gobies in early December 2015 indicates that DO was approaching optimal levels. Therefore, it is likely that environmental conditions within the Mitchell estuary were sufficient to trigger *A. butcheri* spawning and larval populations later in summer.

5.3.6 Human impacts and remediation

As human populations continue to increase in coastal regions, so do the human impacts on estuarine habitats. The negative effects of human impacts can already be observed in the Werribee estuary. During 2014, decreased freshwater flows and increased nutrient inputs may have resulted in another absent year class of *A. butcheri* had the environmental flow not been released to remediate the estuary. However, this release took place just before a naturally high flow event and therefore it is unknown how the system would have responded in the absence of the EFR. Regardless, this remediation practice provided a rare example of an environmental flow maintaining a target species population by creating a trophic level response to altered nutrient and food web dynamics (Rolls et al. 2012, Rolls et al. 2017). This study has provided some important preliminary information as to the timing and magnitude of environmental flows needed in the Werribee estuary. It has been found that NH_4^+ availability is important in the presence of high NO_3^- inputs to stimulate the estuarine food web. Therefore NH_4^+ depletion may be an indicator of EFR timing. Increased chlorophyll *a* concentrations could also be used as an indication of EFR timing as they show the presence of algal blooms. However, high algal biomass (as chlorophyll *a*) was not only observed during the *Cyclotella* sp. bloom, but also the *Euglena* spp. bloom, the latter of which was not negatively associated with decreased copepod abundances. Therefore phytoplankton identification and counts during

chlorophyll *a* peaks would be a better indication of the potential for the bloom to negatively affect higher trophic levels, although expensive and time consuming. Finally, the salinity and flow rate are important indictors of stratification strength and residence times, and are quickly and easily measurable. Extended periods of low flow and a partially mixed upper estuary are undesirable during spring when *A. butcheri* begin spawning. These measures could be particularly useful after a high rainfall event to determine whether increased flows were sufficient to produce stratification or if an EFR is needed to sustain periods of stratification during spring.

In the Mitchell estuary, natural flows, low nutrient inputs, an absence of algal blooms and high phytoplankton and zooplankton productivity reflect the low human impacts in the catchment compared to the Werribee estuary. However, fish larvae populations were generally absent from the estuary except when flushed in from upstream under high flow conditions. Therefore, the least impacted system saw the greatest negative effect of flow on *A. butcheri*. Long periods of stratification under low flow conditions cause depleted DO levels in the bottom water. Flushing events after high flow are important to remove low DO from the system, however salt wedge intrusion reintroduces low DO water in the tip of the salt wedge. Therefore there appears to be a lag between flushing events, salt wedge intrusion and optimal *A. butcheri* DO conditions. Further studies into the timing of these event may inform the timing of environmental flows to regulated catchments that sustain stratified estuaries.

5.4 Recommendations for future work

The potential avenues for future work mainly follow on from important findings in this thesis that could not be further investigated in the scope of this study. A key question raised in this thesis was how the form of nitrogen controls algal species, the effect on secondary production and how these processes interact with stratification. This question has not been fully resolved and therefore a mesocosm experiment could be undertaken to further explore these interactions. Such an experiment would form a link between what was observed in the field studies and the highly controlled bioassay experiments, and also incorporate higher trophic levels and stratification in a controlled way. In such experiments, N concentrations and forms, grazer abundances and stratification strength could be manipulated, either as different treatments or throughout the time span of an experiment. The latter method would eliminate the need to colonise multiple treatments with the same phytoplankton population. As a result, the way in which algal species compete for nitrogen under grazing pressure in various stratification strengths could be evaluated. Additionally and importantly, bottom up effects on copepod and larval fish communities could be further explored using this type of experiment, a limitation of the bioassays undertaken within this study.

If *Cyclotella* sp. continues to form algal blooms in the Werribee estuary, a more targeted approach may be necessary to determine the role of nitrogen forms driving *Cyclotella* sp. productivity and bottom-up effects on calanoid copepods. If ingestion of *Cyclotella* sp. in high volumes does not have a negative impact on calanoid copepod abundance or their reproduction, then an unknown factor caused them to decline in 2014, which may be important for future *A. butcheri* recruitment success. In contrast, if *Cyclotella* sp. is found

to be the probable cause, future monitoring of its abundances could be used to help inform remediation actions and the timing of EFRs. Groundwater inputs measured as ^{222}Rn were found to significantly correlate with *Cyclotella* sp., and therefore ^{222}Rn activity could be used as a proxy measurement of *Cylotella* sp. abundances. This would be a more cost and time effective method than *Cyclotella* sp. identification but further investigation into this link would be required.

The success of the EFR to the Werribee estuary in 2014 is only one example of a successful remediation practice. Further investigation is needed to inform the timing and magnitude of flow releases to the estuary and ensure their continuing success. The monitoring of flow rates, salinity stratification, chlorophyll *a* and NH_4^+ concentrations may be a good indication of the timing of an EFR in the Werribee estuary as mentioned previously. To investigate the magnitude, high rainfall events could be monitored to determine the flow rate required to induce stratification in the upper estuary and the how long stratification is sustained. Harder, but also important would be the monitoring of the larval community, particularly the presence of *A. butcheri* eggs. In the absence of spawning *A. butcheri*, an EFR may be less beneficial, unless its intention is to decrease residence times and remove algal blooms.

Within the Mitchell River estuary low DO below the halocline was hypothesised to have caused the absence of *A. butcheri* larvae during the 2015 sampling season. To test this hypothesis, further studies over a longer time scale (spring and summer) are needed to observe a successful *A. butcheri* spawning season. This will help determine which factors promote spawning and larval survival in the Mitchell estuary, and provide information as to the lag time between salt wedge intrusion and *A. butcheri* spawning. Nutrient

monitoring would also be an important tool when managing the health of the Mitchell estuary due to the apparent sensitivity to increased NO_3^- concentrations.

5.5 Conclusion

This thesis illustrates the complex ways in which hydrodynamics and biogeochemical processes interlink and drive estuarine productivity in two south-east Australian estuaries. By comparing two contrasting systems this project has provided new knowledge on the role of freshwater flows, the structure and function of the halocline, nutrient inputs and cycling, and how these processes impact upon phytoplankton, zooplankton and larval fish dynamics.

This study showed three major findings:

1. An EFR can successfully alter nutrient and food web dynamics and create a trophic level response for the target species *Acanthopagrus butcheri*.

2. Salt wedge intrusion stimulates phytoplankton and zooplankton productivity in a nutrient-poor estuary, but delays spawning in estuarine fish

3. Benthic nutrient recycling is an important source of NH_4^+ in stratified estuarine environments, which supports phytoplankton biomass. However the greatest increase in phytoplankton productivity occurs in the presence of NO_3^-, even in low concentrations.

5.6 References

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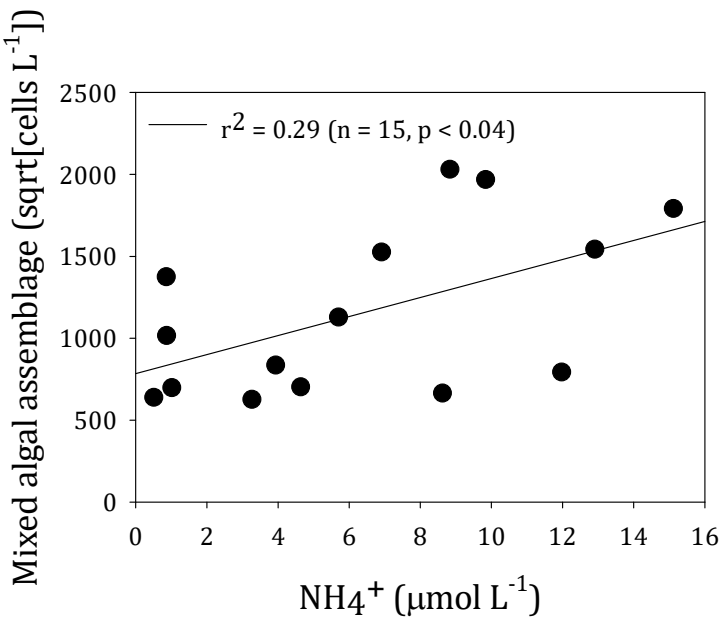
Chapter 6: Appendices

6.1 Supporting information for Chapter 2

a)



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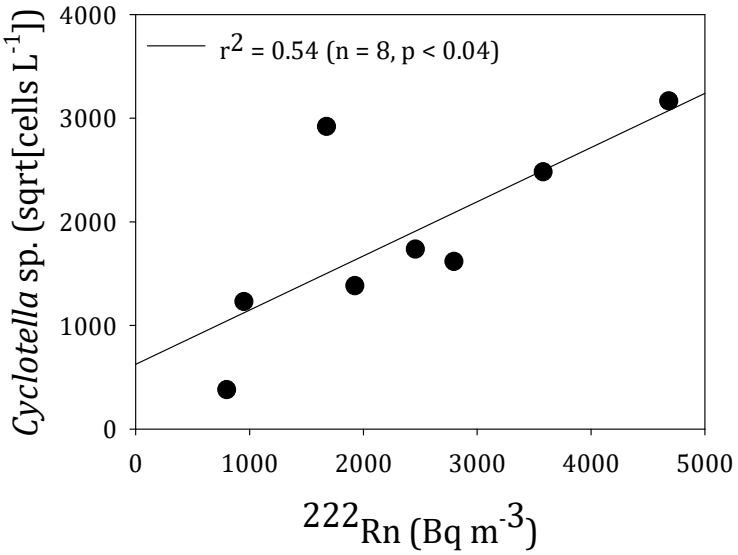

Figure 6.1 Plots of a) the mixed algal assemblage (sqrt[cells L^{-1}]) vs NH_4^+ (µmol L^{-1}), p < 0.04, b) *Cyclotella* sp. (sqrt[cells L^{-1}]) vs bottom water ^{222}Rn (Bq m^{-3}), p < 0.04**.**

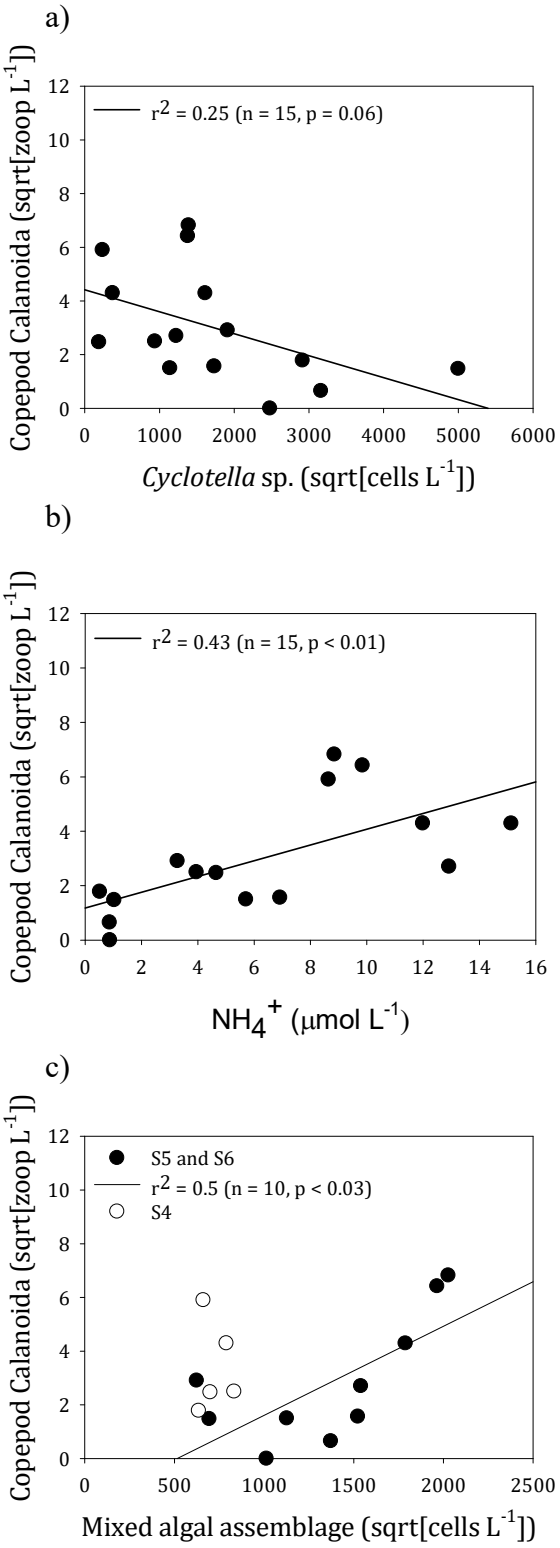

Figure 6.2: Plots of a) Copepod calanoida (sqrt[cells L^{-1}]) vs Cyclotella sp. (sqrt[cells L^{-1}]), p = 0.06, b) Copepod calanoida (sqrt[zoop L^{-1}]) vs NH_4^+ (µmol L^{-1}), p < 0.01, c) Copepod calanoida (sqrt[zoop L^{-1}]) vs the mixed algal assemblage (sqrt[cells L^{-1}]) at S5 and S6, p < 0.001.

Table 6.1: Phytoplankton NMDS codes

	NMDS code
Diatoms	
Achnanthes sp.	dia.ach
Amphora sp.	dia.amp
Bacillaria paxillifera	dia.bac
Ceratoneis closterium	dia.cer
Cocconeis spp.	dia.coc
Cyclotella sp.	dia.cyc
Diploneis sp.	dia.dip
Entomoneis sp.	dia.ent
Fragilaria sp.	dia.fra
Melosira varians	dia.mel
Naviculoid spp.	dia.nav
Nitzschia spp.	dia.nit
Pleurosigma sp.	dia.ple
Synedra sp.	dia.syn
Dinoflagellates	
Gymnodinioid spp.	din.gym
Gyrodinium spp.	din.gyr
Heterocapsa rotundata	din.het
Peridinium sp.	din.per
Chrysophytes	
Calycomonas sp.	chr.cal
Ochromonas spp.	chr.och
Prymnesiophytes	
Chrysochromulina spp.	pry.chr
Cryptophytes	
Campylomonas reflexa	cry.cam
Hemiselmis sp.	cry.hem
Plagioselmis prolonga	cry.pla
Teleaulax acuta	cry.tel
Chlorophytes	
Chlamydomonas/Dunaliella spp.	chl.chl
Kirchneriella sp.	chl.kir
Monoraphidium sp.	chl.mon
Scenedesmus sp.	chl.sce
Stichococcus sp.	chl.sti
Prasinophytes	
Pyramimonas spp.	pra.pyr
Tetraselmis spp.	pra.tet
Euglenophyta	
Euglena spp.	eug.eug
Eutreptiella spp.	eug.eut
Cyanoprokaryota	
Anabaena sp.	cya.ana
Spirulina sp.	cya.spi
Other	
Apedinella spinifera	oth.ape
Mesodinium rubrum	oth.mes

Table 6.2: Phytoplankton water quality NMDS codes and data

Vector	Code	NMDS1	NMDS2	r2	Pr(>r)
Temperature (°C)	Temp.C	-0.2172	0.97613	0.1983	0.569
Salinity (PSU)	sal.psu	-0.8376	-0.5463	0.7472	0.025
DO (%)	DO.%	0.31181	-0.9502	0.6962	0.047
Turbidity (NTU)	Turb.NTU	0.41536	0.90966	0.3378	0.319
NH_4^+ (µmol L^{-1})	NH4.uM	-0.4121	0.91114	0.8265	0.014
FRP (µmol L^{-1})	FRP.uM	-0.9996	0.02765	0.9511	0.001
NO_3^- (µmol L^{-1})	NO3.uM	0.9978	-0.0663	0.3035	0.403
^{222}Rn (Bq m^{-3})	222Rn	0.85775	-0.5141	0.4416	0.215

Table 6.3: Zooplankton NMDS codes

	NMDS code
Calanoid Copepoda	
Paracalanus indicus	cal.para
Gladioferens pectinatus	cal.glad
Acartia tranteri	cal.acar
Cyclopoid Copepoda	
Dioithona rigida	cyc.dio
Oithona nana	cyc.oith
Harpacticoid Copepoda	
Microsetella norvegica	harp.mic
Euterpina acutifrons	harp.eut
Crustacean Larvae	
Copepod Nauplii	cop.naup
Mollusc Larvae	moll.larv
Barnacle Larvae	barn.naup
Crab Zoea	crab.zoea
Annelida	
Polycheate larvae	poly.larv
Rotifera	
Keratella sp.	rot.kera
Brachionus kostei	rot.brac

Table 6.4: Zooplankton water quality NMDS codes and data

Vector	Code	NMDS1	NMDS2	r2	Pr(>r)
Temperature (°C)	Temp.C	0.93253	-0.3611	0.2621	0.441
Salinity (PSU)	sal.psu	-0.6475	-0.7621	0.2856	0.442
DO (%)	DO.%	-0.4632	0.88624	0.5909	0.116
Turbidity (NTU)	Turb.NTU	0.99549	-0.0949	0.0826	0.79
NH_4^+ (µmol L^{-1})	NH4.uM	0.36448	-0.9312	0.7056	0.039
FRP (µmol L^{-1})	FRP.uM	-0.1036	-0.9946	0.353	0.327
NO_3^- (µmol L^{-1})	NO3.uM	0.71833	0.6957	0.4274	0.259
^{222}Rn (Bq m^{-3})	222Rn	-0.1433	0.98967	0.6432	0.082

6.2 Supporting information for Chapter 3

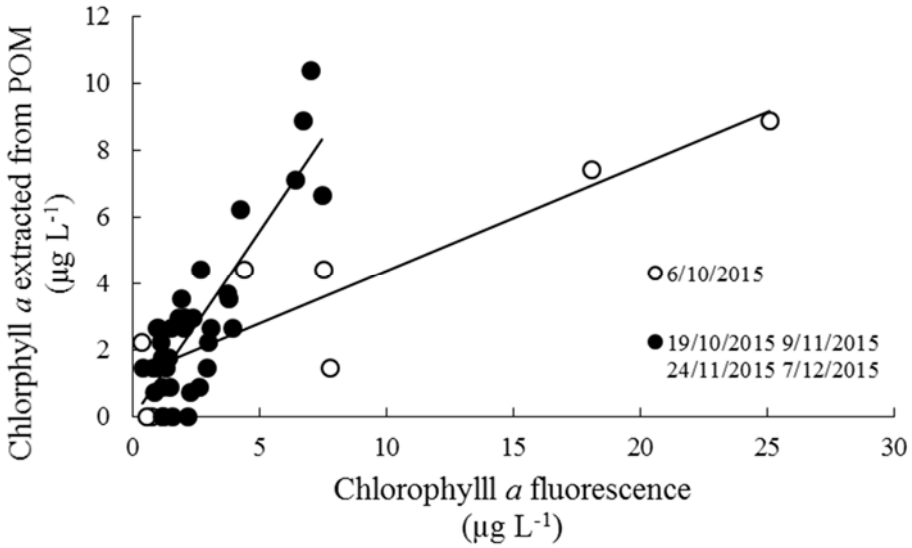

Figure 6.3: Plot of chlorophyll *a* extracted from POM (µg L^{-1}) vs chlorophyll *a* fluorescence (µg L^{-1}) on 6/10/2015 (n = 9, r^2 = 0.80, slope = 0.32, $p < 0.05$) and the remaining dates (n = 35, r^2 = 0.73, slope = 1.12, $p < 0.05$).

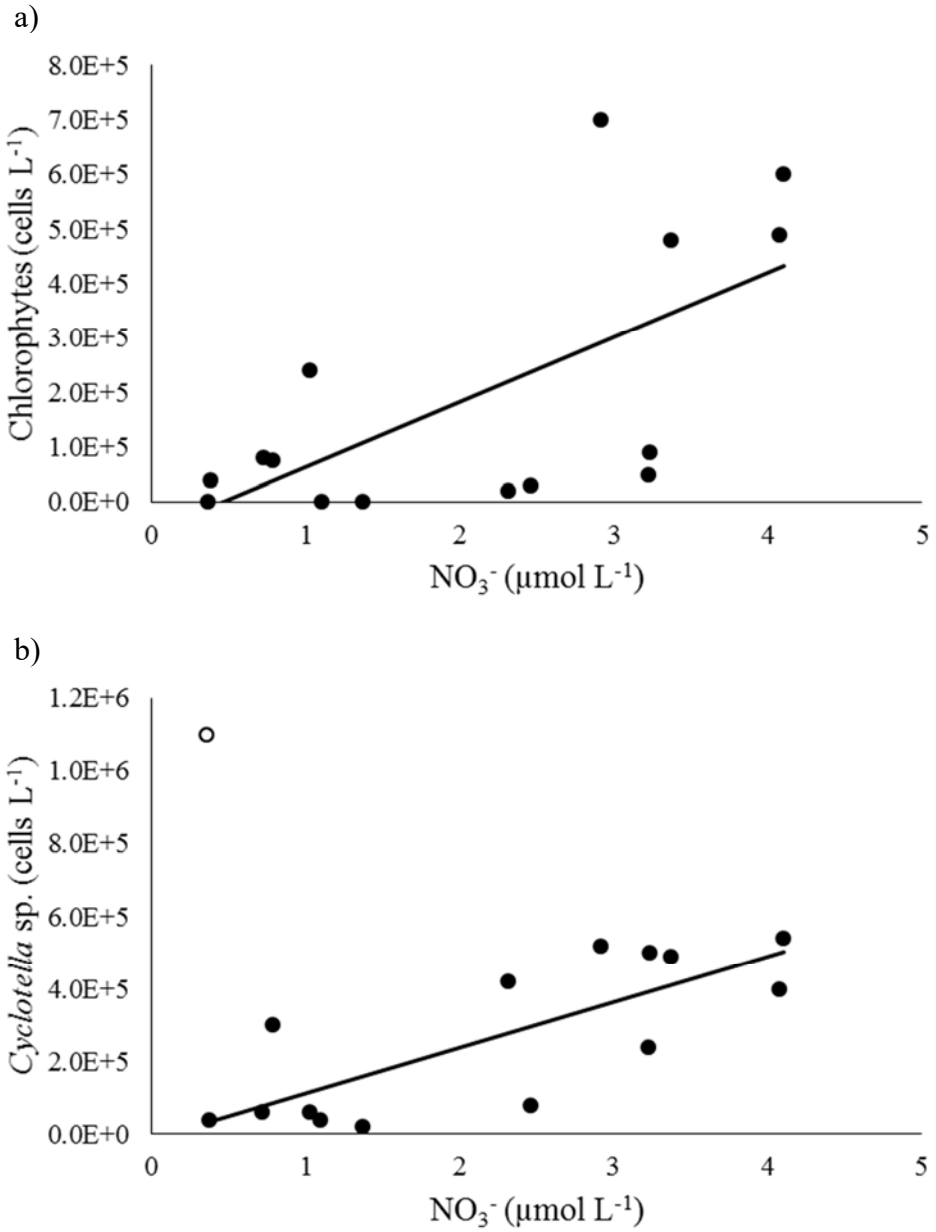

Figure 6.4: a) Plot of total chlorophyte abundance (cells L^{-1}) vs NO_3^- (µmol L^{-1}) ($r^2 = 0.4$, $n = 15$, $p < 0.05$), b) *Cyclotella* sp. abundance (cells L^{-1}) vs NO_3^- (µmol L^{-1}) ($r^2 = 0.6$, $n = 14$, $p < 0.05$, black circles) excluding an outlier from the bottom water on 7/12/2015 (white circle). All data was obtained from S2.

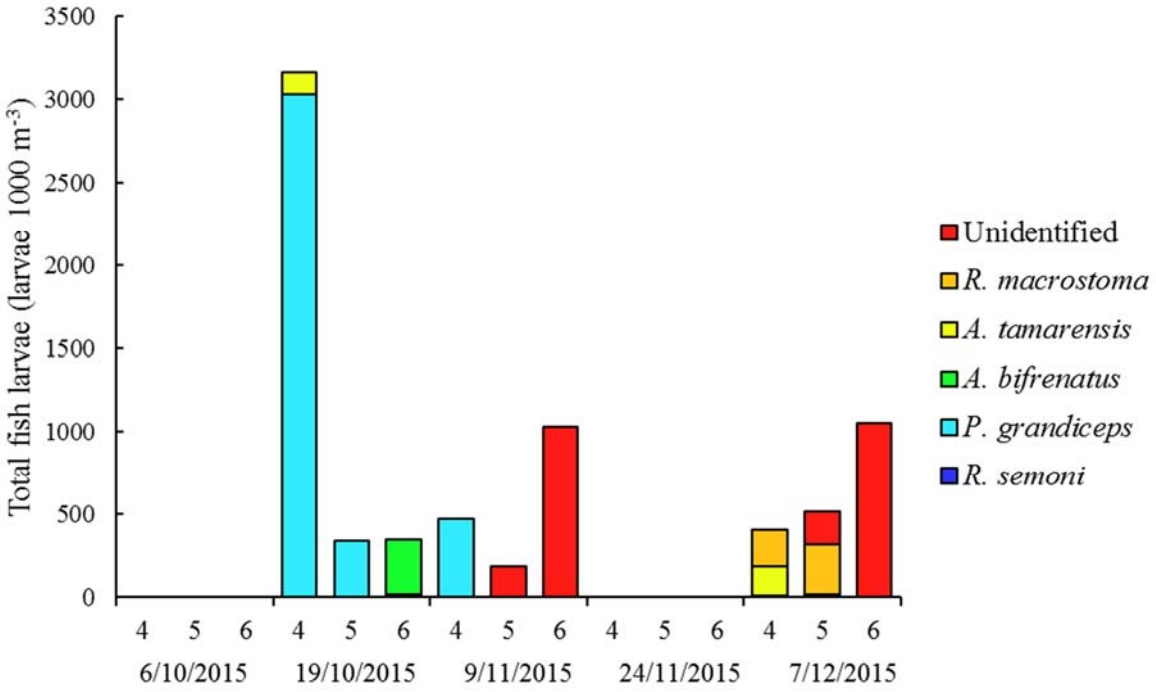

Figure 6.5: Bar plot of total fish larvae abundances (larvae 1000 m^{-3}) at S4, S5 and S6 on each sampling date.

Table 6.5: List of phytoplankton identified and their phytoplankton group.

Diatoms	**Flagellates**
Amphora sp.	**Dinoflagellates**
Aulacoseira granulata	Dinophysis fortii
Bacillaria paxillifera	*Gonyaulax spp.*
Bleakeleya notata	Gymnodinioid spp.
Cerataulina pelagica	*Gyrodinium spp.*
Chaetoceros spp.	*Heterocapsa rotundata*
Cocconeis spp.	*Katodinium glaucum*
Cyclotella sp.	*Oxyrrhis marina*
Cylindrotheca closterium	*Peridinium sp.*
Ditylum brightwellii	**Cryptophytes**
Encyonema sp.	*Campylomonas reflexa*
Entomoneis sp.	*Cryptomonas sp.*
Fragilaria sp.	*Hemiselmis sp.*
Fragilariopsis sp.	*Komma sp.*
Licmophora sp.	*Leucocryptos spp.*
Melosira varians	*Plagioselmis prolonga*
Naviculoid spp.	*Teleaulax acuta*
Nitzschia spp.	**Chrysophytes**
Pleurosigma sp.	*Dinobryon sp.*
Pseudo-nitzschia delicatissima group	*Mallomonas sp.*
Pseudo-nitzschia pungens/multiseries	*Ochromonas spp.*
Skeletonema costatum complex	**Prasinophytes**
Tabellaria sp.	*Nephroselmis sp.*
Synedra sp.	*Pyramimonas spp.*
Tabellaria sp.	*Tetraselmis spp.*
Thalassiosira sp.	**Prymnesiophytes**
Chlorophytes	*Chrysochromulina spp.*
Chlamydomonas/Dunaliella spp.	*Emiliania huxleyi*
Chlorella sp.	*Prymnesium patellifera*
Crucigenia sp.	**Euglenophyta**
Monoraphidium sp.	*Eutreptiella spp.*
Scenedesmus sp.	
Tetrastrum sp.	
Cyanoprokaryota	
Pseudanabaena limnetica	

Table 6.6: Water quality vector data from phytoplankton NMDS (Fig. 6.5).

Vector	Code	NMDS1	NMDS2	r2	Pr(>r)
NH_4^+ (µmol L^{-1})	NH4.uM	0.14546	-0.9894	0.1337	0.447
FRP (µmol L^{-1})	FRP.uM	-0.3473	-0.9378	0.1111	0.509
NO_3^- (µmol L^{-1})	NO3.uM	0.61933	0.78513	0.7729	0.001
Temperature (°C)	Temp.C	0.19037	0.98171	0.3542	0.063
DO (%)	DO.%	-0.4624	0.8867	0.169	0.324
Salinity (PSU)	sal.psu	0.06775	-0.9977	0.3326	0.083
Flow (ML day^{-1})	Flow.MLDay	-0.7973	-0.6036	0.3042	0.119
Turbidity (NTU)	Turb.NTU	-0.6612	0.75024	0.4591	0.029

Table 6.7: List of zooplankton and fish larvae codes presented in the NMDS (Fig. 6.8).

	NMDS code
Calanoid Copepoda	cop.cal
Cyclopoid Copepoda	cop.cyc
Harpacticoid Copepoda	cop.harp
Crustacean Larvae	
Copepod Nauplii	cop.naup
Mollusc Larvae	moll.larv
Copepod Eggs	cop.egg
Polycheate Larvae	poly.larv
Rotifera	rotifer
***Retropinna semoni* Larvae**	R.semoni
***Philipnodon grandiceps* Larvae**	P.grandiceps
Goby Larvae	Total.goby

Table 6.8: Water quality vector data from zooplankton and fish larvae NMDS (Fig. 6.8).

Vector	Code	NMDS1	NMDS2	r2	Pr(>r)
NH_4^+ (µmol L^{-1})	NH4.uM	-0.9884	0.15222	0.0307	0.806
FRP (µmol L^{-1})	FRP.uM	0.21465	-0.9767	0.2121	0.238
NO_3^- (µmol L^{-1})	NO3.uM	0.21807	0.97593	0.079	0.58
Temperature (°C)	temp.oC	0.23232	-0.9726	0.6842	0.003
Chlorophyll *a* (µg L^{-1})	chla.ugL	0.59045	0.80707	0.1086	0.487
DO (%)	do.%	-0.5308	0.84753	0.3312	0.071
Salinity (PSU)	sal.psu	0.97793	-0.2089	0.6042	0.001
Flow (ML day^{-1})	flow.MLday	-0.995	-0.0995	0.7366	0.001

6.3 Supporting information for Chapter 4

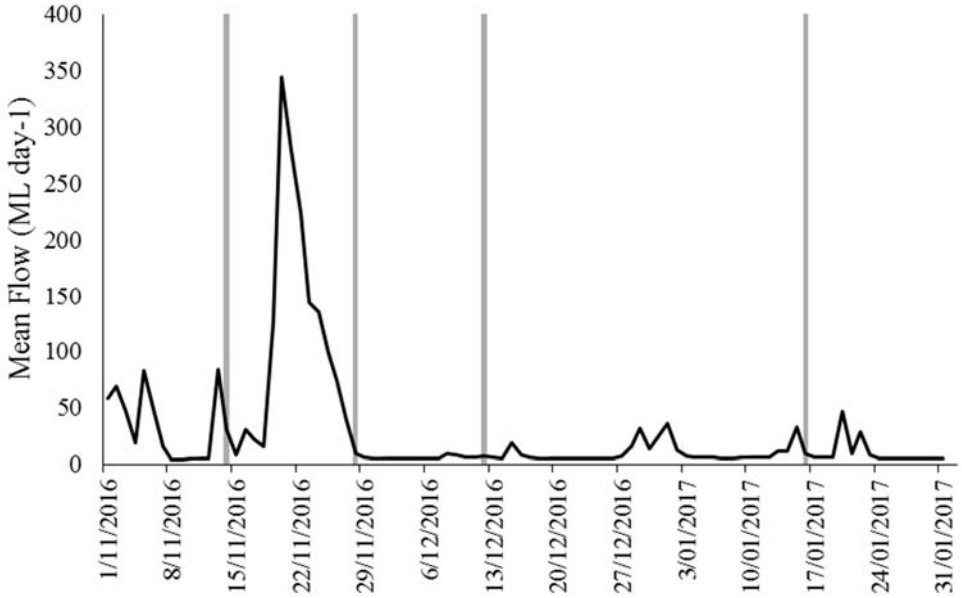

Figure 6.6: River flow (ML day^{-1}) at Cottrell St Ford, Werribee in 2016/17 when sampling for bioassays was undertaken. Grey bars indicate sampling dates.