Seasonal Variation of Zooplankton Nutritional Quality at a Reef Manta Ray (*Mobula alfredi*) Feeding Ground on Ningaloo Reef

Submitted by

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

Alexandra Thornton

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Abstract

Zooplankton provide a fundamental connection between primary producers and higher trophic level consumers, supporting some of the largest marine animals as well as microbial organisms. Therefore, the nutritional resource of zooplankton must be sufficient to support a wide array of marine species. The nutritional quality (lipids, protein and carbohydrates) varies seasonally with changes in species composition, as different organisms store varying amounts of biochemical components. These seasonal variations have direct effects on the marine food web, and those organisms that rely upon the nutritional resource. Zooplankton has a spatiotemporally patchy distribution, with variations in species composition. This is reflected by the variability of each biochemical component when comparing regions around the world.

This study aimed to quantify the seasonal proportions of zooplankton nutritional components at Bateman Bay, Ningaloo Reef in Western Australia, in order to determine what planktivorous fish such as reef manta rays (*Mobula alfredi*) are receiving year round. Feeding manta rays were used to locate patches of plankton, and samples were collected via towing a 300 μm mesh plankton net from the back of a boat. Samples were collected from four sites in autumn and three sites in winter. All samples were analysed for lipids, protein, carbohydrates, total organic carbon and nitrogen (C:N ratio) and biomass.

All nutritional components varied significantly between seasons, and each site showed compositional variability, especially protein. Protein and lipids were significantly higher during autumn than winter, while carbohydrates were higher during winter. The C:N ratio was significantly higher during winter, when phytoplankton abundance was higher. Biomass was larger in winter when there was a greater abundance of portunid crab larvae, eggs and phytoplankton, however was not statistically significant. Environmental variables; temperature, turbidity and tide had no correlation to zooplankton biomass, nutritional value and reef manta ray feeding. However, greater nutritional quantities were found when manta

rays were feeding, and the highest biomass was recorded when the largest feeding aggregation of reef manta rays was observed.

This study has provided insight into the biochemical composition of mixed zooplankton populations around Bateman Bay, Ningaloo Reef in autumn and winter. It appears that manta rays match their distribution to the zooplankton, and nutritional quantities proved to be higher during warmer temperatures. However, additional year-round sampling is recommended for future studies, in order to better understand these preliminary findings. Knowledge of how manta rays rely on areas with high nutritional value will help to explain seasonal variations in reef manta ray visitation, and provide management implications for the conservation of this species.

Introduction

1. Zooplankton

Zooplankton are essential to the functioning of marine food webs because of their high abundance and fundamental ecological roles (Richardson 2008; Le Quéré *et al.* 2016). They provide a connection between primary producers and higher trophic level consumers (Dalsgaard *et al.* 2003; Richardson 2008). Some of the largest animals on the earth (for example whale sharks (Rohner *et al.* 2013a), blue whales (Buchan and Quiñones 2016), megamouth sharks (Tomita *et al.* 2011) and basking sharks (Sims *et al.* 2003)), rely on zooplankton to provide sufficient energy and nutrients for their survival. Zooplankton communities not only support large marine animals but microbial organisms as well. Benthic communities including cnidarians, echinoderms and anemones rely on zooplankton larvae, faecal matter and dead carcasses for sustenance as they gradually fall to the sea floor (Pepper *et al.* 2015; Le Quéré *et al.* 2016). These organisms and animal waste products contain much needed organic carbon (Richardson 2008), nitrogen and phosphorous, which is recycled by microbial organisms (Dubinsky and Jokiel 1994).

Since zooplankton create fundamental links between primary producers and higher order consumers (Dalsgaard *et al.* 2003), imbalances in environmental and biochemical composition can negatively impact consumer growth (Muller-Navarra 2008). Stable isotope analysis has shown high variability in zooplankton community composition between sheltered reef lagoons and oceanic outer reef areas (McCauley *et al.* 2014). These areas are subject to varying oceanographic conditions and therefore different trophic levels (McCauley *et al.* 2014). The mechanical energy behind oceanographic fronts and eddies has important implications for biological activity and productivity (Lebourges-Dhaussy *et al.* 2014). Eddies attract higher trophic level marine animals because they create areas of dense, easy to access prey (Lebourges-Dhaussy *et al.* 2014). A mesoscale cyclonic eddy known as the Capricorn Eddy in the southern Great Barrier Reef (GBR), is believed to greatly influence the manta ray

(*Mobula alfredi*) aggregations at Lady Elliot Island (LEI), where they have been seen foraging in close proximity to the eddy (Weeks *et al.* 2015).

1.1 Zooplankton Biodiversity and Biochemical Components

The nutritional quality of zooplankton (lipids, protein and carbohydrates) varies seasonally due to changes in species composition, which has direct effects on the marine food web and fish that rely upon it (Bascur *et al.* 2017). For most fish a diet of high protein and a large amount of essential fatty acids and lipids is essential for growth and metabolism (Conceição *et al.* 2010), therefore plankton communities that consist of high amounts of lipids suggest a rich source of food (Parrish *et al.* 2005). Where, how and when a particular species feeds determines reproductive success, spatial ecology and nutritional condition, and can influence their evolutionary pattern, such as speciation (Stewart *et al.* 2016). The function of lipids varies between zooplankton taxa, from gelatinous species, which only contain a very small percentage of lipids, to copepods with over 60% of lipid dry mass (Lee *et al.* 2006). Average nutritional composition of zooplankton in the Southern Ocean, Arabian Sea and Equatorial Pacific showed protein was most abundant, comprising approximately 65% of the total biomass, followed by carbohydrates at 19% and lipids at 16% (Hedges *et al.* 2002). The energy stored within zooplankton is transferred to planktivorous fish when consumed (Barroeta *et al.* 2017).

Zooplankton has a spatiotemporally patchy distribution (Jagadeesan *et al.* 2010), and the variability of each biochemical component can be seen when comparing regions around the world. Plankton dynamics differ strongly in high to low latitudes; polar waters are much more productive than tropical or temperate waters, due to strong seasonal cycles (Findlay *et al.* 2006; Ji *et al.* 2010). Phytoplankton biomass (often used as a proxy for zooplankton productivity), in the southern end of the Humboldt Current system, off Chile, has shown inter-seasonal variations due to environmental variables such as temperature and wind stress (Gomez *et al.* 2017). Wind stress influences plankton biomass most significantly in spring and summer, due to solar radiation enhancing phytoplankton's response to seasonal upwelling during this time (Gomez *et al.* 2017). Species composition has also been observed to fluctuate with these seasonal events, which has an effect on primary and secondary production and local fisheries (Gomez *et al.* 2017). Tidal variations also influence zooplankton biomass (Armstrong *et al.* 2016). The average biomass of zooplankton off the Cabo Catoche in the Yucatan Peninsula, Mexico has been reported as 4.5 g m^{-3} at planktivorous fish feeding sites (Motta *et al.* 2010), while average biomass fluctuations of between 0.08 and 1.76 g m^{-3} have been reported in the Arabian Sea (Bhat *et al.* 1993), showing the range of biomass possible in different systems.

Due to the high variability of oceanographic conditions and environmental variables within different marine systems, it is difficult to determine a global average range of the biochemical components of marine plankton (Hedges *et al.* 2002). During the Arabian Sea monsoon season, zooplankton composition was found to consist of 41% protein, 15% carbohydrates and 20% lipids of total dry mass (Jagadeesan *et al.* 2010), whereas during dry season the average biochemical components were 34% protein, 3% carbohydrates and 9% lipids (Bhat *et al.* 1993). Nutritional components of zooplankton around North Stradbroke Island, southern GBR, were calculated as 3% protein, 3% carbohydrates, and 22% lipids (Verlinden 2010). The low protein found was attributed to the mixed sub-tropical zooplankton population (Verlinden 2010). In the tropical Indian Ocean, protein represented 45% of the zooplankton, 7% carbohydrates and 14% lipids (Krishna Kumari and Goswami 1993). Changes in biochemical proportions, vary with seasonal species composition.

1.2 Abundance, Distribution and Species Composition

If all of the organisms were homogeneous throughout the ocean, the nutritional resources would be insufficient to sustain the high level of productivity observed (McManus and Woodson 2012). The ocean is structured horizontally and vertically as a result of thermodynamics, which together with organism behaviour drives the patchy distribution of predators and prey, thus supporting productivity in various marine environments (McManus and Woodson 2012). This structure is well-defined in coastal ecosystems. Zooplankton biomass generally peaks during warmer months due to higher temperatures promoting phytoplankton growth, and provides appropriate spawning conditions for many species (Taylor and Pearce 1999; Tsikliras *et al.* 2010; Gilmour *et al.* 2016). Increased temperatures stimulate nutrient pulses, promoting phytoplankton blooms and zooplankton biomass (Findlay *et al.* 2006). Zooplankton grazing helps to decrease phytoplankton blooms, keeping nutrient levels down (Findlay *et al.* 2006). Zooplankton respond to phytoplankton availability; therefore, zooplankton biomass is often positively correlated to peaks in phytoplankton abundance (Kiorboe and Nielsen 1994; Irigoien *et al.* 2004; Batchelder *et al.* 2012). Autumnal plankton blooms on the GBR, eastern Australia, are produced by increased nutrients, sufficient light availability and vertical mixing from deeper layers (Findlay *et al.* 2006). Studies from the Sea of Japan indicate higher zooplankton abundance in warmer months, with a seasonal variation of species composition (Jo *et al.* 2016). The Bering Sea has shown higher abundance in spring, due to increased temperatures promoting phytoplankton growth, which supports zooplankton blooms in April-May (Coyle *et al.* 2008). Local storms promote mixing, which encourages post-bloom productivity events within the plankton (Coyle *et al.* 2008). Copepod species make up a high percentage of the zooplankton throughout many regions (Coyle *et al.* 2008; Richardson 2008; Jo *et al.* 2016). The zooplankton species composition in the Sea of Japan is mostly made up of copepods, chaetognaths, ostracods and chordates (Jo *et al.* 2016), while copepods are the most abundant zooplankton in the Bering Sea (Coyle *et al.* 2008). Copepods and other crustaceans have a higher nitrogen and protein content than gelatinous species, having approximately 68% and 23% of protein respectively (Jo *et al.* 2016). Due to their abundance, and high nutritional value, they are important drivers of trophic interactions (Ladhar *et al.* 2014).

However, due to increased anthropogenic stressors on the marine environment such as eutrophication, over-fishing and climate change, blooms of gelatinous zooplankton (i.e. cnidarians, tunicates and ctenophores) are increasing around the world (Lynam *et al.* 2006; Jaspers *et al.* 2014). Gelatinous zooplankton have a high water content, and generally have low nutritional quality (Jaspers *et al.* 2014; Wang *et al.* 2015). Among gelatinous zooplankton, chaetognaths have a higher lipid content than ctenophores, tunicates or scyphozoans (Wang *et al.* 2015).

Organisms within the zooplankton community will have varying degrees of nutritional worth at different stages of their life (e.g. larval and egg phases), resulting in seasonal variation with reproduction and spawning events (Guzmán *et al.* 2016; Bascur *et al.* 2017). Due to the high protein content of copepods and euphausiids (Wang and Jeffs 2014) they are generally the dominant species targeted in planktivorous fish-feeding aggregations (Armstrong *et al.* 2016; Bennett *et al.* 2016; McGregor pers. comm.), rather than tunicates or chaetognaths. Horizontal plankton tows conducted within the Ningaloo Marine Park during May 2013, showed that crustaceans dominated the plankton, followed by gelatinous taxa (West 2013). Whale sharks are observed around Ningaloo from March to June every year (Wilson *et al.* 2001) and these seasonal aggregations are believed to coincide with the nutritionally rich zooplankton available at this time (Hanson and McKinnon 2009). The zooplankton composition showed temporal variation, indicating high variability in species dynamics. Vertical plankton tows revealed zooplankton to be more abundant at night, when species of decapod and euphausiid crustaceans vertically migrate from depth, indicating an obvious diurnal pattern (West 2013). Copepods, chaetognaths, salps and eggs did not show an obvious diurnal trend, however contributed significantly to zooplankton assemblages (West 2013).

1.3. Biochemical Composition

1.3.1. Lipids and Fatty Acids

Lipids provide many important functions, such as energy reserves, antioxidants and buoyancy regulation (Kattner *et al.* 2007). Essential fatty acids are needed in animal diets, for growth, survival and reproductive success (Muller-Navarra 2008). Polyunsaturated fatty acids (ω-3 and ω-6) are particularly important for animals as they sustain membrane fluidity and maintain tissue hormones (Muller-Navarra 2008). Lipids in zooplankton have important physiological properties for reproduction, early development and times of dormancy (Kattner *et al.* 2007). By understanding how these critical nutrient levels are transferred from one trophic level to the next, the lipid content of zooplankton can be used as a trophic marker to determine the productivity of a particular environment (Dalsgaard *et al.* 2003).

Lipids are produced by phytoplankton, then consumed by herbivorous zooplankton, which are preyed upon by many larger organisms, transferring energy from primary to secondary and tertiary production (Dalsgaard *et al.* 2003). Particular fatty acids can be traced as they are transferred up the food chain, indicating predator-prey interactions (Dalsgaard *et al.* 2003). Zooplankton consume most of the essential fatty acids they need for survival, growth and reproduction from phytoplankton communities (Sterner and Schulz 1998), as well as other zooplankton. Zooplankton excretion, and biomineralisation of faeces by bacteria, helps to regenerate nitrogen levels in the ocean, and encourage phytoplankton productivity (Richardson 2008). Tropical regions have a high turnover of zooplankton biomass, resulting in lipid-poor organisms, which is common within oligotrophic waters due to their high metabolic rate (Kattner *et al.* 2007). Tropical marine plankton do not need to accumulate lipid stores for times when food is scarce, because low concentrations of prey can be found year round in tropical regions (Barroeta *et al.* 2017). In comparison, zooplankton in high-latitude regions have large lipid stores, as they convert lipid-poor phytoplankton into rich lipid deposits (Kattner and Hagen 1995). Lipid content of fish eggs and larvae in the South Eastern Pacific is lower in summer and higher in winter, due to the need to store lipids during winter for survival in times of dormancy (diapause) (Bascur *et al.* 2017). While seasonal variations in temperate regions are significantly different, they are not as apparent in the tropics; this drives the differences in lipid storage. The changing global climate could have effects on lipid rich zooplankton, as increasing temperatures influence the stability of the water column, productivity, size composition and diversity and trophic efficiency of zooplankton (Richardson 2008). This could prompt a shift in species dominance, affecting the energy transfer between producer and consumer (Kattner *et al.* 2007).

Copepods generally have high lipid content and are therefore an important food resource for many higher trophic organisms (Kattner *et al.* 2007). Lipid stores provide energy throughout the colder months, with some organisms being able to reproduce in winter without needing to feed (Kattner *et al.* 2007). However, many animals rely on lipid-rich plankton and align their reproduction to events with planktonic abundance (Kattner *et al.* 2007). Calanoid copepods are particularly rich in monounsaturated fatty acids, as they consume them from phytoplankton; this in turn is transferred up the food chain to higher trophic animals (Kattner *et al.* 2007), such as whale sharks and manta rays. Therefore, the health of zooplankton will determine the health of their predators (Dalsgaard *et al.* 2003).

1.3.2. Protein

Proteins are responsible for a variety of physiological functions, which create the cell structure in organisms (Harris *et al.* 2000). They are made up of hundreds of smaller units called amino acids, which are essential in animal metabolism. Amino acid chains are formed by specific bonds and are unique to every protein (Harris *et al.* 2000). There are primary structures, formed by pure amino acid chains, and secondary, tertiary and quaternary structures formed by specific amino acid bonds (Harris *et al.* 2000). Proteins are the major component in cell protoplasm (Harris *et al*. 2000). Protein represents on average 32% of the cellular dry weight in phytoplankton (Moreno and Martiny 2017), and is transferred to zooplankton when consumed. The limiting nutrient (C, N or P) in a particular system determines which proteins are regulated and the organisms' capacity to uptake nutrients varies between planktonic lineages (Moreno and Martiny 2017). Various studies have found higher amounts of protein within the plankton during warmer months (Percy 1979; Moncheva *et al.* 2003; Verlinden 2010), which generally coincides with reproductive cycles (Percy 1979).

Zooplankton generally consist of between 10-50% protein (Harris *et al*. 2000). Zooplankton in polar regions consist of a high amount of protein (Percy and Fife 1981), where there is an abundance of protein rich euphausiids (for example Antarctic krill) and copepods (Murphy *et* *al.* 2016). Copepods contain a large amount of protein, which is present year-round in the Sea of Japan (~48%) (Jo *et al.* 2016). Zooplankton in the Southern Ocean has a high protein content (~70%), while those in the Arabian Sea have less protein (~51%) but are higher in carbohydrates and lipids (Hedges *et al.* 2002). Protein was found to be the major component along the west coast of India (~35%), where high amounts of calanoid copepods and fish eggs were present (Jagadeesan *et al.* 2010). Tropical plankton communities can also store high amounts of proteins, but have a lower energetic value than those in high-latitudes (Barroeta *et al.* 2017). The zooplankton around North Stradbroke Island, Queensland contained less than 10% protein (Verlinden 2010). The differences between ocean regions indicate high variability, as different species store varying amounts of nutrients (Jaspers *et al.* 2014).

1.3.3. Carbohydrates

Carbohydrates are a diverse group of compounds, the simplest of which are the sugars which form two large groups: the oligosaccharides and polysaccharides (Harris *et al.* 2000). Carbohydrates are found in abundance in phytoplankton cell walls, contributing over 50% of their biochemical composition (Romankevich 1984; Jo *et al.* 2016), however, are usually only found in small amounts (<10%) in zooplankton (Romankevich 1984; Harris *et al.* 2000). Chitin, a type of carbohydrate, forms the exoskeletons of crustaceans and consists of amino-sugars, a form of mucopolysaccharides (Harris *et al.* 2000). Chitin has no nutritional value, however is a very common bio-polymer within the ocean. Carbohydrates are ecologically significant as they are used to create new compounds via metabolic processes (Romankevich 1984), are rich in carbon (Moreno and Martiny 2017), and assist with energetic requirements (Ingole and Parulekar 1995).

One of the earlier determinations of carbohydrate content within zooplankton was by Brandt (1898); suggesting zooplankton comprising mostly of copepods has a carbohydrate content of approximately 20%, which is much higher than those found in more recent studies (Raymont and Krishnaswamy 1960; Raymont and Conover 1961; Romankevich 1984). Raymont and Krishnaswamy (1960) suggested zooplankton from the English Channel to consist of a low amount of carbohydrates (<5%), which increases when the organisms are actively feeding, and lowers when starved. Carbohydrates within zooplankton assemblages around North Stradbroke Island increase during spring to approximately 15% of dry weight, which has been attributed to local phytoplankton blooms (Verlinden 2010). Carbohydrate levels of zooplankton have also been observed to increase during spring and summer in Conception Bay, Newfoundland (Choe *et al.* 2003). This can be attributed to the varying life stages of planktonic organisms such as maturing copepods being present during warmer months, and immature organisms during colder months, coinciding with a lower carbohydrate percentage (Choe *et al.* 2003).

1.3.4. Carbon: Nitrogen Ratio

The ratio of carbon to nitrogen (C:N) within planktonic organisms influences the nutrient cycling of marine food webs (Vrede *et al.* 2004). This affects the structure and functioning of food webs, having repercussions for organism growth efficiency (Vrede *et al*. 2004). C:N ratios show significant interspecies variation, and vary within different life stages (Vrede *et al.* 2004; DeLorenzo Costa *et al.* 2006). C:N ratios positively correspond to lipid content, where female copepods experience less lipids after reproducing, therefore have lower C:N ratios (DeLorenzo Costa *et al.* 2006). High C:N ratios typically correspond with high carbon values and low nitrogen.

High-latitudes generally contain much higher carbon percentages than sub-tropical and tropical regions with up to 70% carbon and C:N ratios up to 10 (Ikeda and McKinnon 2011), whereas tropical regions typically experience lower C:N ratios of approximately 3-4 and <40% organic carbon (Ikeda and McKinnon 2011). Tropical zooplankton experience a much more stable environment than those in polar regions, therefore less variation in C:N ratios is usually found. C:N ratios have been found to generally increase during spring, where warmer temperatures promote phytoplankton blooms, increasing carbon into the system (Escribano *et al.* 2007; Kamburska and Fonda-Umani 2009; Verlinden 2010).

1.4. Impacts of Climate Change on Plankton Composition and Distribution

Rising temperature due to global climate change is considered to be one of the most important factors affecting the future of marine ecosystems. Global oceanic circulation is becoming more stratified due to higher concentrations of atmospheric carbon dioxide (CO_2) (Bopp *et al.* 2001). Zooplankton from polar, temperate and tropical marine systems are affected by these rising CO_2 levels (Walther *et al.* 2002; Smith *et al.* 2016). Zooplankton abundance has been found to be less in areas with high CO_2 levels, however nutritional quality, such as fatty acid quantity is not as affected (Smith *et al.* 2016). This raises the question for future studies, whether the quality of food will be enough even if the biomass is less (Smith *et al.* 2016).

Rising temperature has a direct impact on the decline of phytoplankton biomass, which is correlated with lower mesozooplankton abundance and rapid deterioration of zooplankton communities (Bopp *et al.* 2001; Richardson and Schoeman 2004; Garzke *et al.* 2015). Climate change will also affect ecosystem services provided by planktonic organisms, such as oxygen production, carbon sequestration and biogeochemical cycling (Richardson and Schoeman 2004). Variations in marine productivity have direct effects on fish stocks, impacting higher order organisms and human populations that rely upon them (Bopp *et al.* 2001).

Copepod abundance and size has been found to be significantly less in warmer temperatures (Garzke *et al.* 2015). Mature copepods are not surviving in water bodies experiencing warming; instead an abundance of the earlier nauplius larval stage have been found (Garzke *et al.* 2015). This can be attributed to a decline in copepod reproduction and an acceleration in the hatching process due to rising temperature (Garzke *et al.* 2015). Increasing temperatures cause a higher daily mortality rate in copepods compared to colder environments (Breteler *et al.* 1995). Global warming could lead to a reduction in lipid rich copepods of subarctic ecosystems, with no foreseeable prospects for replacement (Beaugrand 2009; Kattner and Hagen 2009).

Krill (*Euphausia superba*) in the Southern Ocean, a keystone species, supports a wide variety of higher trophic animals (Walther *et al.* 2002). Climate change has had dramatic effects on krill reproduction, impacting their recruitment by a reduction in sea ice near the Antarctic Peninsula (Walther *et al.* 2002). The decline in krill recruitment has negative consequences on marine food webs, causing potential shifts in population dynamics (Walther *et al.* 2002). In southern California, macrozooplankton abundance has decreased by 80% since 1951, due to warming surface waters and an increase in the temperature difference of the thermocline (Roemmich and McGowan 1995). As the ocean becomes more stratified upwelling is reduced, due to the warming of the thermocline; upper layers receive less nutrients and more light, which leads to less production and a decrease in zooplankton (Roemmich and McGowan 1995).

Climate change has caused many species to shift their biogeography in response to changes in temperature, acidity, dissolved oxygen and nutrient availability (Barton *et al.* 2016; Fogarty *et al*. 2017). For example, the East Australian Current transports larvae from tropical waters to southern temperate waters, many of which do not survive during winter (Fogarty *et al*. 2017). However, global warming could provide favourable winter conditions, producing more permanent populations of tropical species in temperate waters (Fogarty *et al.* 2017). Plankton communities in North America have shifted poleward and are predicted to continue shifting due to anthropogenic induced climate change (Barton *et al.* 2016). It is predicted that primary production will decrease globally, which will significantly alter ocean food webs (Steinacher *et al.* 2010). Enhanced stratification, reduced mixing, and slowed circulation as a consequence of climate change decreases nutrient concentrations, which weaken the energy flows between trophic groups that rely upon each other for survival (Steinacher *et al.* 2010; Barton *et al.* 2016).

Planktivores will be directly affected by a shift in plankton distribution as they match their movements to that of their prey. The change in zooplankton reproduction and recruitment could have negative impacts on the amount of essential nutrients planktivorous fish receive, potentially affecting their health, reproduction and survival.

1.5. Planktivorous Elasmobranchs

Planktivorous fish aggregations appear to coincide with local seasonal events, such as coral spawning at Ningaloo Reef, Western Australia (Taylor 1996), copepod blooms in Mexico (Nelson and Eckert 2007), abundances of krill (*Euphausia diomedeae*) in the Philippines (Rohner *et al.* 2017), fish spawning in Belize (Heyman *et al.* 2001), and planktonic blooms in Mozambique (Rohner *et al.* 2013b). There are 14 recognised species of filter feeding elasmobranchs – 11 devil ray species of the genus Mobula, which includes both manta ray species *Mobula birostris* and *Mobula alfredi*, the whale shark (*Rhincodon typus*), the basking shark (*Cetorhinus maximus*) and the megamouth shark (*Megachasma pelagios*), all of which predominantly feed on zooplankton (Motta *et al.* 2010; Couturier *et al.* 2012; Bennett *et al.* 2016).

In Baja California, Mexico, whale sharks are seen aggregating to feed on zooplankton from June to November, corresponding to an abundance of copepod-rich plankton (contributing up to 85% of the composition of the zooplankton) (Nelson and Eckert 2007). There appears to be a minimum density that some large planktivorous fish require before feeding takes place, which was no less than 10,000 individuals m^{-3} (Nelson and Eckert 2007).

Most studies on the diet of large planktivorous fish have been limited to shallow and coastal waters, however there are studies questioning where these animals get the majority of their prey (Bennett *et al.* 2016). Recent studies have suggested a large proportion of their diet is demersal zooplankton (Couturier *et al.* 2013; Burgess *et al.* 2016; Rohner *et al.* 2017). Acoustic and satellite telemetry has suggested that *M. alfredi* reside within shallow coastal regions during the day, but leave at night, posing the question of where they spend their time when they leave the coast (Dewar *et al.* 2008; Jaine *et al.* 2014). It has been suggested that they obtain the majority of their food during night-time (Rohner *et al.* 2013a). As manta rays appear to spend some of their time foraging at depth (Couturier *et al.* 2013; Stewart *et al.* 2016), when they are observed on the surface, it has been proposed they are grazing or basking, until they are able to dive again to forage for the rich abundance of prey (Stewart *et al.* 2016).

Episodes of increased planktonic biomass, and fish spawning events in tropical oligotrophic waters, generally coincide with visiting planktivores such as manta rays and whale sharks (Rohner *et al.* 2013a; Bennett *et al.* 2016). During October-May on the GBR, a large pulse of zooplankton is fed into the Whitsunday Islands by strong tidal currents (Bennett *et al.* 2016). In order to maximise the benefit of these nutritional pulses, predators must match their distribution to that of their prey, making them sensitive to prey variability (Barnett and Semmens 2012). Gliwicz and Maszczyk (2016) found that a variable prey distribution provides planktivorous predators with greater feeding efficiency rather than a homogenous prey distribution; this could be attributed to the amount of time and energy it takes to feed within a pulse of food (Gliwicz and Maszczyk 2016).

1.5.1 Manta rays

Manta rays, family *Mobulidae*, are filter feeding planktivores distributed throughout tropical and sub-tropical regions around the world (Jaine *et al.* 2014). There has been increased interest in manta ray ecotourism, due to their potential to generate significant economic benefits, particularly in coastal communities and developing countries (Anderson *et al.* 2011; Couturier *et al.* 2012). Due to fishing pressures and the low fecundity of these species, both *Mobula alfredi* and *Mobula birostris* have been listed as Vulnerable to Extinction on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Marshall *et al.* 2011; Couturier *et al.* 2012). Studies on feeding ecology are crucial in understanding where, how and when a particular species feeds, which influences their evolutionary pattern, such as speciation, and determines reproductive success, spatial ecology and nutritional condition (Stewart *et al.* 2016). This information can be used to implement management plans for the protection and conservation of vulnerable animals.

Until recently, the genus *Manta* was believed to be monospecific, however a review of the taxonomy of the genus showed there are two distinct species: *Manta birostris* (Marshall *et al.* 2009), an oceanic migratory species, and *Manta alfredi* (Krefft 1868), a predominantly tropical species found around coral reefs and islands (Couturier *et al.* 2011). The genus *Manta* has recently been subject to taxonomic review, and the two species of manta ray have been reallocated to the genus *Mobula*; the two species are now recognised as *Mobula birostris* and *Mobula alfredi* (White *et al.* 2017). *Mobula alfredi* is smaller, with a disc width of up to 5 m (Gadig and Neto 2014) and can weigh up to 800 kg (Kitchen-Wheeler 2013), while *M. birostris* has a disc width of up to 6.7 m and weighs up to 1,400 kg (Dewar *et al.* 2008). Manta rays are filter feeders, using their cephalic lobes to funnel water into their mouths and filter out their planktonic prey through modified gill rakers (Dewar *et al.* 2008).

Mobula birostris are usually found feeding in oceanic channels or surface slicks of a planktonic bloom (Wilson *et al.* 2001; Dewar *et al.* 2008). Observations of *M. birostris* feeding in captivity determined the motivational behaviour behind their cephalic lobes (Ari and Correia 2008). When food is not present, the lobes are curled up or partially open indicating the manta has no incentive to feed, or is ‘tasting’ the water column in search of food. However, when there is an abundance of plankton the manta unfurls its cephalic lobes, creating a scoop to funnel prey into their mouths (Ari and Correia 2008). Mobulid gill plates have adapted morphologically to filter out a range of prey sizes, from larger euphausiids (krill) to smaller calanoid copepods (Stewart *et al.* 2016). Studies at Lady Elliot Island (LEI), the southernmost coral cay of the Great Barrier Reef (GBR), have shown reef manta rays will only feed when zooplankton is at a minimum prey density threshold of 11.2 mg m^{-3} (Armstrong *et al.* 2016). The size and composition of the plankton was found to have no effect on feeding behaviour (Armstrong *et al.* 2016).

There is minimal information on *M. alfredi* diets, as gut content analysis is ethically difficult to obtain when dealing with a vulnerable species (Bennett *et al.* 2016). However, it is understood they mainly feed on zooplankton (including copepods, chaetognaths, krill, fish larvae and fish eggs) (Couturier *et al.* 2013; Bennett *et al.* 2016; Rohner *et al.* 2017). It has been suggested that the *M. alfredi* filtration system has a lower limit for prey size, with a minimum length of approximately 1 mm, indicating they rely on mesozooplankton (Bennett *et al.* 2016). Species of the sub-class Copepoda were found to dominate the gut contents of a *M. alfredi* specimen landed off the Whitsundays, eastern Australia (Bennett *et al.* 2016). Specifically, calanoid copepods were found within the gut, as they contribute significantly to the GBR zooplankton composition (Sale *et al.* 1976; Bennett *et al.* 2016), along with a large proportion of chaetognaths (Bennett *et al.* 2016).

Plankton becomes concentrated along oceanographic fronts, eddies and upwellings, which provide important foraging opportunities for planktivorous organisms, influencing their distribution and feeding behaviour (Jaine *et al.* 2014). Like other large planktivorous fish, it is believed that manta rays seek out their prey and aggregate to areas with high planktonic abundance (Anderson *et al.* 2011). Many coastal regions are areas rich with plankton, generally associated with upwellings that vertically transport nutrients up from the depths (Parra Venegas *et al.* 2011). At Ningaloo Reef, Western Australia, zooplankton becomes concentrated along the coast, potentially due to the combined influence of ocean currents and swell (McGregor pers. comm.). Fish and coral spawn can also create extensive slicks within the reef lagoon from prevailing winds (Taylor and Pearce 1999; McGregor pers. comm). Manta rays are often seen at Ningaloo feeding close to the beach on patches of plankton. In Mexico, around the northeast coast of the Yucatan Peninsula, manta rays have been observed feeding alongside other planktivorous fish (Parra Venegas *et al.* 2011). These feeding aggregations have been attributed to highly productive waters from tropical coastal upwellings (Parra Venegas *et al.* 2011). Offshore plankton occurs in low concentrations when compared to coastal areas, which would imply a less productive food source for large planktivorous fish (Parra Venegas *et al.* 2011). Large coastal aggregations of planktivores such as manta rays and whale sharks are often correlated with spawning events (Parra Venegas *et al.* 2011). Around the remote Palmyra Atoll, in the Northern Line Islands of the central Pacific Ocean, local manta ray populations receive the majority of their energetic requirements from nutrient rich zooplankton within the atoll’s lagoon, rather than offshore (McCauley *et al.* 2014).

Based on long term manta ray sightings at LEI, individuals have been found most commonly feeding approximately two hours before low tide, which coincides with times of high planktonic biomass (Armstrong *et al.* 2016). Similarly, a greater proportion of manta rays have been found feeding in cooler waters, where there is a higher biomass of zooplankton (Armstrong *et al.* 2016). Manta rays have been observed less frequently in stronger currents compared to milder currents and more frequently during the second quarter of the lunar cycle, indicating that tides have a significant influence on the incidence of sightings (Rohner *et al.* 2013b). As zooplankton accumulates during high tide, they have been classified as 'active drifters' rather than 'passive particles' (Wiafe and Frid 1996). Zooplankton density is often greatest at the thermocline, which is supported by studies of *Mobula birostris’* diving behaviour. It was found that these animals spend much of their time at depth throughout the year, which can be directly related to prey density (Stewart *et al.* 2016).

Manta rays around LEI are most commonly observed in large numbers during winter, however due to a unique sequence of events, the largest recorded manta ray feeding aggregation in Australia was seen off LEI in the summer of 2013. Tropical Cyclone Oswald caused a mass influx of nutrients from river run off, upwellings, extensive eddy activity and convergent fronts, triggering plankton blooms (Weeks *et al.* 2015). Approximately 150 manta rays were seen feeding along the oceanographic front, where biological productivity is concentrated into areas rich in nutrients, attracting aggregations of planktivores and other species (Weeks *et al*. 2015).

Mobula alfredi have a much shorter roaming distance than *M. birostris*, which are more migratory. *M. alfredi* have not been observed to travel outside a home range of approximately 500 km (Couturier *et al.* 2011). Deakos *et al.* (2011) suggested that *M. alfredi* do not travel great distances over deep water, but are most commonly found near coastal regions, such as Komodo, Indonesia (Dewar *et al.* 2008), Hawaii (Deakos *et al.* 2011), Mozambique (Marshall *et al.* 2011) and Ningaloo Reef, Australia (Sleeman *et al.* 2007). As *M. alfredi* remain within a relatively small region they are heavily influenced by fluctuations in local environmental parameters (Rohner *et al.* 2013b). Temperature can be a predictor of *Mobula alfredi* presence: they are rarely seen in temperatures below 18°C and generally reside within tropical systems (Rohner *et al.* 2013b). In Mozambique, sightings of *M. alfredi* are higher in summer, coinciding with reproduction and birthing (Marshall and Bennett 2010; Rohner *et al.* 2013a). Studies in Mozambique found temperature had a significant influence on manta ray aggregations, warmer waters having a positive effect (Rohner *et al.* 2013a), contrasting what was found at LEI (Armstrong *et al.* 2016). This variability can complicate any potential data on aggregation trends or sightings (Rohner *et al.* 2013a). Even though *Mobula alfredi* are observed year round, they show clear seasonal patterns as they follow their prey (Rohner *et al.* 2013a). Observations off North Stradbroke Island, eastern Australia, revealed mantas visited during warmer months when plankton is in high abundance (Verlinden 2010).

1.6. Ningaloo Reef

Ningaloo Reef extends approximately 260 km along the west coast of Australia, south from the north-west Cape (Sleeman *et al.* 2007). Ningaloo is a fringing reef that partially encloses a shallow lagoon, and is the only sizeable reef system on the west coast of a continent (Wilson *et al*. 2002). The reef is protected by the Ningaloo Marine Park, and was recognised as a World Heritage Area in 2011 (Ningaloo Coast World Heritage Team 2017). Bateman Bay is a large embayment north of the small coastal town of Coral Bay, situated within the central part of Ningaloo Reef. Manta rays can be found within Bateman Bay year round, as zooplankton gets funneled into the area via the Cardabia Passage (McGregor pers. comm.).

The dominant current off the Western Australian coast is the Leeuwin Current (LC), a warm low salinity current that flows poleward, sustaining life and coral growth on the Ningaloo Reef (Taylor and Pearce 1999). Its strength and poleward flow suppresses nutrient rich upwellings along the Western Australian continental shelf (Rousseaux *et al.* 2012). During autumn and winter the LC flows at its strongest (Rousseaux *et al.* 2012) and in summer, as it weakens, the weaker northward flowing Ningaloo Current dominates. The Mixed Layer Depth (MLD) is a physical parameter given to the upper layer of the ocean, where temperature, salinity and density have been fairly homogenised by wind, waves and solar radiation. The MLD deepens during autumn as a response to the strengthening of the LC, increasing surface nutrients and chlorophyll-*a* concentrations (Rousseaux *et al.* 2012). Chlorophyll-*a* is often used to measure the productivity of the ocean and phytoplankton abundance (Sleeman *et al.* 2007), which peaks during autumn due to the MLD being at its deepest (Rousseaux *et al.* 2012).

For most of the year, Bateman Bay is subject to prevailing south-easterly trade winds throughout the night and morning until the south-westerly sea breeze takes over each afternoon (Taylor and Pearce 1999). Even though the prevailing winds are southerly, the LC is able to overcome them as it continues to flow south (Rousseaux *et al.* 2012). When the LC weakens during summer, and the south-westerly winds grow stronger, wind-driven local currents dominate the region, flowing northwards. Within the lagoon, the flow is driven by the winds in a northerly direction, affecting local transport.

The LC system favours coastal downwelling due to its poleward flow alongside the Ningaloo boundary current (Zhang *et al.* 2016), which negatively affects coastal nutrient levels and productivity (Hanson *et al.* 2005). The LC has a significant influence on the transport of organisms and biological activity (Taylor and Pearce 1999). The current flows most weakly at the Ningaloo Peninsula, where there are episodic southerly winds strong enough to change the direction of the along-shelf current (from southward to northward) generating transient coastal upwelling, promoting plankton blooms (Zhang *et al.* 2016). The continental shelf along the Ningaloo coast is steeper than most shelves with an eastern boundary current system (i.e.

Peru, Benguela, California and Northwest Africa) (Chavez and Messié 2009; Pitcher *et al.* 2010; Zhang *et al.* 2016), meaning any upwellings generally come from the middle of the water column rather than closer to the sea-bed (Xu *et al.* 2016; Zhang *et al.* 2016). The supply of nutrients to the Ningaloo system is most likely from deeper layers mixing into the surface waters, as there are few river inputs (Rousseaux *et al.* 2012). Due to the transient nature of these upwelling episodes, the benthic coral growth is able to thrive because nutrients are limited and temperature stresses are controlled (Zhang *et al.* 2016). Fluctuations in the strength of the LC influence the production and distribution of plankton year round (Sleeman *et al.* 2007). Waves from the outer reef bring in richly oxygenated waters, which flow back out via gaps in the reef crest, creating a well-mixed system that supports a diverse array of organisms (Taylor and Pearce 1999; Sleeman *et al.* 2007).

During autumn, high concentrations of surface nutrients and chlorophyll-*a* have been measured, which drive planktonic blooms at this time of year (Rousseaux *et al.* 2012). It is believed there is an influx of nitrogen to the Ningaloo system during this time, which is caused by a combination of cooling surface waters and the acceleration of the LC, causing nutrients from deep water to mix with the upper layers of the water column (Rousseaux *et al.* 2012). The combination of the warm LC and cooling surface waters causes the thermocline to breakdown, promoting mixing (Price *et al.* 1986).

During summer an anti-clockwise eddy has been observed forming off the LC, south of Point Cloates, which has important implications for organisms such as zooplankton and coral spawn (Taylor and Pearce 1999). The eddy has the potential to transport and mix biota from northern Ningaloo Reef, across the continental shelf and back into the lagoon area via the Ningaloo Current (Taylor and Pearce 1999). This counter-current is strongest during March and April, which is peak coral spawning time (Taylor and Pearce 1999). Eddies are recognised as important for oligotrophic waters, as they increase productivity and nutrients in the region (Lebourges-Dhaussy *et al.* 2014). The upwelling in the centre of cyclonic eddies transports nutrient-rich waters into the euphotic zone (Lebourges-Dhaussy *et al.* 2014). The eddy observed off the LC is anti-cyclonic (Taylor and Pearce 1999), which causes central downwelling, but concentrates material at the surface (Bakun 2006). Zooplankton biomass has been observed to be higher in cyclonic eddies, as opposed to anti-cyclonic ones, under stable conditions (Lebourges-Dhaussy *et al*. 2014). It has been suggested that the Ningaloo Current transports large amounts of protein from coral spawn and larvae throughout the Ningaloo system during this time of year, which is important for the reef's survival (Taylor and Pearce 1999). Many other organisms have been observed to spawn at a similar time, which boosts protein content within the zooplankton as millions of eggs are dispersed (Taylor and Pearce 1999). The Ningaloo Current is predominantly driven by the southerly winds, and therefore is limited to surface waters; however, it is strong enough to generate cold water upwellings, enhancing planktonic productivity and biomass (Woo *et al.* 2006; Sleeman *et al.* 2007).

Whale sharks aggregate to Ningaloo Reef between March and June every year (Wilson *et al.* 2001; Sleeman *et al.* 2007). Their arrival is believed to coincide with zooplankton productivity events, such as fish and coral spawning, which promote plankton production (Sleeman *et al.* 2010). This also corresponds with large offshore schools of krill and fish that travel down from northern Ningaloo (Sleeman *et al.* 2007). The strength of the LC is dependent on the El Niño Southern Oscillation (ENSO), which describes the change in atmospheric pressure gradient between the central Pacific and northeastern Indian ocean (Wilson *et al*. 2001). When the pressure is higher over the Pacific Ocean than the Indian, the Leeuwin Current is stronger, due to a rise in sea level around Indonesia, where the Indonesian through-flow pushes warm water down the west Australian coast. Wilson *et al*. (2001) found that ENSO correlated with whale shark aggregations off Ningaloo, suggesting their movements are influenced by fluctuations in the LC's strength. The LC provides directional cues and is a method of active transport for many large migratory animals (Sleeman *et al.* 2007).

Manta ray abundance and distribution has been attributed to oceanographic 'bottom up' processes that influence food availability (Sleeman *et al.* 2007). Variables such as bathymetry, sea surface temperature (Couturier *et al.* 2011), and cholorophyll-*a* concentrations have shown correlations in zooplankton distribution and abundance (Sleeman *et al.* 2007). It follows that zooplankton can govern the abundance and distribution of planktivorous fish. Zooplankton abundance has been used as a surrogate measurement to determine megafauna distribution (Sleeman *et al.* 2007). As manta rays target these pockets of zooplankton abundance, whether they are present or not could be an indicator of seasonal ecosystem health (McGregor pers. comm.). From this we can gauge how well the Ningaloo system can support large planktivorous fish.

Manta rays occur in the Ningaloo waters year round, however have been observed to be most abundant in May (McGregor pers. comm.). More than 800 individuals have been identified from the Bateman Bay region, some remaining all year round, while others are seen more sporadically (McGregor pers. comm.). Unlike other planktivores, both manta ray species have shown patterns of residency in their home regions such as, Indonesia, Mozambique, Philippines, Mexico (Stewart *et al.* 2016) and Australia (Armstrong *et al*. 2016; McGregor pers. comm). Gelatinous species have been observed in abundance at Ningaloo during winter, so nutritional quantity can be expected to be lower at this time, which corresponds with fewer observed manta ray feeding aggregations (McGregor pers. comm.). Copepods and chaetognaths dominate the zooplankton communities during warmer temperatures, coinciding with frequent manta ray sightings (McGregor pers. comm.). Measuring the available biochemical composition of prey items helps us understand how much nutrition the animals within a certain region are receiving. Preliminary research on zooplankton distribution and species composition has been used as a guide to determine where and when seasonal zooplankton aggregations may occur.

1.7. Aims

The focus of this study is on the composition and variability of mesozooplankton (300 μm – 1000 μm) communities within Bateman Bay, Ningaloo Reef. The aim is to quantify the nutritional value (lipid, protein and carbohydrates) these zooplankton populations are providing to planktivorous fish such as manta rays. This research aims to add to preliminary work done on zooplankton distribution and composition, by investigating the seasonal nutritional quality. It is hypothesised that zooplankton nutritional quantity will be higher in autumn, when an abundance of nutritionally rich organisms is found.

2. Materials and Methods

2.1. Description of Study Area

Bateman Bay

Bateman Bay (23°05'32.1"S, 113°46'45.6"E) is a large north facing embayment, situated between Point Maud and Bruboodjoo Point, within the Ningaloo Reef Marine Park (Figure 1).

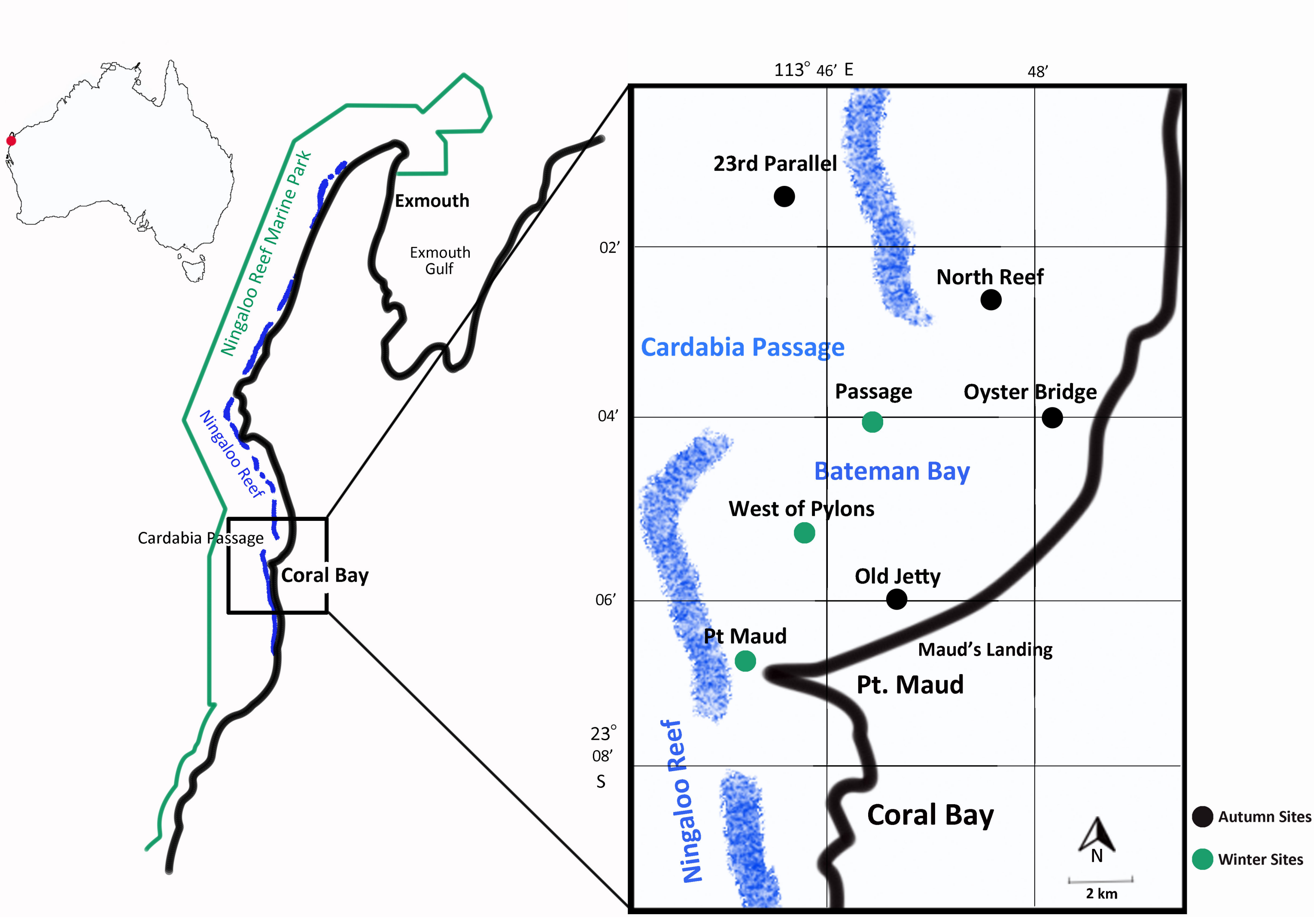

Figure 1. Bateman Bay, Ningaloo Reef. Map of Ningaloo Marine Park (indicated left, by green boxed area) along the Northwest Cape, showing the Coral Bay region (inset left), and map of Bateman Bay (zoomed right), showing manta ray aggregation areas used for the autumn (black), and winter (green) sample sites. Blue shaded area indicates the Ningaloo Reef crest.

Bateman Bay is semi-enclosed and is approximately 5 km wide, from the coastline to the reef crest. Unlike other sheltered lagoon areas along the Ningaloo Reef, Bateman Bay is open to the ocean via a gap in the reef crest where the Cardabia Passage, at >15 m deep and 5 km wide, brings increased wave energy into the bay (McGregor pers. comm). A variety of habitats occur within Bateman Bay, from shallow sand flats to patchy reef and the deep Cardabia Passage. This area supports a wide variety of animals including predatory pelagic fish such as tiger sharks, billfish and regular aggregations of reef manta rays (*Mobula alfredi*). The oceanography of the region influences the ecology within Bateman Bay, which is suggested by the presence of these predatory fish (Fitzpatrick and Penrose 2002).

Bateman Bay is adjacent to where the Leeuwin and Ningaloo Currents mix, therefore is influenced by their movements and the prevailing winds as organisms and larvae are dispersed around the bay. The oceanography of the lagoon is influenced by waves, tide and wind. Tides within the Ningaloo Reef are predominantly semi-diurnal, with a spring tide of approximately 2 m. The tides significantly influence wave-induced currents within the Ningaloo lagoon (Taebi *et al*. 2011). The shallow reefs of Ningaloo are separated periodically by channels that allow water exchange between the open ocean and the lagoon (Taebi *et al.* 2011). The currents inside the lagoon, driven by waves flow alongshore before exiting out via these gaps in the reef crest or deeper channels. The water is forced north by the prevailing southerly winds, past Point Maud and out through the Cardabia Passage. This occurs all year round, and can be influenced by swell pumping in through the Cardabia Passage, which pushes up against the beach in Bateman Bay.

Autumn Sample Sites

Four sites were sampled within the region in autumn, three within Bateman Bay and one outside North Reef (Figure 1). Zooplankton is often washed in via the Cardabia Passage, ending up in abundant pockets at these sites, therefore they are significant manta ray aggregation areas (McGregor pers. comm). One site occurs outside the reef crest (23rd Parallel), adjacent to the passage, and is influenced by oceanic currents and the ‘spur and groove’ flow of water in and out of the reef lagoon. Manta rays forage along the beach for food carried in by local currents, and aggregate to nearby cleaning stations; ecologically significant locations where manta rays and large fish come to get cleaned of parasites by small cleaner wrasse. Several known cleaning stations reside within Bateman Bay, all of which are located in areas where manta rays are frequently observed feeding. The zooplankton assemblages sampled inside Bateman Bay are from local seasonal pulses that attract manta ray feeding aggregations as certain individuals come inside the lagoon to be cleaned or reproduce (McGregor pers. comm.). When mantas are not observed inside the reef, they are regularly feeding offshore and at depth (Jaine *et al.* 2014). Zooplankton samples were collected when manta rays were observed feeding on the surface, and if none were present, the site where they were last observed was sampled. Although species were not quantified, microscopy observations of species present in the samples was recorded.

The sites inside Bateman Bay are influenced by different oceanography to the 23rd Parallel site, as they are relatively more protected. Local currents are influenced by wind stress, which prevails from a south-westerly direction, transporting zooplankton in a similar course, depending on the strength of the wind and current. North Reef, situated just inside the reef crest, and home to a cleaning station, is adjacent to the Cardabia Passage. Old Jetty is north of another significant cleaning station; Point Maud. The fourth site used in this study was Oyster Bridge, also an important manta ray cleaning station. This site is quite deep for the lagoon (~9 m); a limestone shelf that runs parallel to the coastline. Manta ray aggregations are common here as there is an abundance of zooplankton, directed in by the Cardabia Passage through to Oyster Bridge.

Winter Sample Sites

Three sites were sampled within the Bateman Bay region during winter (Figure 1). The Cardabia Passage (P) was one, as manta rays have been observed feeding there, as zooplankton is washed in and out of the gap in the reef. The second site occurs west of the Old Jetty site, and is referred to as West of Pylons (WP) in this study. The third site occurs off Point Maud (PtM), where manta rays have been observed tasting the water column around the full moon in August, as this is a spawning time for crabs (McGregor pers. comm).

Prevailing winds and swell during winter can be stronger, therefore can have a stronger effect on zooplankton distribution. Portunid crabs have been observed spawning around the full moon in August, and large aggregations of manta rays have followed.

Due to the time constraints of this project only autumn and winter were sampled. All sites are areas where manta rays are often seen feeding, therefore were chosen as areas to sample zooplankton nutritive quality to determine what planktivores, such as manta rays, are consuming within Bateman Bay. The sample site was chosen daily, using information from local tour operators and a local spotter plane, to determine the whereabouts of feeding manta rays. Preliminary work has identified seasonal variations in plankton biomass and species composition (McGregor pers. comm.); those observations were used to guide this study.

Numbers of feeding manta rays were determined from a spotter plane, used by local tour operators. Sampling was conducted off a tour vessel, and a smaller research vessel. Sampling on the tour vessel, Utopia, made locating manta rays easier due to having access to the local spotter plane. However, when sampling on the research vessel, locating feeding manta rays was more difficult as there was no direct access to the spotter plane, and we had to rely on reports on where they were last observed.

A pilot study was conducted in March to work out project logistics, experimental design and practice using the sampling equipment. Preliminary data was used from this study to work out a routine for analysis and improve on any issues that occurred.

2.2. Zooplankton Collection

Zooplankton was collected by obliquely towing a 300 μm mesh plankton net, with a 50 cm diameter, at a speed of ~2-3 knots, for four minutes. To achieve random sampling and account for any zooplankton dispersal this was repeated four times at each site. Sites were chosen each day, in accordance with where manta rays were observed feeding. The plankton net was equipped with a calibrated General Oceanics 2030R flowmeter, to calculate the volume of water filtered through the net during each tow. Environmental parameters were also recorded; turbidity, tide, water temperature, wind and current direction. Turbidity was estimated using a tape measure held between two people, who swam away from each other until just visible, and the distance recorded. Tides were recorded using data from Willy Weather (WillyWeather 2017), which is supplied by the Bureau of Meteorology. Water temperature was recorded using an Aqua Lung i450T dive computer, wind speed and direction using a handheld thermo-anemometer (Dick Smith Electronics Air Speed Temperature Meter, Q1301) and the direction of the local current determined by a drifter, when I was in the water. Zooplankton was collected in a cod end, fitted at the base of the plankton net. After each tow, any plankton caught on the net was rinsed down into the cod end, using a squeeze bottle, then samples were collected and separated out into 300 μm and 1000 μm size classes using meshed containers, and then transferred by rinsing down the containers with the squeeze bottle, into pre-labelled, 100 mL Nasco Whirl-Paks. Each sample was then put on ice until fieldwork was finished and samples were able to be processed in the laboratory. After each tow the net and cod end were rinsed in seawater, without the cod end attached, along with each mesh container.

2.3. Sample Processing

Each sample was separated into 300 μm and 1000 μm size classes to separate larger taxa, such as chaetognaths and salps, from smaller organisms, like copepods. Each sample was filtered into 100 mL whirl packs, which were processed at the end of each sample day at the Coral Bay Research Station. Each 100 mL sample was filtered onto pre-weighed Whatman 47 mm GFC glass fiber filter papers, using a 47 mm filter tower in preparation for analysis. After filtering, all samples were weighed again to obtain wet weight, and frozen until they were taken back to Murdoch University for processing. Once filtered, each filter paper was folded in half to prevent any sample from falling out and kept frozen in small zip lock bags for future analysis.

Preservation

For biochemical analysis, ideally samples should be preserved at -80°C to stop enzymatic activity (Harris *et al.* 2000). Due to the limitation of being in the field at Coral Bay, samples were filtered then preserved at -20°C. Once taken back to Murdoch University, they were stored at -80°C, until they were freeze dried (HETO*SICC*-CD4 Freeze Dryer) in preparation for biochemical analyses. After freeze drying samples for at least 24 hours, they were stored in a -20°C freezer.

Preparation for Biochemical Analysis

For the evaluation of protein, lipids, carbohydrates and total organic carbon and nitrogen, samples were divided into four equal amounts and freeze dried. For autumn, there were four samples per site for each component, and in winter six per site for each component. Samples being analysed for lipids, protein and carbohydrates were transported to the Algae R & D Centre at Murdoch University where they were stored in a -20°C freezer. Six samples per season were randomly selected, and prepared for total organic carbon and nitrogen, and analysed by the Marine and Freshwater Research Laboratory (MAFRL) at Murdoch University.

Biomass

Filter papers were weighed individually before filtering the zooplankton samples after which wet weights were recorded. All samples (n=45) were freeze dried for at least 24 hours and individually weighed to obtain dry weights. The weight of each filter paper was subtracted from the dry weights. Each dry weight (g) was divided by the volume of water filtered (m^3) through the net (calculated from the calibrated flowmeter), and expressed as g/m^3 of dry mass.

2.4. Biochemical Analysis

Lipids

The proportion of lipids present within the zooplankton was determined using the extraction method of Bligh and Dyer (1956). Lipids were extracted from samples by adding a chloroform:

methanol: deionised water solvent. After drying the samples with liquid nitrogen, 2 mL of the chloroform solvent was added and each sample crushed using a glass rod. A further 3.7 mL of the chloroform solvent was added and the mixture further homogenised. In order to separate the liquid from solid, the samples were centrifuged for 10 minutes at 3500 rpm (Centurion Scientific-PRO-VET.MULTI). Centrifuging was repeated if necessary. This extraction procedure was performed twice, to ensure 99% recovery of lipids (Linford 1965). Once separated, the lipid solution was transferred into larger glass test tubes and 3 mL deionised water and 3 mL chloroform added. Each sample was mixed using a vortex, then more deionised water and chloroform was added and mixed again before storing in a refrigerator overnight. The following day the lipid solution had fully separated into two phases; the lower phase containing the lipids. The lipid layer was extracted using a Pasteur pipette attached to a syringe, transferred into small glass vials (previously weighed), and placed under nitrogen gas on a hot plate at 38°C to dry. Once dried, each sample was weighed individually to determine the total amount of lipids present in the zooplankton. Lipids are expressed as a percentage of total lipids per g of dry weight used for the extraction. A more detailed protocol for lipid determination is outlined in Appendix 1.

Protein

The amount of protein in the zooplankton was determined using a Bio-rad protein assay kit (Bradford 1976). A dye (Coomassie® Brilliant Blue G-250), was added to the sample in 10 mL centrifuge tubes. As the dye sticks to the proteins in the solution, the amount of protein is proportional to the intensity of the blue colour. One mL of each sample was pipetted into 1 mL glass cuvettes, and the absorbance of each solution measured at 595 nm using a spectrophotometer (Thermo Scientific, Biomate 36). To determine the proportion of protein in the zooplankton, the sample result was compared to a standard curve of five known protein concentrations, that were pre-prepared using Bovine Serum Albumin (Figure 2). Protein is expressed a percentage of total protein per mg of dry weight used for the homogenate. A more detailed procedure for protein determination is outlined in Appendix 2.

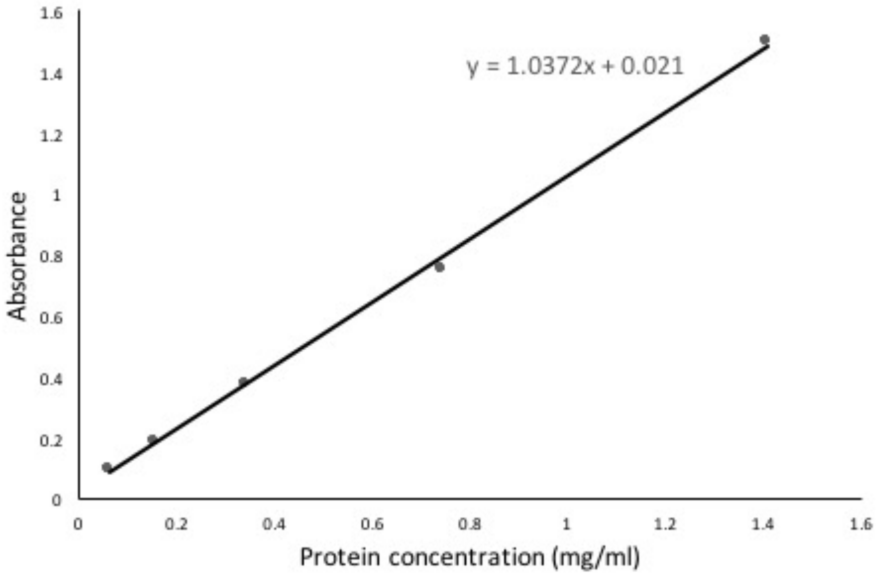

Figure 2. **Standard Protein Concentrations**. Absorbance of five different concentrations of bovine serum albumin used as a protein standard, measured at 595 nm with a spectrophotometer.

Carbohydrate

The proportion of carbohydrates in the samples was calculated once total lipid and protein were known. An estimate for the total carbohydrate percentages present in the zooplankton was provided by taking the sum of lipid and protein for each sample minus 100. Ash content was not calculated from dry weights; 3.5% was estimated from the salinity of seawater (35 ppt), and subtracted from carbohydrate estimates.

Carbon and Nitrogen

Filtered and preserved samples (six from each season) were freeze dried and provided to MAFRL for total nitrogen (TN) and total organic carbon (TOC) analyses. There are two separate methods for TN and TOC, therefore filters containing the sample evenly spread across it, were cut into two equal halves. TN was analysed as ammonium ion following Kjeldalh digestion with sulphuric acid, and run on a Lachat QC8500 Flow Injection Analyser. For TOC, hydrochloric acid (HCl), was used to remove any inorganic carbon present, then the filter was ashed in a furnace at 900°C. The carbon dioxide (CO_2) produced was passed through an IR detector (Shimadzu TOC SSM solid sample module), which gave the percentage of organic carbon.

2.5. Statistical Analysis

Each biochemical component within the zooplankton samples (lipid, protein and carbohydrate) was expressed as a percentage of the sample. Predictor variables were season and site, while response variables were lipid, protein, carbohydrate, and C:N ratio. Seasons were decided based on preliminary knowledge of high planktonic productivity (McGregor pers. comm.), and kept within the time constraints of this project: autumn and winter. Sites were defined using preliminary data on manta ray aggregation areas (McGregor pers. comm.), with enough space between them to allow for possible variations in zooplankton composition. Initially 300 and 1000 μm size classes were also going to be compared, however due to insufficient samples in both sizes, they were combined for statistical analyses.

Data normality was assessed using a linear regression model and Q-Q plot to visualize the spread; this information was used to decide which statistical test was appropriate and whether a transformation was necessary. Homogeneity of variance and normal distribution are the primary assumptions when performing ANOVAs, and the data was not normally distributed. Log and square root transformations were performed; however, the data still did not meet the assumptions for ANOVA. Therefore, in order to test the significance of nutritional variation within season and site, and the C:N ratio for both seasons, a series of non-parametric, Chi-square tests was executed.

A Chi-square test was performed to determine the influence of season and site on the amount of lipid, protein and carbohydrate in the zooplankton. An additional Chi-square test was used to test for significance of season on the C:N ratio of the zooplankton. Due to the small sample size, the normality assumption was not met to perform linear regression models on environmental parameters (turbidity, tides, water temperature, wind and current direction) with biochemical components. Therefore, these were compiled into a table, and compared to biochemical component percentages.

The number of feeding manta rays was compared to nutritional value of each response variable each season and across sites to gauge whether this had any effect on their presence or absence. However, no correlation was found, therefore observational data was compiled with environmental variables to assist with explaining nutritional value with feeding manta ray presence or absence.

Zooplankton biomass was also compared to the biochemical components of the plankton for season and site, with reference to whether there were manta rays present or not, to determine if manta rays prefer abundance over nutritional value or vice versa. All statistical tests were based on a 95% confidence interval, and analyses were performed using the open-source software R (version 1.0.136).

3. Results

Zooplankton samples were collected from four sites in autumn (n= 20 per biochemical component) and three in winter (n= 18 per biochemical component). Sites were chosen based on where manta rays were spotted feeding on the day of sampling, therefore sites were different in the two seasons sampled. Feeding status was determined on whether the animals had their cephalic lobes unfurled.

Environmental variables and observations were recorded for each sample day and collated into a table to compare manta ray abundance to any changes in the surrounding environment. For biochemical analyses, four samples from each site in autumn were used for each component, and six samples for each component, from each site in winter.

3.1. Environmental Variables and Observations

Seasons

The only environmental variable that noticeably changed seasonally was sea surface temperature (SST) (Table 1). The local current ran in a northerly direction in both seasons and wind speed and direction changed daily. Manta rays were mostly observed feeding after midday, and when plankton samples were visually dense. Zooplankton abundance appeared to change daily, as one sample day would yield a lot of plankton, when the next there would be almost none, which seemed to correlate with manta ray presence (Table 1). The largest feeding aggregation observed during this study was after a crab spawning event, after the full moon in August, where the plankton samples were almost 100% portunid crab larvae, and more than 15 manta rays were surface feeding.

The plankton was noticeably different in winter from autumn; calanoid copepods were abundant in autumn, however harpacticoid copepods and more phytoplankton and algal detritus was present in the winter samples. At the Point Maud (PtM) site an abundance of diatoms was observed in the samples.

Table 1. Environmental variables of each season and site, including observations during sampling, and manta ray presence or absence.

Site	Sample date Am-before midday Pm-after midday	Season	SST (°C)	Turbidity (m)	Wind (km/hr and direction)	Current (direction)	Tide (m) ebb/flood	Observations and Manta Ray presence or absence and species composition (Utopia – tour vessel, Get Nudi – research vessel)
OB1	16/5 pm	Autumn	25	8	22-24 SW	N	1.2 ebb	0 mantas feeding at time of sampling, however 1 seen in the morning, calanoid copepods present. (Get Nudi)
OB2	22/5 pm	Autumn	25	5	15 SE	N	1.05 flood (low tide)	4+ mantas barrel feeding close to shore, difficult to sample due to swell, dense small pocket of copepods where mantas were, minimal gelatinous taxa. 1 tow was ~99% calanoid copepods. (Get Nudi)
NR	19/5 pm	Autumn	26	15	18 SW	N	1.5 ebb (just after high tide)	8 mantas surface feeding in small area, just before new moon, less copepods more chaetognaths, appendicularians and krill in sample. (Get Nudi)
NR	21/5 pm	Autumn	26	5	19 SW	N	1.1 flood (just after low tide)	1 manta surface feeding, copepods, krill, juv fish and eggs in sample, however less than previous day. Lots of algae present. (Get Nudi)
23P	17/5 pm	Autumn	26	12	15 E	NE	1.6 m ebb (high tide)	4 mantas feeding outside reef, noticeably different plankton composition, lots of chaetognaths, appendicularians. No mantas inside the bay on this day, therefore sampled outside. Perhaps plankton was taken outside the reef via local currents. (Get Nudi)
OJ	18/5 pm	Autumn	25	5	14 SSW	NE	1.5 m ebb (just after high tide)	2 mantas surface feeding, lots of calanoid copepods in sample, much less chaetognaths than outer reef site yesterday. (Get Nudi)
PtM	7/8 am	Winter	23	10	10 NE	NE	1.47 ebb (high tide)	No mantas in am, lots of eggs present in sample. Plankton not very dense. (Utopia)
PtM	7/8 pm	Winter	23	8	6 NE	NE	0.66 ebb (full moon)	4 mantas 'tasting' in pm, seems to be they're waiting for portunid crabs to spawn after full moon. Lots of diatoms and barnacle moults in sample (Utopia)
PtM	13/8 am	Winter	22	7 (overcast)	5 NW	N	0.85 flood	No mantas that we could see, overcast day, some spotted in area yesterday, no spotter plane today. (Get Nudi)
P	13/8 am	Winter	22	10	5 NW	N	1.07 flood	No mantas present. All tows done before high tide, minimal plankton. (Get Nudi)
P	14/8 pm	Winter	22	8 (overcast)	15 W	N	1.18 flood	1 pregnant manta tasting, but not feeding. Large swell this day, not much present in plankton. Only 1 tow due to being on tour vessel.
P	5/8 pm	Winter	22	8	15 E	N	0.86 ebb	No mantas feeding. Crab spawning to happen after full moon. Not much in the plankton. (Utopia)
WP	11/8 am	Winter	23	15	16 ENE	N	1.54 flood (almost high)	15 mantas in area. Located crab spawn, water thick with portunid crab spawn. Very dense, towed right next to mantas. (Utopia)
WP	16/8 am	Winter	22	10	5 NW	N	1.01 flood	No mantas in area. Spotter plane saw them outside reef but too much swell to tow there. Not much plankton in sample. (Utopia)

Sites

A lot of variability was observed between sites; the outer reef site (23P) had a noticeably different species composition to the samples inside the reef. Biomass was lower towards the outer reef (Figure 3), but protein was high. There were less calanoid copepods towards the reef crest and more chaetognaths. The plankton seemed to be densest closer to the beach, where there was an abundance of calanoid copepods at Oyster Bridge (OB) and Old Jetty (OJ). The local current flowed in a northerly direction for all sites. Turbidity varied between sites and sample days, where highly turbid areas and overcast days made it difficult to locate manta rays. The majority of observed manta ray feeding aggregations occurred just after high tide, except during winter at West of Pylons (WP) during the crab spawn, when the tide was almost high, however plankton was dense therefore manta rays most likely continued feeding until after high tide.

3.2. Biomass

Seasons

Mean biomass was 0.021 g/m^3 in autumn and higher in winter with a mean of 0.036 g/m^3 (Figure 3), but was not significantly different between seasons (χ^2 = 0.690, (44), P > 0.05).

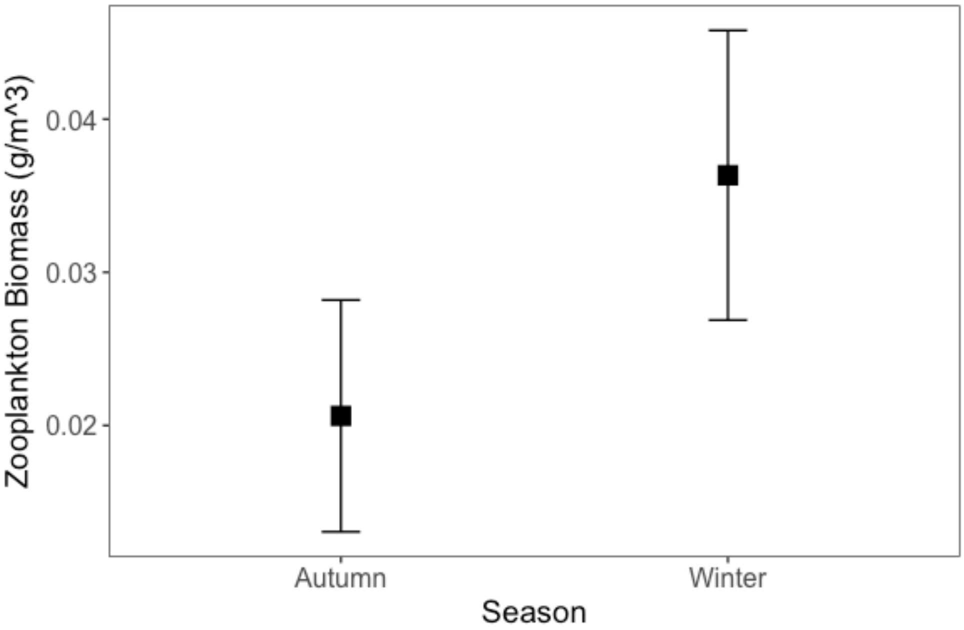

Figure 3. **Zooplankton Biomass per Season.** Mean biomass (g/m^3) of zooplankton for both seasons at Bateman Bay.

Sites

Biomass showed some variability between sites (Figure 4) with the highest mean value (0.041 g/m^3) at West of Pylons (WP) site in winter. The lowest mean value (0.012 g/m^3) was in autumn at the North Reef site. However, there was no significant difference between sites.

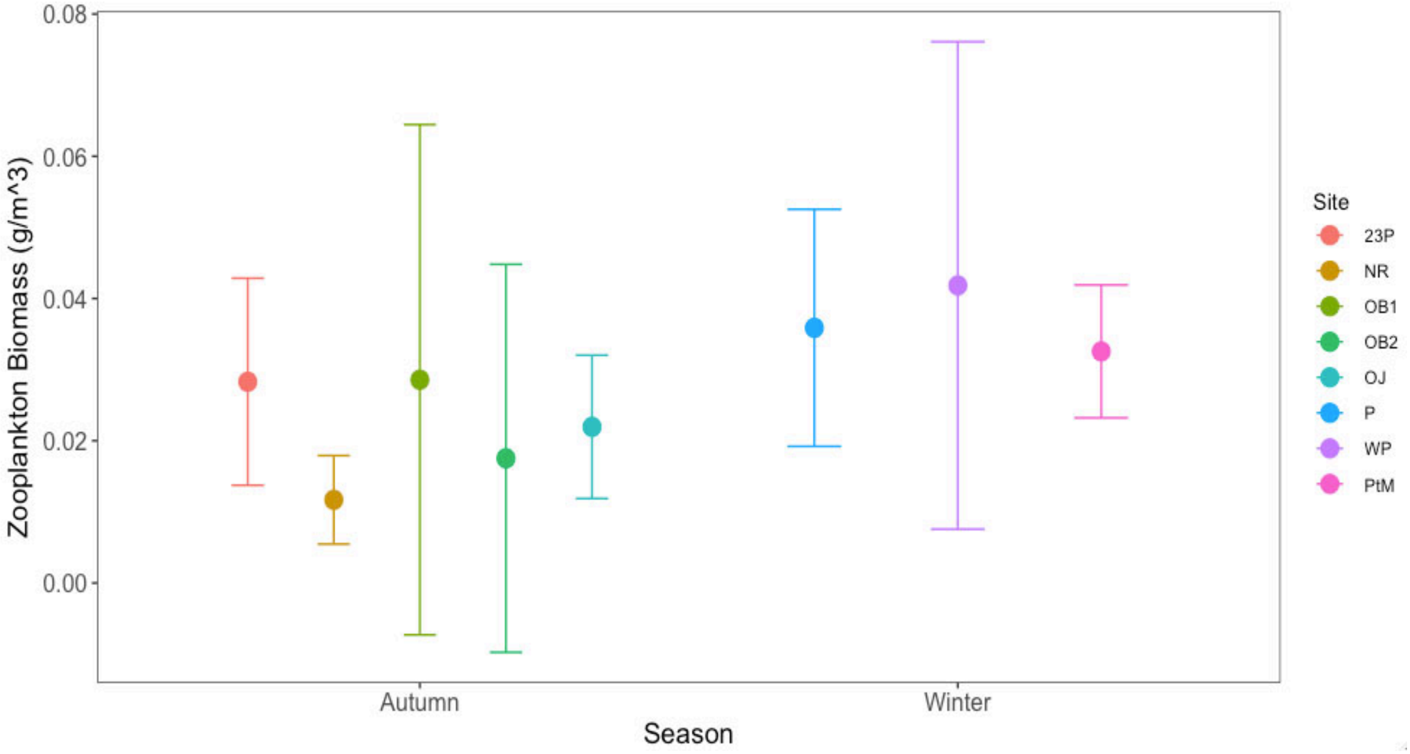

Figure 4. Zooplankton Biomass per Site. Mean weight (g/m^3) of zooplankton biomass for all sites and seasons. Sites ordered from north to south of Bateman Bay (Sites are: 23P- 23rd Parallel, NR- North Reef, OB1- Oyster Bridge background sample, OB2- Oyster Bridge 2, OJ- Old Jetty, P- Passage, WP- West of Pylons, PtM- Point Maud).

3.3. Carbon: Nitrogen Ratio

The mean C:N ratio was 3.66 for autumn and 8.86 for winter (Figure 5), which showed a significant difference between seasons ($\chi^2 = 35.65$, (11), $P < 0.01$).

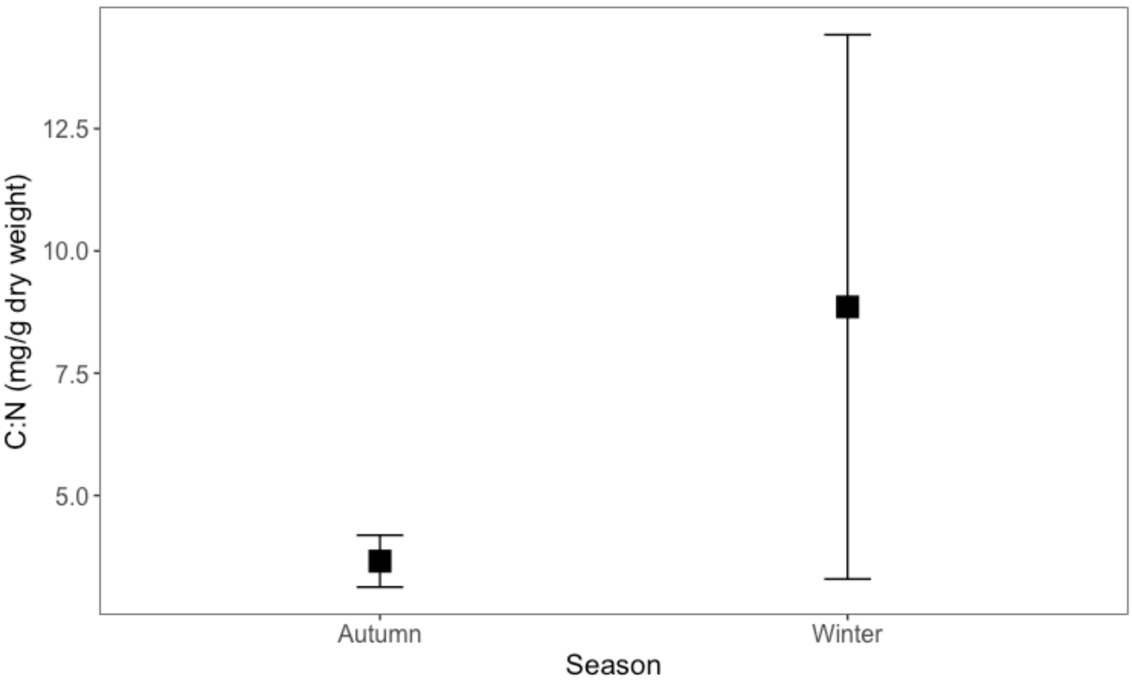

Figure 5. C:N Ratio per Season. Mean (±SE) C:N ratio (mg/g dry weight) of zooplankton sampled in autumn and winter.

Mean total organic carbon was 30.70% of dry weight of the samples for autumn, and 16.63% for winter, while mean total nitrogen content was 8.85% of the samples for autumn, and 3.02% for winter.

3.4. Nutritional Components

Carbohydrates formed the largest mean contribution for all samples (60.89%), followed by protein (31.22%), then lipids (4.39%), with the remaining 3.5% being an estimate of ash content from the salinity of seawater (35ppt). Carbohydrates were higher than other organic components for both seasons and all sites except for North Reef (NR) in autumn, where protein was highest (82.02%). Lipid percentages were low over both seasons and all sites.

Seasons

All components showed significant differences between seasons (Table 2). The mean protein and lipid content was higher in autumn than winter (Table 3), while carbohydrates were higher in winter than autumn (Table 3 and Figure 6). Even though higher in autumn, lipids were low for both seasons (Figure 6).

Table 2. Chi-square results of protein, carbohydrate and lipid proportions for both seasons.

Component	Test Statistic	Degrees of Freedom	Critical Value	P-value
Carbohydrate	390.83	39	54.53	<0.01
Protein	941.07	39	56.94	<0.01
Lipid	72.26	37	52.19	<0.01

Table 3. Mean proportions (%) of all organic components for each season sampled.

Season	Carbohydrate	Protein	Lipid	Ash	Total
Autumn	50.81	39.30	6.39	3.5	100
Winter	79.56	15.28	1.66	3.5	100

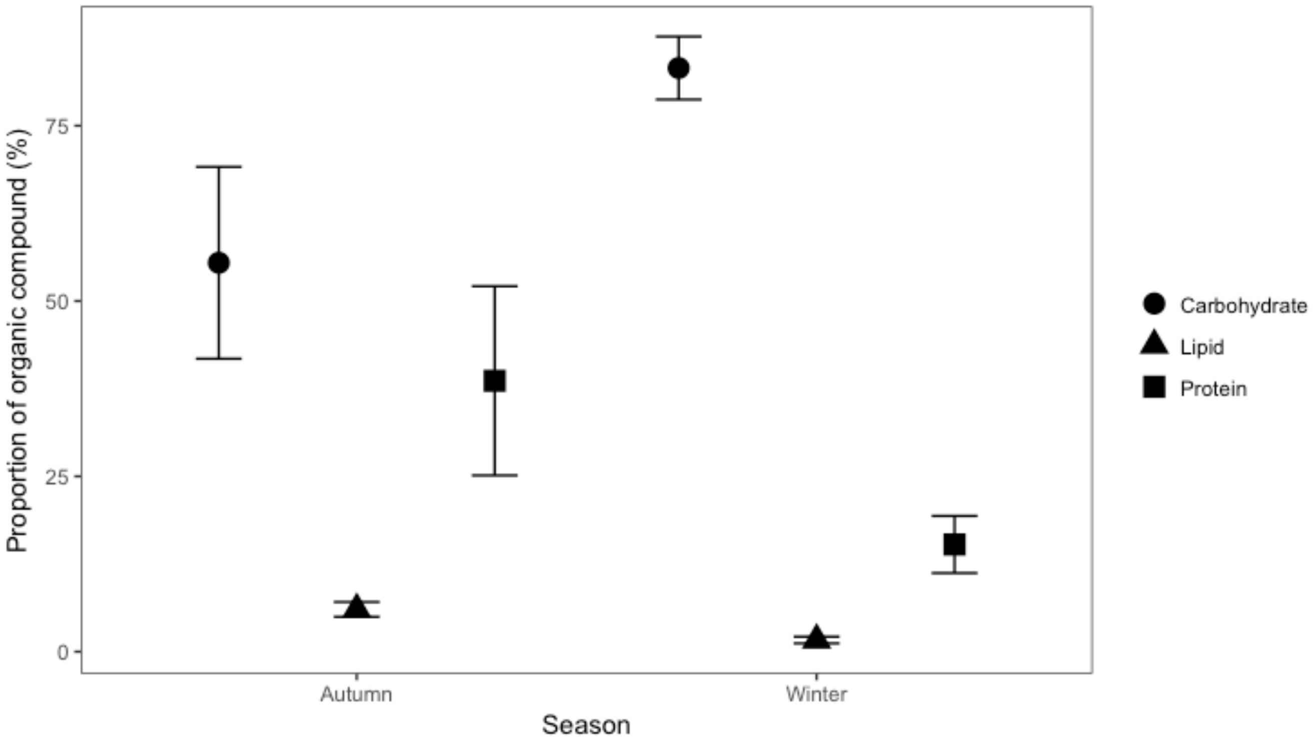

Figure 6. Proportion of Organic Components per Season. Mean proportion (%±SE) of organic components (lipids, protein, carbohydrates) of zooplankton sampled in autumn and winter.

Sites

Carbohydrates contributed the highest percentage at all sites, except North Reef (NR) where protein had the highest percentage (Figure 7). Carbohydrates and protein contributed similar proportions at 23rd Parallel (23P). When protein was high, carbohydrates were low, and vice versa; except at 23P where they did not greatly vary. North Reef and 23P showed the highest variability in protein and carbohydrate weight compared to the other sites (Figure 7). Lipids had the lowest proportion at all sites, while protein remained fairly constant over all sites, except NR where it showed a higher proportion (Figure 7).

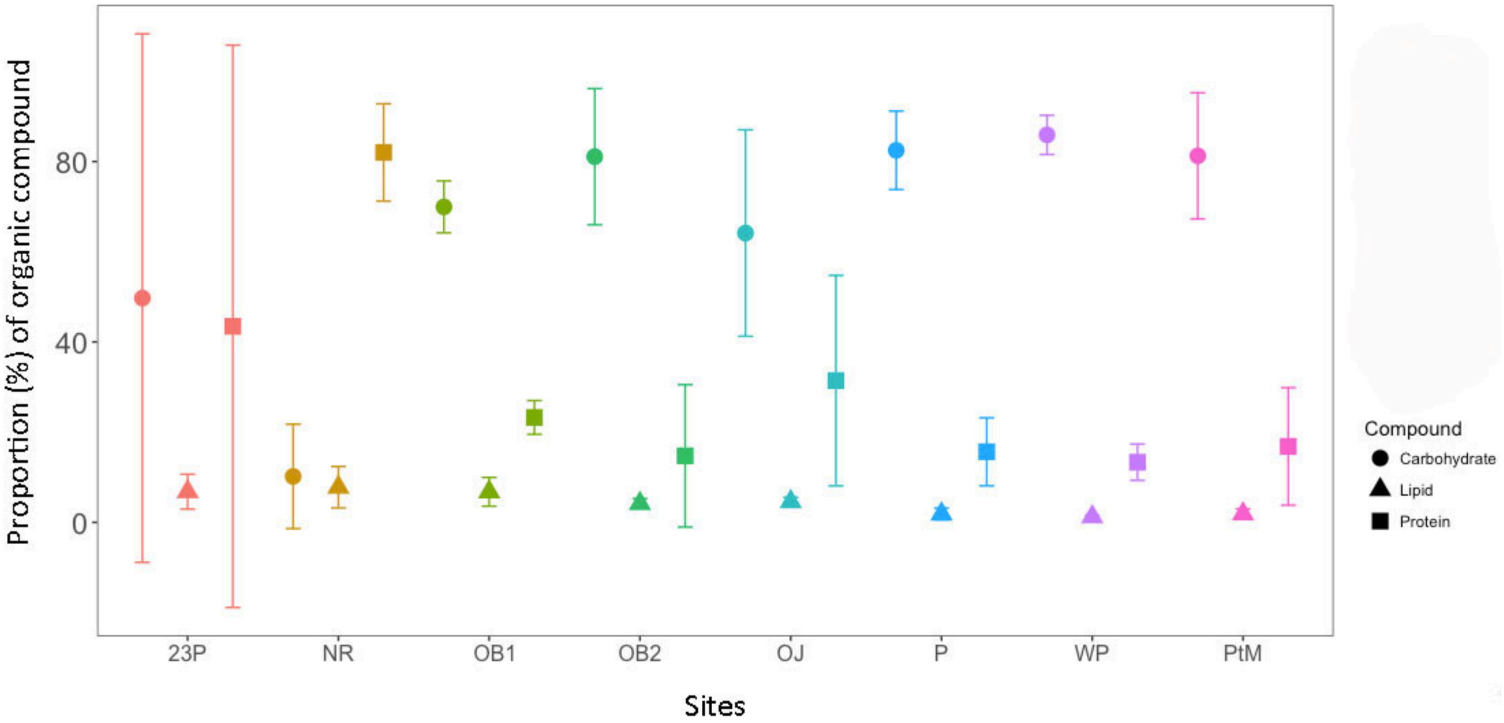

Figure 7. Proportions of Organic Components per Site. Mean proportion (±SE) of organic components (lipids, Protein, carbohydrates) for all sites. First five sites (23P, NR, OB1, OB2 and OJ) are from autumn, while the last three sites (P, WP and PtM) are from winter. Sites are represented by colour and are in order from north to south, while components are represented by shape.

3.5. Link to Manta Ray Feeding Aggregations

No real correlation was found between nutritional value and feeding manta ray abundance. Manta rays were observed feeding at NR, when protein contributed the highest nutritional percentage (Table 4). They were also observed feeding when protein was high as 23P and OJ. The first day Oyster Bridge was sampled (OB1) was intended as a background sample as no manta rays were feeding during time of sampling. However, a high amount of protein was found in these samples compared to when manta rays were feeding the next day this site was sampled (OB2).

No correlation was found between manta ray abundance and zooplankton biomass (Table 4), however, the largest feeding aggregation was observed at WP, when zooplankton biomass was highest (Table 4).

Table 4. Summary of mean biomass values (g/m^3), mean organic component proportions (%) and manta rays feeding at time of sampling, for each season and site.

Season	Site	Biomass (g/m^3)	Lipids (%)	Protein (%)	Carbohydrates (%)	Manta Rays feeding
Autumn	23P	0.0283	6.80	43.47	46.23	4
Autumn	NR	0.0117	7.80	82.02	6.69	8
Autumn	OB1	0.0286	6.78	36.64	53.08	0
Autumn	OB2	0.0175	4.17	14.75	77.58	4
Autumn	OJ	0.022	4.58	31.42	64.86	2
Winter	P	0.0359	1.86	15.65	78.99	0
Winter	WP	0.0418	1.22	13.34	81.93	15
Winter	PtM	0.0326	1.89	16.84	77.77	0

4. Discussion

The aims of this project were to quantify the seasonal nutritional components of mixed zooplankton populations around Bateman Bay, Ningaloo Reef. Zooplankton was sampled where reef manta rays (*Mobula alfredi*) were observed feeding, to discern whether there was any relationship between nutritional quantities and manta ray abundance. It was hypothesised that nutritional quality be higher during autumn than winter, when an abundance of nutritionally rich crustaceans dominates zooplankton assemblages.

4.1. Environmental Variables and Observations

Different community structures can be found with different seasons, as environmental variables such as light, temperature and turbidity vary seasonally (Moncheva *et al.* 2003). Seasonal changes in the environment can influence zooplankton reproduction, growth, population size, niche requirements and for some species can change the timing and duration of seasonal dormancy (Ji *et al.* 2010). Environmental conditions also do not perfectly repeat every season and year, as the environmental conditions and organism responses constantly vary (Ji *et al.* 2010). Only two seasons were sampled in this study, and there was no significant difference in environmental variables. Other studies have found greater variations in zooplankton abundance, distribution, species and chemical composition in spring and summer (Choe *et al.* 2003; Coyle *et al.* 2008; Verlinden 2010; Gomez *et al.* 2017), as warmer temperatures encourage phytoplankton blooms, which support zooplankton populations (Coyle *et al.* 2008). Even though the Ningaloo system generally favours downwelling, which negatively affects nutrient levels (Hanson *et al.* 2005), transient coastal upwelling can occur north of Bateman Bay during warmer months. Here the LC flows weakest and strong episodic winds are able to change the direction of the along-shelf current from south to north, increasing productivity and nutrient levels (Zhang *et al.* 2016). If this study were to be repeated, also sampling spring and summer would be beneficial to see an annual environmental cycle, and the influences it may have on planktonic productivity.

It is widely recognised that pelagic marine organisms are influenced by a variety of oceanographic and atmospheric variables (Sleeman *et al.* 2010). The importance of these variables is relative to each species, and is dependent on the spatial scale on which these processes operate and the functional importance of this scale to the animal (Sleeman *et al*. 2010). Variations in zooplankton phenology are frequently associated with environmental anomalies; water temperature being a major factor (Mackas *et al.* 2012). Sea surface temperature was approximately 4°C lower at Bateman Bay in winter than autumn, which could affect the zooplankton species composition and distribution. A higher abundance of calanoid copepods was observed during autumn, suggesting the water temperature was appropriate for these particular organisms. During the lower winter temperatures fewer calanoid copepods were present; instead, harpacticoid copepods were present, which were not seen in the autumn samples. Temperature has a significant influence on copepod development, reproduction and distribution, and they rely on these environmental cues to reproduce and seek out food resources (Breteler *et al.* 1995). Some copepods are able to respond to density gradients and food availability, displaying hierarchical foraging behaviours, suggesting they are influenced by oceanographic changes, which can lead to a patchy plankton distribution (Woodson *et al.* 2005). This implies copepods are able to pick up on oceanographic cues in order to locate areas abundant with food (McManus and Woodson 2012).

Zooplankton respond to their surrounding physical environment, which influences their distribution (Lumeran 2016), and their populations can vary temporally within days to years (Mackas *et al.* 2012). The spatial distribution is determined by interactions between environmental physical processes and the organisms' swimming and behavioural characteristics (McManus and Woodson 2012). Plankton behaviour can vary from passive particles to actively being able to determine their position in the water column (McManus and Woodson 2012). The distribution of plankton within Bateman Bay appeared to be influenced by the dominant currents, and depending on the swimming ability of the organisms, current strength, and prevailing swell, would form a surface slick or sink below the surface.

Zooplankton are susceptible to the seasonal fluctuations of the upper-ocean, such as currents, storms, wind and temperature (Mackas *et al.* 2012). These variations can be extreme and persistent, influencing seasonal population sizes and determining reproduction, growth and dormancy in zooplankton communities (Mackas *et al.* 2012). Ocean currents and prevailing winds were stronger during winter, which could influence the plankton distribution around Bateman Bay. The local current ran in a northerly direction for both seasons, where greater biomass was found at the most northern site (23P) during autumn and at the West of Pylons site in winter, as portunid crab larvae were transported northwards towards the passage. Zooplankton could have been transported from Bateman Bay outside the reef due to strong winter winds and current, where perhaps a larger number of planktivorous fish could be feeding. The local spotter plane reported manta ray feeding aggregations outside the reef on two days during the winter field trip, however due to the large swell, it was not practical to take samples.

A different species composition was observed towards the reef crest than inside the bay. An abundance of chaetognaths and appendicularians were present outside the reef crest and at NR. Chaetognaths mainly prey upon copepods and fish larvae, and can have an impact on zooplankton and fish species composition (De Souza *et al.* 2014), which could explain the different composition observed at 23P. Appendicularian abundance is correlated with water temperature (Uye and Ichino 1995), whereas copepod distribution is related to food availability (Folt and Burns 1999). Zooplankton species were not quantified in this study; however, a higher abundance of copepods was observed inside the bay during autumn. Temperature was slightly warmer at NR and 23P than the sites closer to the beach, which could affect species composition.

The biological productivity on the Ningaloo Reef fluctuates with the strength of the Leeuwin Current, as it influences changes in the food web and availability of prey (Sleeman *et al.* 2010). As the Leeuwin Current slows down from September to April, the cooler northward flowing Ningaloo Current takes over, strengthened by the south-westerly winds. This current is believed to influence coastal upwelling in the region due to Eckman transport (Holloway and Nye 1985; Taylor and Pearce 1999; Holloway 2001). Therefore, if this study were to be conducted in the future, also sampling spring and summer, could provide greater variation in food web composition due to the changes in dominant oceanographic processes.

4.2. Biomass

Biomass was found to be highest at WP, which coincided with the largest feeding aggregation observed in this study. Carbohydrates were also high, while protein and lipids were low, suggesting manta rays preferred quantity and carbohydrates, over energetic quality in this particular circumstance. Armstrong *et al.* (2016) found manta rays at LEI feeding when biomass was highest, which was in February. Biomass in this study was not significantly different between the seasons sampled, however other studies (Taylor and Pearce 1999; Tsikliras *et al.* 2010; Armstrong *et al.* 2016; Gilmour *et al.* 2016), have found biomass to be higher in spring and summer. Therefore, perhaps also sampling spring and summer could show greater variation between seasonal biomass. There is a strong intraseasonal fluctuation in plankton biomass, which is affected by upwelling periods, times of downwelling or relaxation periods (Gomez *et al.* 2017). Increased solar radiation enhances the response of phytoplankton to increased nutrients during upwelling periods (Gomez *et al.* 2017). Warmer temperatures promote phytoplankton growth and provide more appropriate conditions for many species to reproduce (Taylor and Pearce 1999; Findlay *et al.* 2006; Tsikliras *et al.* 2010; Gilmour *et al.* 2016). Due to the constantly changing environmental variables, biomass fluctuates seasonally and regionally, which is supported by other studies where biomass is variable across different regions (Bhat *et al.* 1993; Motta *et al.* 2010; Armstrong *et al.* 2016).

Body-size and temperature also affect plankton biomass, which could explain variations in productivity (Sterner and Schulz 1998). Tide appeared to influence zooplankton biomass as manta rays were mostly observed feeding on zooplankton after high tide, confirming reports by Wiafe and Frid (1996), who found zooplankton accumulates during high tide. This is different from findings at the southern GBR, where higher zooplankton biomass was observed before low tide (Armstrong *et al.* 2016). Tides have been found to have significant influences on manta ray sightings (Rohner *et al*. 2013b), as the zooplankton is distributed according to the tides, so the planktivores match their distribution to that of their prey. Tide and temperature had a significant influence on zooplankton biomass at LEI and manta rays were observed foraging when biomass was at its highest (Armstrong *et al*. 2016).

A prey density threshold of 11.2 mg/m^3 was calculated for reef manta rays at LEI (Armstrong *et al.* 2016). The mean biomass from all samples in this study was over this critical threshold. If the population of manta rays on the Ningaloo Reef require a similar threshold, this suggests there was enough plankton present during autumn and winter. However, perhaps the species composition or nutritional quality was not adequate when manta rays were not feeding. A larger proportion of phytoplankton and detritus was observed in the winter samples, which would affect the biomass. Therefore, if this study were to be repeated in the future, quantifying the species composition alongside biomass and nutritional quality would be beneficial to see whether manta rays need a certain nutritional threshold as well as density.

4.3. Carbon: Nitrogen Ratio

Zooplankton residing in tropical and subtropical regions generally contain a much lower proportion of carbon than those at high-latitudes (Ikeda and McKinnon 2011). Zooplankton in polar regions can contain up to 70% organic carbon, and a C:N ratio of up to 10, as planktonic organisms accumulate large lipid stores, rich in carbon (Ikeda 1974). However, zooplankton in tropical and subtropical oceans do not accumulate lipid deposits, therefore contain less carbon (<40%) and a C:N ratio of approximately 3-4 (Ikeda 1974; Ikeda and McKinnon 2011). Carbon and nitrogen proportions on the GBR were found to be 38.7% organic carbon and 10.3% nitrogen, with a C:N ratio of 3.8 (Ikeda and McKinnon 2011). The

C:N ratio found at Bateman Bay in autumn had a mean value of 3.7, which is consistent with other studies in low latitudes (Ikeda 1974; Gorsky *et al.* 1988; Ikeda and McKinnon 2011).

The C:N ratio found in winter was relatively high for this sub-tropical region, at 8.9. Even though the ratio was high, the mean percentage of total organic carbon present in winter was 16.63%, characteristically low of oligotrophic waters. High C:N ratios also generally indicate larger lipid stores, which was not found in this study, where lipid content was very low in winter. However, oligotrophic zooplankton generally have low lipid stores and lower carbon, as they do not accumulate lipid reserves. The increase in C:N ratio indicates a high amount of phytoplankton was present during winter, which is consistent with the higher amount of carbohydrates found within the samples, and characteristic of phytoplankton. The high C:N ratio observed in winter can perhaps be explained by the small sample size. A large amount of variance was observed between sites and sample days during winter, which is reflected in the large standard error. Therefore, more data for this season would be beneficial, to establish whether the high C:N ratio in winter is typical.

Different species experience variations in chemical composition due to different life strategies and fluctuations in their surrounding environment (DeLorenzo Costa *et al.* 2006). C:N ratios are positively correlated with lipid content (Delorenzo Costa *et al*. 2006); low carbon composition indicates high amounts of protein, but low lipid content, which is consistent with the results found in autumn in this study. Omnivorous zooplankton have considerably higher C:N ratios than herbivorous species (Delorenzo Costa *et al*. 2006), suggesting there were also more omnivorous species present, alongside phytoplankton during winter, according to the higher C:N ratio found. Also, species that do not exhibit diapause are more likely to adopt an omnivorous feeding strategy (Lee *et al.* 2006). Higher C:N ratios suggest there is a low amount of nitrogen in the zooplankton, resulting in lower levels of protein (Ikeda 1974). The high C:N ratio found in winter, along with lower levels of protein, suggests low nitrogen content, reflected by a mean nitrogen percentage of 3.02%.

C:N ratios were found to increase significantly during spring in other studies, where local phytoplankton blooms have caused an increase of carbon into the system (Escribano *et al.* 2007; Kamburska and Fonda-Umani 2009; Verlinden 2010). Therefore, it would be beneficial to also sample spring and summer to establish whether there is an increase of C:N ratio during warmer temperatures, and whether it coincides with foraging planktivores such as manta rays. It is likely that the Leeuwin Current and local oceanography around Bateman Bay would influence the influx of phytoplankton to the region, altering the C:N ratio of the zooplankton.

4.4. Nutritional Components

A different biochemical composition was found at the outer reef sites compared to inside the bay, as waves from the outer reef bring in richly oxygenated waters, which flow back out via gaps in the reef crest (Taylor and Pearce 1999; Sleeman *et al.* 2007). Plankton swarms have been observed within 50 m of the shore within Bateman Bay, and offshore in deeper channels. The Mixed Layer Depth deepens in autumn, as the Leeuwin Current increases in strength, causing an increase in surface nutrients and chlorophyll-*a* concentration (Rousseaux *et al*. 2012), which could be the reason why protein and lipid proportions were higher during autumn.

Lipids

The amount of lipids within the zooplankton was very low at all sites and both seasons when compared to other studies (Harris *et al*. 2000; Hedges *et al*. 2002; Verlinden 2010), implying the zooplankton population around this time of year at Bateman Bay has poor energy stores. Lipid percentages were significantly higher during autumn (6.39%), meaning more nutritionally rich plankton, however were still <10%, which is low according to some studies (Harris *et al*. 2000; Hedges *et al*. 2002; Choe *et al*. 2003; Verlinden 2010).

High lipid content is generally characteristic of high-latitude and temperate plankton, as they undergo seasonal diapause during the autumn and winter months (Lee *et al.* 2006). It is necessary for these zooplankton to accumulate large lipid stores needed for reproduction and energy in periods of low productivity (Lee *et al.* 2006). However, it is unlikely that zooplankton communities around Bateman Bay would enter a diapause phase, as most studies describe species in high-latitudes (Donnelly *et al.* 1994; Fiksen 2000; Lee *et al.* 2006), and few tropical species have been observed exhibiting diapause. This is most likely due to the sea surface temperature in tropical regions, which has a relatively small seasonal change, unlike temperate or polar waters. Tropical zooplankton, including gelatinous species, usually do not accumulate lipid stores, resulting in low lipid content (Lee *et al.* 2006), which would explain the low lipid content found in this study. The low lipid content may also be due to relatively high primary production, where sufficient food is available year round to the zooplankton, therefore removing the need to accumulate lipid stores (Jo *et al.* 2016). Oligotrophic waters generally support lipid-poor organisms due to the high turn-over of zooplankton and their high metabolic rate (Kattner *et al.* 2007).

During summer an anti-clockwise eddy forms off the LC, south of Point Cloates, increasing nutrients in the Ningaloo region. The eddy has the potential to transport biota into the lagoon area via the Ningaloo Current (Taylor and Pearce 1999). Eddies are important in oligotrophic systems as they enhance productivity and nutrients in the region (Lebourges-Dhaussy *et al.* 2014). As a result, perhaps lipid content would increase within the zooplankton during summer. However, sampling was not conducted in summer in this study, which may explain the low lipid proportions, as downwelling is more likely during cooler months due to the southerly direction and strength of the LC.

Each site had a noticeably different species composition, which influences nutritional proportions; different species store varying amounts of biochemical components, and therefore have variable nutritional value (Jaspers *et al.* 2014). Herbivorous zooplankton generally has higher lipid stores than carnivorous species (DeLorenzo Costa *et al.* 2006), suggesting a higher abundance of carnivorous zooplankton may have been present in the zooplankton around Bateman Bay, during autumn and winter. Other studies suggest lipid proportions peak during spring (Hedges *et al*. 2002; Verlinden 2010), therefore additional sampling in spring could show higher lipid proportions around Bateman Bay.

Protein

Protein levels were high in both seasons sampled, and significantly higher during autumn. Zooplankton generally consists of 10-50% protein (Harris *et al*. 2000), which is consistent with the values in this study (15.28% in winter and 39.30% in autumn). The high protein content of zooplankton recorded in this study in autumn might be related to the abundance of copepods, as copepods and euphausiids are particularly high in protein (Murphy *et al.* 2016; Jo *et al.* 2016). High proportions of protein have been reported where copepods have been the dominant species (Jagadeesan *et al.* 2010), and the higher protein levels observed in this study were found at sites where copepods were present (i.e. OB, NR and OJ). An abundance of calanoids was observed during autumn, and harpacticoids during winter, which could contribute to the differences in protein levels. Greater amounts of protein within zooplankton are generally observed when species are highly productive (Percy 1979), which could also explain the protein quantities observed in autumn for Bateman Bay, as warmer temperatures are favoured by many species for reproduction. Female copepods also have lower lipid content after reproducing (Lee *et al.* 2006), which supports the high amount of protein but low amount of lipids, if they were reproducing. In support of these findings, protein was also found to contribute the highest percentage of biochemical components in various other studies (Bhat *et al.* 1993; Krishna Kumari and Goswami 1993; Hedges *et al.* 2002; Choe *et al.* 2003; Jagadeesan *et al.* 2010).

Tropical plankton communities tend to store large amounts of protein, however have lower energetic value than those at high latitudes, as they don’t accumulate large lipid stores. Individuals within the zooplankton will also have varying degrees of nutritional components, at different times of their life phases, resulting in seasonal variation depending on reproduction and spawning events (Guzmán *et al.* 2016; Bascur *et al.* 2017). Each site and season showed variations in species composition, supporting the variability of protein percentages.

March through to April each year is when the corals spawn on the Ningaloo Reef, coinciding with visiting whale sharks. Protein and other nutrients increase within the region as the Ningaloo Current transports spawn and larvae throughout the system (Taylor and Pearce 1999). Therefore, there could still have been spawn and larvae within the region during May when we sampled the area. Even though copepods are predominantly herbivorous, they have been observed feeding on fish eggs and larvae (Turner *et al.* 1985; Gliwicz *et al.* 1993). Therefore, it can be assumed that some species also feed upon coral spawn; it is likely that their reproduction is timed to match this rich food supply, therefore boosting copepod abundance. Furthermore, if additional sampling were completed during the spawning time, one could expect an increase in protein and other nutrients during this time.

Carbohydrates

Carbohydrate content was high within the zooplankton for both seasons and all sites except North Reef, where it was low (6.69%). Carbohydrates within zooplankton are usually found to be <10% (Romankevich 1984), therefore the high carbohydrate content in this study (50.81% in autumn and 79.56% in winter) is quite unusual. Chitin, a type of carbohydrate, forms the exoskeletons of crustaceans (Harris *et al*. 2000), of which there were many in the winter samples. Other studies have separated chitin from other carbohydrates (Raymont *et al.* 1964; Donnelly *et al.* 1994), which was not done in the current study. An abundance of crustacean carapaces was observed in the samples, which would explain the high carbohydrate content. The composition at NR mainly consisted of chaetognaths, appendicularians and eggs which do not have an exoskeleton, thus low in carbohydrates.

Carbohydrates can contribute over 50% of phytoplankton composition (Jo *et al.* 2016). Hence, if the majority of the composition was made up of phytoplankton, which was observed in the winter samples, this would explain the high percentages (79.56% in winter and 50.81% in autumn). Carbohydrate sugars, oligosaccharides and polysaccharides, are found in abundance in phytoplankton cell walls, therefore the winter samples are most likely comprised of a large amount of phytoplankton and crustacean exoskeletons. High carbohydrates could also be attributed to local phytoplankton blooms, which would explain if there was a high amount of phytoplankton in the composition. Mature copepods also have a high amount of carbohydrates (Choe *et al.* 2003), which were present during both seasons.

The highest percentage of carbohydrates was at the WP site in winter, where the composition was almost all portunid crab larvae, whose exoskeletons would contribute to the high carbohydrate content. All the sites with highest carbohydrate percentages were areas where a lot of crustaceans were observed in the samples, apart from winter, where a larger proportion of samples included phytoplankton.

4.5. Link to Manta Ray Feeding Aggregations

Manta rays are able to adjust their feeding strategies to the life history of their prey (Couturier *et al.* 2013), which has been suggested by this study. Manta rays appear to target specific spawning events of planktonic crustaceans, and they are observed aggregating seasonally to Bateman Bay, to forage on these particular assemblages (McGregor pers. comm.). They have been observed feeding on the surface or sea floor during the day, and satellite-tracked manta rays have shown they spend long periods of time at depth, which could be associated with foraging activities (Jaine *et al.* 2014). It has been suggested that manta rays feed on demersal plankton, which has larger individuals than pelagic plankton, thus a greater biomass (Couturier *et al*. 2013). *Mobula alfredi* reside within coastal regions during the day, where they surface feed and receive warmth from sunlight; but mounting research suggests they obtain the majority of their food at depth, when they forage at night, presumably where they receive most of their nutritional needs (Dewar *et al.* 2008; Rohner *et al.* 2013a; Jaine *et al.* 2014; Stewart *et al.* 2016). Manta ray feeding aggregations are often seen at the surface, however the food consumed during these short pulses of plankton may not be enough to sustain such a large animal. Hence, a large proportion of their diet may be sourced from depth

(Couturier *et al*. 2013). Few manta rays were found feeding during winter, suggesting they were foraging elsewhere, presumably following the nutritionally rich zooplankton. Nutritional value of plankton was found to be less in winter, and more phytoplankton present, perhaps explaining why manta rays were not as abundant.

Upwelling is more common north of Bateman Bay, therefore manta rays could be travelling up and down the coast searching for pockets of plankton. Depending on the zooplankton distribution, they could be foraging at night, when some species of crustaceans migrate vertically from depth (West 2013). This winter had an uncommonly low number of manta ray feeding aggregations (McGregor pers. comm. 2017), therefore sampling consecutive years as a comparison would be beneficial. Episodes of increased planktonic biomass, generally coincide with planktivorous feeding aggregations, therefore the animals could be foraging elsewhere (Rohner *et al.* 2013a; Bennett *et al.* 2016).

Fluctuations of environmental variables were not found to have any particular effect on manta ray presence, perhaps due to insufficient variation between the seasons sampled. However, animals in higher trophic levels greatly depend on synchronizing their movements with seasonal pulses of primary production in order to obtain sufficient food resources (Ji *et al.* 2010). Changes in environmental conditions can cause a divergence in this synchronization between trophic groups, resulting in limited food availability, hence a decline in recruitment of the organisms that rely upon that particular food resource (Edwards and Richardson 2004). Changes in copepod reproduction and growth can result in an inadequate supply of lipids needed for energy stores in higher trophic animals (Ji *et al.* 2010). Zooplankton have evolved to exploit patches of dense prey as they undertake vertical migrations, which planktivores follow. Changes in density caused by variations in temperature and salinity influence the spatial confines of vertical migration (McManus and Woodson 2012). Larger predators undertake vertical and horizontal migrations in order to exploit the patchiness and nature of how zooplankton is dispersed (McManus and Woodson 2012).

Whale sharks aggregate to Ningaloo between March and May every year (Taylor 1996; Wilson *et al.* 2001), coinciding with high numbers of manta ray sightings. The occurrence of these planktivores is believed to coincide with productivity events, as they aggregate to feed upon the abundant supply of food (Sleeman *et al.* 2010). Due to the high protein content of copepods (Wang *et al.* 2015), they are generally targeted by planktivorous fish feeding aggregations (Armstrong *et al.* 2016; Bennett *et al.* 2016). This was also observed in this study: where manta rays were feeding, there was generally a large proportion of copepods within the samples.

Sampling exactly where the manta rays were feeding proved difficult in certain areas, i.e. when they were feeding in very shallow water adjacent to the beach at Oyster Bridge. Some samples in these areas showed high variability in zooplankton abundance, and nutritional quantities proved to be far less than expected due to abundance of copepods observed in some samples. This shows that the zooplankton had a very patchy distribution, which was at times difficult to follow. This patchiness was also observed at North Reef, where an abundance of krill, chaetognaths and appendicularians was sampled one day alongside eight feeding manta rays. Two days later the same site was sampled, and only one manta ray was observed feeding, whilst the samples yielded far less zooplankton but more phytoplankton. These observations show that manta rays appear to be sensitive to where the most abundant, nutritionally rich food resources are, and match their distribution to that of their preys (Barnett and Semmens 2012).

4.6. Recommendations for Future Studies on the Nutritional Quality of Zooplankton

Future studies should address spring and summer in addition to autumn and winter, in order to compare the annual nutritional variability. Ideally, sampling should span more than one year, to determine differences in environmental variables as seasonal changes do not perfectly repeat in the same way every year. Chlorophyll-*a* concentration can be useful in measuring phytoplankton productivity in the region (Rousseaux *et al.* 2012), therefore it is recommended that this also be quantified and related to zooplankton nutritional values. It has been suggested that zooplankton from depth provide greater nutrients and biomass to planktivorous fish such as manta rays, and they in fact forage at depth during the night when various planktonic organisms vertically migrate. Therefore, sampling zooplankton assemblages at depth as well as surface communities could be useful to compare any differences in nutritional value. While broad estimates of dominant zooplankton species were obtained during this study, quantifying the different species present in the zooplankton would be beneficial to discern if there are particular species that provide planktivores with a greater nutritional resource than others. Furthermore, quite a lot of variability was observed within the samples and between sites, reflected by the variation in nutritional component percentages; increasing the number of sample replicates would make the outcome more statistically robust.

Essential fatty acids are important for animal growth, survival and reproductive success (Sterner and Schulz 1998; Muller-Navarra 2008), therefore analysing lipids for different fatty acid classes would provide further detail into the nutritional values of the zooplankton. Polyunsaturated fatty acids (ω-3 and ω-6) are important for animals as they sustain membrane fluidity and tissue hormones (Muller-Navarra 2008), and certain fatty acids are transferred up the food chain to higher trophic consumers (Dalsgaard *et al.* 2003).

4.7. Conclusion

This study has provided some insight into the seasonal variation of zooplankton nutritional value at manta ray feeding grounds on the Ningaloo Reef, Western Australia. Zooplankton assemblages have a greater nutritional value during autumn, when warmer temperatures provided more appropriate conditions for certain planktonic organisms. Protein content was high for both seasons, which is consistent with other studies and typical of tropical zooplankton communities. Lipid quantities were very low for both autumn and winter, however characteristic of zooplankton within oligotrophic waters, as organisms do not undertake diapause, and therefore do not need to accumulate large lipid stores. Carbohydrates were uncharacteristically high for zooplankton, however could be explained by the large amount of crustaceans and phytoplankton within the plankton composition, which hold high carbohydrate value. The C:N ratio was significantly higher during winter, coinciding with the high amount of carbohydrates and phytoplankton; however autumn ratios were found to be consistent with other studies from oligotrophic regions.

Environmental variables such as temperature, tides, winds and currents influence the patchy distribution of zooplankton. A greater amount of seasonal environmental data is needed to statistically determine to what extent these factors affect zooplankton around Bateman Bay. Tide appeared to have a positive effect on zooplankton biomass and manta ray presence. In most cases, a higher abundance of manta ray feeding aggregations occurred when nutritional value was highest. Even though zooplankton species were not quantified in this study, observational data provided some understanding of the varying compositions present, due to the different environmental conditions of each site and season. Zooplankton biomass also appeared to affect manta ray foraging behaviour, as the greater biomass supported the largest feeding aggregation observed in this study, however nutritional value was lower than other sites. It appears that manta rays match their distribution to the zooplankton, however additional year-round sampling is required to better understand these preliminary findings.

References

Anderson, R. C., Adam, M. S., and Goes, J. I. (2011). From monsoons to mantas: Seasonal distribution of Manta alfredi in the Maldives. *Fisheries Oceanography* **20**, 104–113. doi:10.1111/j.1365-2419.2011.00571.x

Ari, C., and Correia, J. P. (2008). Role of sensory cues on food searching behavior of a captive Manta birostris (Chondrichtyes, Mobulidae). *Zoo Biology* **27**, 294–304. doi:10.1002/zoo.20189

Armstrong, A. O., Armstrong, A. J., Jaine, F. R. A., Couturier, L. I. E., Fiora, K., Uribe-Palomino, J., Weeks, S. J., Townsend, K. A., Bennett, M. B., and Richardson, A. J. (2016). Prey density threshold and tidal influence on reef manta ray foraging at an aggregation site on the Great Barrier Reef. *PloS one* **11**, e0153393. doi:10.1371/journal.pone.0153393

Bakun, A. (2006). Fronts and eddies as key structures in the habitat of marine fish larvae: opportunity, adaptive response and competitive advantage. *Scientia Marina* **70S2**, 105–122. doi:10.3989/scimar.2006.70s2105

Barnett, A., and Semmens, J. M. (2012). Sequential movement into coastal habitats and high spatial overlap of predator and prey suggest high predation pressure in protected areas. *Oikos* **121**, 882–890. doi:10.1111/j.1600-0706.2011.20000.x

Barroeta, Z., Olivar, M. P., and Palomera, I. (2017). Energy density of zooplankton and fish larvae in the southern Catalan Sea (NW Mediterranean). *Journal of Sea Research* **124**, 1–9. doi:10.1016/j.seares.2017.04.008

Barton, A. D., Irwin, A. J., Finkel, Z. V., and Stock, C. A. (2016). Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities. *Proceedings of the National Academy of Sciences* **113**, 2964–2969. doi:10.1073/pnas.1519080113

Bascur, M., Guzmán, F., Mora, S., and Urzúa, Á. (2017). Seasonal changes in the biochemical composition of females and offspring of red squat lobster, Pleuroncodes monodon (Decapoda, Munididae), from the Southeastern Pacific. *Marine Ecology* **38**, 1–13. doi:10.1111/maec.12419

Batchelder, H. P., Mackas, D. L., and O'Brien, T. D. (2012). Spatial-temporal scales of synchrony in marine zooplankton biomass and abundance patterns: A world-wide comparison. *Progress in Oceanography* **97**–**100**, 15–30. doi:10.1016/j.pocean.2011.11.010

Beaugrand, G. (2009). Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. *Deep-Sea Research Part II: Topical Studies in Oceanography* **56**, 656–673. doi:10.1016/j.dsr2.2008.12.022

Bennett, M. B., Coman, F. F., Townsend, K. A., Couturier, L. I. E., Jaine, F. R. A., and Richardson, A. J. (2016). A historical and contemporary consideration of the diet of the reef manta ray (Manta alfredi) from the Great Barrier Reef, Australia. *Marine and Freshwater Research* **1935**, 993–997. doi:http://dx.doi.org/10.1071/MF16046

Bhat, K. L., Sreepada, R. A., and Ansari, Z. A. (1993). Biochemical composition of zooplankton from the northern arabian sea. *Pakistan Journal of Marine Sciences* **2**, 17–22.

Bopp, L., Monfray, P., Aumont, O., and Dufresne, J. (2001). Potential impact of climate change on marine export production. *Global Biogeochemical* **15**, 81–99. doi:10.1029/1999GB001256

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254. doi:10.1016/0003-2697(76)90527-3

Breteler, W. C. M. K., Gonzalez, S., and Schogt, N. (1995). Development of Pseudocalanus elongatus temperature and food conditions. *Marine Ecology Progress Series* **119**, 99–110. Available at: http://www.int-res.com/abstracts/meps/v119/p99-110/

Buchan, S. J., and Quiñones, R. A. (2016). First insights into the oceanographic characteristics of a blue whale feeding ground in northern Patagonia, Chile. *Marine Ecology Progress Series* **554**, 183–199. doi:10.3354/meps11762

Burgess, K. B., Couturier, L. I. E., Marshall, A. D., Richardson, A. J., Weeks, S. J., and Bennett, M. B. (2016). Manta birostris, predator of the deep? Insight into the diet of the giant manta ray through stable isotope analysis. *Royal Society Open Science* **3**, 160717. doi:10.1098/rsos.160717

Chavez, F. P., and Messié, M. (2009). A comparison of eastern boundary upwelling ecosystems. *Progress in Oceanography* **83**, 80–96. doi:10.1016/j.pocean.2009.07.032

Choe, N., Deibel, D., Thompson, R. J., Lee, S. H., and Bushell, V. K. (2003). Seasonal variation in the biochemical composition of the chaetognath Parasagitta elegans from the hyperbenthic zone of Conception Bay, Newfoundland. *Marine Ecology Progress Series* **251**, 191–200. doi:10.3354/meps251191

Conceição, L. E. C., Yúfera, M., Makridis, P., Morais, S., and Dinis, M. T. (2010). Live feeds for early stages of fish rearing. *Aquaculture Research* **41**, 613–640. doi:10.1111/j.1365-2109.2009.02242.x

Couturier, L. I. E., Jaine, F. R. A., Townsend, K. A., Weeks, S. J., Richardson, A. J., and Bennett, M. B. (2011). Distribution, site affinity and regional movements of the manta ray, Manta alfredi (Krefft, 1868), along the east coast of Australia. *Marine and Freshwater Research* **62**, 628–637. doi:10.1071/MF10148

Couturier, L. I. E., Marshall, A. D., Jaine, F. R. A., Kashiwagi, T., Pierce, S. J., Townsend, K. A., Weeks, S. J., Bennett, M. B., and Richardson, A. J. (2012). Biology, ecology and conservation of the Mobulidae. *Journal of Fish Biology* **80**, 1075–1119. doi:10.1111/j.1095-8649.2012.03264.x

Couturier, L. I. E., Rohner, C. A., Richardson, A. J., Marshall, A. D., Jaine, F. R. A., Bennett, M. B., Townsend, K. A., Weeks, S. J., and Nichols, P. D. (2013). Stable isotope and signature fatty cid analyses suggest reef manta rays feed on demersal zooplankton. *PLoS ONE* **8**. doi:10.1371/journal.pone.0077152

Coyle, K. O., Pinchuk, A. I., Eisner, L. B., and Napp, J. M. (2008). Zooplankton species composition, abundance and biomass on the eastern Bering Sea shelf during summer: The potential role of water-column stability and nutrients in structuring the zooplankton community. *Deep-Sea Research Part II: Topical Studies in Oceanography* **55**, 1775–1791. doi:10.1016/j.dsr2.2008.04.029

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., and Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* **46**, 225–340. doi:10.1016/S0065-2881(03)46005-7

Deakos, M. H., Baker, J. D., and Bejder, L. (2011). Characteristics of a manta ray Manta alfredi population off Maui, Hawaii, and implications for management. *Marine Ecology Progress Series* **429**, 245–260. doi:10.3354/meps09085

DeLorenzo Costa, A., Durbin, E. G., and Mayo, C. A. (2006). Variability in the nutritional value of the major copepods in Cape Cod Bay (Massachusetts, USA) with implications for right whales. *Marine Ecology* **27**, 109–123. doi:10.1111/j.1439-0485.2006.00087.x

Dewar, H., Mous, P., Domeier, M., Muljadi, A., Pet, J., and Whitty, J. (2008). Movements and site fidelity of the giant manta ray, Manta birostris, in the Komodo Marine Park, Indonesia. *Marine Biology* **155**, 121–133. doi:10.1007/s00227-008-0988-x

Donnelly, J., Torres, J., Hopkins, T., and Lancraft, T. (1994). Chemical composition of

Antarctic zooplankton during austral fall and winter. *Polar Biology* **14**, 171–183. doi:https://doi.org/10.1007/BF0024052

Dubinsky, Z., and Jokiel, P. L. (1994). Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. *Pacific Science* **48**, 313–324.

Edwards, M., and Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* **430**, 881–884. doi:10.1038/nature02808

Escribano, R., Hidalgo, P., González, H., Giesecke, R., Riquelme-Bugueño, R., and Manríquez, K. (2007). Seasonal and inter-annual variation of mesozooplankton in the coastal upwelling zone off central-southern Chile. *Progress in Oceanography* **75**, 470–485. doi:10.1016/j.pocean.2007.08.027

Fiksen, Ø. (2000). The adaptive timing of diapause - a search for evolutionarily robust strategies in Calanus finmarchicus. *ICES Journal of Marine Science* **57**, 1825–1833. doi:10.1006/jmsc.2000.0976

Findlay, H. S., Yool, A., Nodale, M., and Pitchford, J. W. (2006). Modelling of autumn plankton bloom dynamics. *Journal of Plankton Research* **28**, 209–220. doi:10.1093/plankt/fbi114

Fogarty, H., Burrows, M., Pecl, G., Robinson, L., and Poloczanska, E. (2017). Are fish outside their usual ranges early indicators of climate-driven range shifts? *Global Change Biology* **23**, 2047–2057. doi:http://dx.doi.org/10.1111/gcb.13635

Folt, C. L., and Burns, C. (1999). Biological drivers of zooplankton patchiness. *Trends in Ecology and Evolution* **14**, 300–305. doi:10.1016/S0169-5347(99)01616-X

Gadig, O. B. F., and Neto, D. G. (2014). Notes on the feeding behaviour and swimming pattern of Manta alfredi (Chondrichthyes, Mobulidae) in the Red Sea. *Acta Ethologica* **17**, 119–122. doi:10.1007/s10211-013-0165-1

Garzke, J., Ismar, S. M. H., and Sommer, U. (2015). Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance. *Oecologia* **177**, 849–860. doi:10.1007/s00442-014-3130-4

Gilmour, J., Speed, C. W., and Babcock, R. (2016). Coral reproduction in Western Australia. *PeerJ* **4**, e2010. doi:10.7717/peerj.2010

Gliwicz, Z. M., and Maszczyk, P. (2016). Heterogeneity in prey distribution allows for higher food intake in planktivorous fish, particularly when hot. *Oecologia* **180**, 383–399. doi:10.1007/s00442-015-3485-1

Gliwicz, Z. M., Stibor, H. (1993). Egg predation by copepods in Daphnia brood cavities. *Oecologia* **95**, 295–298. doi:10.1007/bf00323503

Gomez, F. A., Spitz, Y. H., Batchelder, H. P., and Correa-Ramirez, M. A. (2017). Intraseasonal patterns in coastal plankton biomass off central Chile derived from satellite observations and a biochemical model. *Journal of Marine Systems* **174**, 106–118. doi:10.1016/j.jmarsys.2017.05.003

Gorsky, G., Dallot, S., Sardou, J., Fenaux, R., Carré, C., and Palazzoli, I. (1988). C and N composition of some northwestern Mediterranean zooplankton and micronekton species. *Journal of Experimental Marine Biology and Ecology* **124**, 133–144. doi:10.1016/0022-0981(88)90116-5

Guzmán, F., Olavarría, L., and Urzúa, Á. (2016). Seasonal variation in reproductive parameters of the squat lobster Pleuroncodes monodon from a South Pacific population. *Invertebrate Reproduction & Development* **60**, 137–144. doi:10.1080/07924259.2016.1174155

Hanson, C. E., and McKinnon, A. D. (2009). Pelagic ecology of the Ningaloo region, Western Australia: Influence of the Leeuwin Current. *Journal of the Royal Society of Western Australia* **92**, 129–137.

Hanson, C. E., Pattiaratchi, C. B., and Waite, A. M. (2005). Sporadic upwelling on a downwelling coast: Phytoplankton responses to spatially variable nutrient dynamics off the Gascoyne region of Western Australia. *Continental Shelf Research* **25**, 1561–1582. doi:10.1016/j.csr.2005.04.003

Harris, R., Wiebe, P., Lenz, J., Skjoldal, H. R., and Huntley, M. (2000). 'ICES zooplankton methodology manual'. (San Diego Academic Press.)

Hedges, J. I., Baldock, J. A., Gelinas, Y., Lee, C., Peterson, M. L., and Wakeham, S. G. (2002). The biochemical and elemental compositions of marine plankton: A NMR perspective. *Marine Chemistry* **78**, 47–63. doi:10.1016/S0304-4203(02)00009-9

Heyman, W. D., Graham, R. T., Kjerfve, B., and Johannes, R. E. (2001). Whale sharks Rhincodon typus aggregate to feed on fish spawn in Belize. *Marine Ecology Progress Series* **215**, 275–282. doi:10.3354/meps215275

Holloway, P. E. (2001). A regional model of the semidiurnal internal tide on the Australian North West Shelf. *Journal of Geophysical Research* **106**, 19625–19638. doi:10.1029/2000JC000675

Holloway, P., and Nye, H. (1985). Leeuwin current and wind distributions on the southern part of the Australian North West Shelf between January 1982 and July 1983. *Marine and Freshwater Research* **36**, 123. doi:http://dx.doi.org/10.1071/mf9850123

Ikeda, T. (1974). Nutritional ecology of marine zooplankton. *Memoirs of the Faculty of Fisheries Hokkaido University* **22**, 1–97. Available at: http://hdl.handle.net/2115/21857

Ikeda, T., and McKinnon, A. D. (2011). Metabolism and chemical composition of zooplankton and hyperbenthos from the Great Barrier Reef waters , North Queensland , Australia. **7**, 8–19. Available at: http://www.plankton.jp/PBR/issue/vol07_1/pbr0701_008.pdf

Ingole, B. S., and Parulekar, A. H. (1995). Biochemical composition of zooplankton. *Indian Journal of Marine Sciences* **24**, 73–75. Available at: http://drs.nio.org/drs/bitstream/handle/2264/2295/Indian_J_Mar_Sci_24_73.pdf?sequence=2&isAllowed=y

Irigoien, X., Huisman, J., and Harris, R. P. (2004). Global biodiversity patterns of marine phytoplankton and zooplankton. *Nature* **429**, 863–867. doi:10.1038/nature02593

Jagadeesan, L., Arivuselvan, N., Thirumaran, G., Anantharaman, P., and Balasubramanian, T. (2010). Biomass and biochemical composition of zooplankton along the Arabian sea, west coast of India. *Advance Journal of Food Science and Technology* **2**, 96–99.

Jaine, F. R. A., Rohner, C. A., Weeks, S. J., Couturier, L. I. E., Bennett, M. B., Townsend, K. A., and Richardson, A. J. (2014). Movements and habitat use of reef manta rays off eastern Australia: Offshore excursions, deep diving and eddy affinity revealed by satellite telemetry. *Marine Ecology Progress Series* **510**, 73–86. doi:10.3354/meps10910

Jaspers, C., Acuña, J. L., and Brodeur, R. D. (2014). Interactions of gelatinous zooplankton within marine food webs. *Journal of Plankton Research* **37**, 985–988. doi:10.1093/plankt/fbv068

Ji, R., Edwards, M., MacKas, D. L., Runge, J. A., and Thomas, A. C. (2010). Marine plankton phenology and life history in a changing climate: Current research and future directions. *Journal of Plankton Research* **32**, 1355–1368. doi:10.1093/plankt/fbq062

Jo, N., Kang, J. J., Park, W. G., Lee, B. R., Yun, M. S., Lee, J. H., Kim, S. M., Lee, D., Joo, H. T., Lee, J. H., Ahn, S. H., and Lee, S. H. (2016). Seasonal variation in the biochemical compositions of phytoplankton and zooplankton communities in the southwestern East/Japan Sea. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 0–1. doi:10.1016/j.dsr2.2016.12.001

Kamburska, L., and Fonda-Umani, S. (2009). From seasonal to decadal inter-annual variability of mesozooplankton biomass in the Northern Adriatic Sea (Gulf of Trieste). *Journal of Marine Systems* **78**, 490–504. doi:10.1016/j.jmarsys.2008.12.007

Kattner, G., and Hagen, W. (2009). ‘Lipids in marine copepods: Latitudinal characteristics and perspective to global warming’ Eds M. T. In Arts, M. T. Brett, and M. Kainz. (Lipids in Aquatic Ecosystems. Springer: New York.)

Kattner, G., and Hagen, W. (1995). Polar herbivorous copepods - different pathways in lipid biosythesis. *ICES Journal of Marine Science* **52**, 329–335. doi:10.1016/1054-3139(95)80048-4

Kattner, G., Hagen, W., Lee, R. F., Campbell, R., Deibel, D., Falk-Petersen, S., Graeve, M., Hansen, B. W., Hirche, H. J., Jónasdóttir, S. H., Madsen, M. L., Mayzaud, P., Müller-Navarra, D., Nichols, P. D., Paffenhöfer, G.-A., Pond, D., Saito, H., Stübing, D., and Virtue, P. (2007). Perspectives on marine zooplankton lipids. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 1628–1639. doi:10.1139/f07-122

Kiorboe, T., and Nielsen, T. . (1994). Regulation of zooplankton biomass and production in a temperate coastal ecosystem. *Limnology and Oceanography* **39**, 493–507. doi:10.4319/lo.1994.39.3.0493

Kitchen-Wheeler, A.-M. (2013). The behaviour and ecology of Alfred mantas (Manta Alfredi) in the Maldives. Available at: https://theses.ncl.ac.uk/dspace/handle/10443/1936

Krishna Kumari, L., and Goswami, S. C. (1993). Biomass and Biochemical Composition of Zooplankton From Northwest Bay of Bengal During January 1990. *Indian Journal of Marine Sciences* **22**, 143–145.

Ladhar, C., Ayadi, H., Denis, F., Tastard, E., and Sellami, I. (2014). The effect of environmental factors on the fatty acid composition of copepods and Artemia in the Sfax solar saltern (Tunisia). *Biochemical Systematics and Ecology* **56**, 237–245. doi:10.1016/j.bse.2014.06.005

Lebourges-Dhaussy, A., Huggett, J., Ockhuis, S., Roudaut, G., Josse, E., and Verheye, H. (2014). Zooplankton size and distribution within mesoscale structures in the Mozambique Channel: A comparative approach using the TAPS acoustic profiler, a multiple net sampler and ZooScan image analysis. *Deep-Sea Research Part II: Topical Studies in Oceanography* **100**, 136–152. doi:10.1016/j.dsr2.2013.10.022

Lee, R. F., Hagen, W., and Kattner, G. (2006). Lipid storage in marine zooplankton. *Marine*

Ecology Progress Series **307**, 273–306. doi:Doi 10.3354/Meps307273

Linford, E. (1965). Biochemical studies on marine zooplankton: II. Variations in the lipid content of some Mysidacea. *ICES Journal of Marine Science* **30**, 16–27. doi:https://doi.org/10.1093/icesjms/30.1.16

Lumeran, B. T. (2016). Spatial distribution of marine planktons off the Coast of Sitra, Kingdom of Bahrain. *Journal of US-China Public Administration* **13**. doi:10.17265/1548-6591/2016.05.004

Lynam, C. P., Gibbons, M. J., Axelsen, B. E., Sparks, C. A. J., Coetzee, J., Heywood, B. G., and Brierley, A. S. (2006). Jellyfish overtake fish in a heavily fished ecosystem. *Current Biology* **16**, 1976. doi:10.1016/j.cub.2006.09.012

Mackas, D. L., Greve, W., Edwards, M., Chiba, S., Tadokoro, K., Eloire, D., Mazzocchi, M. G., Batten, S., Richardson, A. J., Johnson, C., Head, E., Conversi, A., and Peluso, T. (2012). Changing zooplankton seasonality in a changing ocean: Comparing time series of zooplankton phenology. *Progress in Oceanography* **97**–**100**, 31–62. doi:10.1016/j.pocean.2011.11.005

Marshall, A. D., and Bennett, M. B. (2010). Reproductive ecology of the reef manta ray Manta alfredi in southern Mozambique. *Journal of Fish Biology* **77**, 169–190. doi:10.1111/j.1095-8649.2010.02669.x

Marshall, A. D., Compagno, L. J. V, and Bennett, M. B. (2009). Redescription of the genus Manta with resurrection of Manta alfredi (Krefft, 1868) (Chondrichthyes; Myliobatoidei; Mobulidae). *Zootaxa*, 1–28. doi:10.3161/000345409X484856

Marshall, A. D., Dudgeon, C. L., and Bennett, M. B. (2011). Size and structure of a photographically identified population of manta rays Manta alfredi in southern Mozambique. *Marine Biology* **158**, 1111–1124. doi:10.1007/s00227-011-1634-6

McCauley, D. J., DeSalles, P. A., Young, H. S., Papastamatiou, Y. P., Caselle, J. E., Deakos, M. H., Gardner, J. P. A., Garton, D. W., Collen, J. D., and Micheli, F. (2014). Reliance of mobile species on sensitive habitats: A case study of manta rays (Manta alfredi) and lagoons. *Marine Biology* **161**, 1987–1998. doi:10.1007/s00227-014-2478-7

McManus, M. A., and Woodson, C. B. (2012). Plankton distribution and ocean dispersal. *Journal of Experimental Biology* **215**, 1008–1016. doi:10.1242/jeb.059014

Moncheva, S., Gorinstein, S., Shtereva, G., Toledo, F., Arancibia-Avila, P., Goshev, I., and Trakhtenberg, S. (2003). Biomass, protein- and carbohydrate-composition of phytoplankton in Varna Bay, Black Sea. *Hydrobiologia* **501**, 23–28. doi:10.1023/A:1026229100626

Moreno, A. R., and Martiny, A. C. (2017). Ecological stoichiometry of ocean plankton. **10**, 1–27. doi:10.1146/annurev-marine-121916-063126

Motta, P. J., Maslanka, M., Hueter, R. E., Davis, R. L., de la Parra, R., Mulvany, S. L., Habegger, M. L., Strother, J. A., Mara, K. R., Gardiner, J. M., Tyminski, J. P., and Zeigler, L. D. (2010). Feeding anatomy, filter-feeding rate, and diet of whale sharks Rhincodon typus during surface ram filter feeding off the Yucatan Peninsula, Mexico. *Zoology* **113**, 199–212. doi:10.1016/j.zool.2009.12.001

Muller-Navarra, D. C. (2008). Food web paradigms: The biochemical view on trophic interactions. *International Review of Hydrobiology* **93**, 489–505. doi:10.1002/iroh.200711046

Murphy, E. J., Cavanagh, R. D., Drinkwater, K. F., Grant, S. M., Heymans, J. J., Hofmann, E. E., Hunt, G. L., and Johnston, N. M. (2016). Understanding the structure and functioning of polar pelagic ecosystems to predict the impacts of change. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20161646. doi:10.1098/rspb.2016.1646

Nelson, J. D., and Eckert, S. A. (2007). Foraging ecology of whale sharks (Rhincodon typus) within Bahia de Los Angeles, Baja California Norte, Mexico. *Fisheries Research* **84**, 47–64. doi:10.1016/j.fishres.2006.11.013

Ningaloo Coast World Heritage Team (2017). The Ningaloo Coast, West Australia - Overview - World Heritage places. Available at: http://www.environment.gov.au/heritage/places/world/ningaloo%0D [accessed 5 August 2017]

Parra Venegas, R. de la, Hueter, R., Cano, J. G., Tyminski, J., Remolina, J. G., Maslanka, M., Ormos, A., Weigt, L., Carlson, B., and Dove, A. (2011). An unprecedented aggregation of whale sharks, Rhincodon typus, in Mexican coastal waters of the Caribbean sea. *PLoS ONE* **6**, e18994. doi:10.1371/journal.pone.0018994

Parrish, C. C., Thompson, R. J., and Deibel, D. (2005). Lipid classes and fatty acids in plankton and settling matter during the spring bloom in a cold ocean coastal environment. *Marine Ecology Progress Series* **286**, 57–68. doi:10.3354/meps286057

Pepper, R. E., Jaffe, J. S., Variano, E., and Koehl, M. A. R. (2015). Zooplankton in flowing water near benthic communities encounter rapidly fluctuating velocity gradients and accelerations. *Marine Biology* **162**, 1939–1954. doi:10.1007/s00227-015-2713-x

Percy, J. A. (1979). Seasonal changes in organic composition and caloric content of an Arctic marine amphipod, Onisimus (Boeckosimus) affinis H.J. Hansen. *Journal of Experimental Marine Biology and Ecology* **40**, 183–192. doi:10.1016/0022-0981(79)90044-3

Percy, J. A., and Fife, F. J. (1981). The biochemical composition and energy content of Arctic marine macrozooplankton. *Arctic* **34**, 307–313.

Pitcher, G. C., Figueiras, F. G., Hickey, B. M., and Moita, M. T. (2010). The physical oceanography of upwelling systems and the development of harmful algal blooms. *Progress in Oceanography* **85**, 5–32. doi:10.1016/j.pocean.2010.02.002

Price, J., Weller, R., and Pinkel, R. (1986). Diurnal cycling: Observations and models of the upper ocean response to diurnal heating, cooling, and wind mixing. *Journal of Geophysical Research* **94**, 8411. doi:http://dx.doi.org/10.1029/jc091ic07p08411

Le Quéré, C., Buitenhuis, E. T., Moriarty, R., Alvain, S., Aumont, O., Bopp, L., Chollet, S., Enright, C., Franklin, D. J., Geider, R. J., Harrison, S. P., Hirst, A. G., Larsen, S., Legendre, L., Platt, T., Prentice, I. C., Rivkin, R. B., Sailley, S., Sathyendranath, S., Stephens, N., Vogt, M., and Vallina, S. M. (2016). Role of zooplankton dynamics for Southern Ocean phytoplankton biomass and global biogeochemical cycles. *Biogeosciences* **13**, 4111–4133. doi:10.5194/bg-13-4111-2016

Raymont, J., Austin, J., and Linford, E. (1964). Biochemical studies on marine zooplankton: I. The biochemical composition of Neomysis integer. *ICES Journal of Marine Science* **28**, 354–363. doi:http://dx.doi.org/10.1093/icesjms/28.3.354

Raymont, J. E. G., and Conover, R. J. (1961). Further investigations on the carbohydrate content of marine zooplankton. *Limnology and Oceanography* **6**, 154–164. doi:10.4319/lo.1961.6.2.0154

Raymont, J., and Krishnaswamy, S. (1960). Carbohydrates in some marine planktonic animals. *Journal of Marine Biology Ass. UK* **39**, 239–248. doi:10.1017/S002531540001328X

Richardson, A. (2008). In hot water: zooplankton and climate change. ICES Journal of Marine. *ICES Journal of Marine Science* **65**, 279–295. doi:10. 1093/icesjms/fsn028

Richardson, A. J., and Schoeman, D. S. (2004). Climate impact on plankton ecosystems in the Northeast Atlantic. *Science* **305**, 1609–1612.

Roemmich, D., and McGowan, J. (1995). Climatic warming and the decline of zooplankton in the California Current. *Science* **267**, 1324–1326. Available at: http://www.jstor.org.libproxy.murdoch.edu.au/stable/2885994

Rohner, C. A., Burgess, K. B., Rambahiniarison, M., Stewart, J. D., and Richardson, A. J. (2017). Mobulid rays feed on euphausiids in the Bohol Sea. *Royal Society Open Science* **4**, 161060. doi:http://dx.doi.org/10.1098/rsos.161060

Rohner, C. A., Couturier, L. I. E., Richardson, A. J., Pierce, S. J., Prebble, C. E. M., Gibbons, M. J., and Nichols, P. D. (2013a). Diet of whale sharks Rhincodon typus inferred from stomach content and signature fatty acid analyses. *Marine Ecology Progress Series* **493**, 219–235. doi:10.3354/meps10500

Rohner, C. A., Pierce, S. J., Marshall, A. D., Weeks, S. J., Bennett, M. B., and Richardson, A. J. (2013b). Trends in sightings and environmental influences on a coastal aggregation of manta rays and whale sharks. *Marine Ecology Progress Series* **482**, 153–168. doi:10.3354/meps10290

Romankevich, E. . (1984). Geochemistry of organic matter in the ocean. *Journal of the Marine Biological Association of the United Kingdom* **65**, 334–842. doi:doi.org/10.1017/S0025315400052711

Rousseaux, C. S. G., Lowe, R., Feng, M., Waite, A. M., and Thompson, P. A. (2012). The role of the Leeuwin Current and mixed layer depth on the autumn phytoplankton bloom off Ningaloo Reef, Western Australia. *Continental Shelf Research* **32**, 22–35. doi:10.1016/j.csr.2011.10.010

Sale, P., McWilliam, P., and Anderson, D. (1976). Composition of the near-reef zooplankton at Heron Reef, Great Barrier Reef. *Marine Biology* **34**, 59–66. doi:http://dx.doi.org/10.1007/bf00390788

Sims, D. W., Southall, E. J., Richardson, A. J., Reid, P. C., and Metcalfe, J. D. (2003). Seasonal movements and behaviour of basking sharks from archival tagging: No evidence of winter hibernation. *Marine Ecology Progress Series* **248**, 187–196. doi:10.3354/meps248187

Sleeman, J. C., Meekan, M. G., Fitzpatrick, B. J., Steinberg, C. R., Ancel, R., and Bradshaw, C. J. A. (2010). Oceanographic and atmospheric phenomena influence the abundance of whale sharks at Ningaloo Reef, Western Australia. *Journal of Experimental Marine Biology and Ecology* **382**, 77–81. doi:10.1016/j.jembe.2009.10.015

Sleeman, J. C., Meekan, M. G., Wilson, S. G., Jenner, C. K. S., Jenner, M. N., Boggs, G. S., Steinberg, C. C., and Bradshaw, C. J. A. (2007). Biophysical correlates of relative abundances of marine megafauna at Ningaloo Reef, Western Australia. *Marine and Freshwater Research* **58**, 608–623. doi:10.1071/MF06213

Smith, J. N., De'ath, G., Richter, C., Cornils, A., Hall-Spencer, J. M., and Fabricius, K. E. (2016). Ocean acidification reduces demersal zooplankton that reside in tropical coral reefs. *Nature Climate Change* **6**, 1124–1129. doi:10.1038/nclimate3122

De Souza, C. S., Luz, J. A. G., and Mafalda Junior, P. O. (2014). Relationship between spatial distribution of chaetognaths and hydrographic conditions around seamounts and islands of the tropical southwestern Atlantic. *Anais da Academia Brasileira de Ciencias* **86**, 1151–1165. doi:10.1590/0001-3765201420130101

Steinacher, M., Joos, F., Frolicher, T. ., Bopp, L., Cadule, P., Cocco, V., Doney, S. C., Gehlen, M., Lindsay, K., Moore, J. K., Schneider, B., and Segschneider, J. (2010). Projected 21st century decrease in marine productivity: a multi-model analysis. *Biogeosciences* **7**, 979–1005. Available at: https://www.biogeosciences.net/7/979/2010/bg-7-979-2010.pdf

Sterner, R. W., and Schulz, K. L. (1998). Zooplankton nutrition: Recent progress and a reality check. *Aquatic Ecology* **32**, 261–279. doi:10.1023/A:1009949400573

Stewart, J. D., Hoyos-Padilla, E. M., Kumli, K. R., and Rubin, R. D. (2016). Deep-water feeding and behavioral plasticity in Manta birostris revealed by archival tags and submersible observations. *Zoology* **119**, 406–413. doi:10.1016/j.zool.2016.05.010

Taylor, J. G. (1996). Seasonal occurrence, distribution and movements of the whale shark, Rhincodon typus, at Ningaloo reef, western Australia. *Marine and Freshwater Research* **47**, 637–642. doi:10.1071/MF9960637

Taylor, J. G., and Pearce, A. F. (1999). Ningaloo reef currents: Implications for coral spawn dispersal, zooplankton and whale shark abundance. *Journal of the Royal Society of Western Australia* **82**, 57–65.

Tomita, T., Sato, K., Suda, K., Kawauchi, J., and Nakaya, K. (2011). Feeding of the megamouth shark (Pisces: Lamniformes: Megachasmidae) predicted by its hyoid arch: A biomechanical approach. *Journal of Morphology* **272**, 513–524. doi:10.1002/jmor.10905

Tsikliras, A. C., Antonopoulou, E., and Stergiou, K. I. (2010). Spawning period of Mediterranean marine fishes. *Reviews in Fish Biology and Fisheries* **20**, 499–538.

doi:10.1007/s11160-010-9158-6

Turner, J., Tester, P., and Hettler, W. (1985). Zooplankton feeding ecology A laboratory study of predation on fish eggs and larvae by the copepods Anomalocera ornata and Centropages typicus. *Marine Biology* **90**, 1–8. doi:http://dx.doi.org/10.1007/bf00428208

Uye, S. ichi, and Ichino, S. (1995). Seasonal variations in abundance, size composition, biomass and production rate of Oikopleura dioica (Fol) (Tunicata: Appendicularia) in a temperate eutrophic inlet. *Journal of Experimental Marine Biology and Ecology* **189**, 1–11. doi:10.1016/0022-0981(95)00004-B

Verlinden, N. (2010). Seasonal variation of zooplankton nutritional quality in a manta ray (Manta alfredi) aggregation area. The University of Queensland.

Vrede, T., Dobberfuhl, D. R., Kooijman, S. A. L. M., Elser, J. J., Ecology, S., and May, N. (2004). Fundamental connections among organism C : N : P stoichiometry , macromolecular composition , and growth. *Ecology* **85**, 1217–1229.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J. M., Hoegh-Guldberg, O., and Bairlein, F. (2002). Ecological responses to recent climate change. *Nature* **416**, 389–395. doi:10.1038/416389a

Wang, M., and Jeffs, A. G. (2014). Nutritional composition of potential zooplankton prey of spiny lobster larvae: A review. *Reviews in Aquaculture* **6**, 270–299. doi:10.1111/raq.12044

Wang, M., Mackenzie, A. D., and Jeffs, A. G. (2015). Lipid and fatty acid composition of likely zooplankton prey of spiny lobster (Jasus edwardsii) phyllosomas. *Aquaculture Nutrition* **21**, 385–400. doi:10.1111/anu.12164

Weeks, S. J., Magno-Canto, M. M., Jaine, F. R. A., Brodie, J., and Richardson, A. J. (2015). Unique sequence of events triggers manta ray feeding frenzy in the Southern Great Barrier Reef, Australia. *Remote Sensing* **7**, 3138–3152. doi:10.3390/rs70303138

West, L. (2013). Diurnal variation in zooplankton communities and its relationship to whale shark (Rhincodon typus) movements at Ningaloo Marine Park, Western Australia. Murdoch University.

White, W. T., Corrigan, S., Yang, L., Henderson, A. C., Bazinet, A. L., Swofford, D. L., and Naylor, G. J. P. (2017). Phylogeny of the manta and devilrays (Chondrichthyes: Mobulidae), with an updated taxonomic arrangement for the family. *Zoological Journal* *of the Linnean Society* **82**, 6573. doi:10.1093/zoolinnean/zlx018

Wiafe, G., and Frid, C. L. J. (1996). Short-term temporal variation in coastal zooplankton communities: the relative importance of physical and biological mechanisms. *Journal of Plankton Research* **18**, 1485–1501. doi:10.1093/plankt/18.8.1485

WillyWeather (2017). Coral Bay Tide Times, WA 6701. Available at: https://tides.willyweather.com.au/wa/gascoyne/coral-bay.html [accessed 2 August 2017]

Wilson, S.G., Pauly, T., Meekan, M. G. (2002). Marine Freshwater data in coastal waters off Ningaloo Reef , Western Australia. *Marine and Freshwater Research* **53**, 1005–1015.

Wilson, S. G., Taylor, J. G., and Pearce, A. F. (2001). The seasonal aggregation of whale sharks at Ningaloo Reef, Western Australia: Currents, migrations and the El Niño/ Southern Oscillation. *Environmental Biology of Fishes* **61**, 1–11. doi:10.1023/A:1011069914753

Woo, M., Pattiaratchi, C., and Schroeder, W. (2006). Dynamics of the Ningaloo Current off Point Cloates, Western Australia. *Marine and Freshwater Research* **57**, 291–301. doi:10.1071/MF05106

Woodson, C. B., Webster, D. R., Weissburg, M. J., and Yen, J. (2005). Response of copepods to physical gradients associated with structure in the ocean. *Limnology and Oceanography* **50**, 1552–1564. doi:10.4319/lo.2005.50.5.1552

Xu, J., Lowe, R. J., Ivey, G. N., Jones, N. L., and Zhang, Z. (2016). Ocean transport pathways to a world heritage fringing coral reef: Ningaloo reef, Western Australia. *PLoS ONE* **11**, 1–26. doi:10.1371/journal.pone.0145822

Zhang, Z., Lowe, R., Ivey, G., Xu, J., and Falter, J. (2016). The combined effect of transient wind-driven upwelling and eddies on vertical nutrient fluxes and phytoplankton dynamics along Ningaloo Reef, Western Australia. *Journal of Geophysical Research* **121**, 4994–5016. doi:10.1002/2016JC011791

Appendix 1. Procedure to determine total lipid content within zooplankton, using Bligh and Dyer (1956) method for lipid extraction.

1. Prepare freeze dried samples into 10 mL centrifuge tubes, add 1 teaspoon liquid nitrogen.
2. Prepare a solvent of methanol: chloroform: deionised water (2:1:0.8).
3. Add 2 mL of solvent to each tube containing the samples and crush using a glass rod. Add a further 3.7 mL of solvent to each tube and homogenise well using a glass rod, to give a final volume of 5.7 mL.
4. Centrifuge at 3500 rpm, for 10 mins, or until the pellet has separated from the liquid.
5. Transfer the supernatant into 20 mL glass tubes with screw caps.
6. Add a further 5.7 mL to the 10 mL plastic centrifuge tubes and vortex, to re-suspend and homogenise the pellet with the solvent.
7. Centrifuge again at 3500 rpm for 10 mins, until the pellet has separated from the liquid.
8. Transfer the supernatant to the glass tubes containing the original extract, and reseal tubes.
9. Add 3 mL of deionised water to each 20 mL glass tube, and vortex.
10. Add 3 mL of chloroform to each 20 mL glass tube and mix by vortexing.
11. Reseal tubes and place them in a refrigerator for at least 24 hours, for phase separation to occur (the bottom phase being the one with the lipids).
12. Remove samples from refrigerator after at least 24 hours.
13. Once the samples are at room temperature, carefully remove the upper layer using a Pasture pipette connected to a syringe and transfer to a waste container.
14. When the top layer has been removed, add 8 drops of toluene to each sample, now just containing the chloroform layer, to remove any amount of water remaining.
15. Remove the toluene layer using the Pasture pipette and discard into waste container.
16. Transfer the chloroform layer into clean, dry, pre-weighed 10 mL glass vials.
17. Place the vials under a stream of ultra pure nitrogen (N_2) gas for evaporation on a heating plate at 38°C.
18. Once evaporated, immediately weight the vials with the lipids inside.

Appendix 2. Standard procedure to determine the protein concentration within the zooplankton, using the Bio-rad protein assay kit, and spectrophotometer.

1. Prepare dye reagent, by diluting 1-part dye reagent concentration with 4 parts deionised water (DI). Filter through Whatman #1 filter paper, then add DI.
2. Prepare 5 dilutions of a protein standard, using Bovine Serum Albumin (BSA), in 10 mL centrifuge tubes 0.09375, 0.1875, 0.375, 0.75 and 1.5 mg/mL was used here.
3. Prepare freeze dried zooplankton samples in 10 mL centrifuge tubes, add liquid nitrogen.
4. After 10 mins, liquid nitrogen should be dry, add 5 mL of pure water and crush samples using a glass rod, centrifuge for 10 mins.
5. Pipette 0.1 mL of each standard and sample solution into clean centrifuge tubes.
6. Add 5 mL of diluted dye reagent to each tube and vortex.
7. Incubate at room temperature for 5 mins.
8. Using a spectrophotometer, absorbance of each sample was measured at 595 nm.
    - If the absorbance measured at a higher concentration to the standard, then sample was diluted; add 1 mL of sample and 1 mL of pure water to a clean centrifuge tube, then measure again.