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Benthic Infauna of Mangrove Forests: Dissolved Oxygen and Environmental Settings Determine Their Community Composition and Function

Thesis submitted by

Carlo Mattone
BSc James Cook University
MAppSC James Cook University

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Abstract

Despite their importance in regulating many ecological processes the benthic infauna of mangrove forests are poorly understood. The anoxic nature of the sediments in which mangroves grow is likely to influence the dissolved oxygen (DO) dynamics of water in mangrove forests, and in turn influence the way benthic organisms utilize mangrove habitats. Despite this, little research has focused on understanding DO dynamics of the overlying water during tidal inundation and in the remnant pools formed after the tide ebbed and how this affects biological interactions. Therefore this study aimed to fill this gap and to assess the extent to which DO and environmental settings influence benthos composition and distribution in tropical *Rhizophora stylosa* mangrove forests. We assessed the DO patterns at various distances within an estuarine *Rhizophora stylosa* forest in tropical north Queensland over a total of 32 tidal cycles encompassing multiples seasons and tidal ranges. There were substantial fluctuations in DO, often varying from normoxic (100% saturation) to hypoxic (<10% saturation) within the same tidal cycle. A number of factors and their interactions influenced DO dynamics, in particular: tidal height, time of the day, tidal phase and distance from the forest edge. For instance, during spring nocturnal tide DO was relatively high during both flooding and ebbing phase, however during diurnal tide the water would become oversaturated in DO during the ebbing phase. On the other hand, during neap tide DO would exhibit a declining profile during both diurnal and nocturnal tides. Moreover during tidal disconnection the remnant pools within the forest quickly became anoxic, and low DO saturations are likely to affect how nekton and benthic organisms utilize mangrove forest. The trend for repeated declines to low DO levels correlates with a paucity of benthic taxa within estuarine *R. stylosa* forests, generally dominated by DO tolerant species such as sipunculids and capitellids. Furthermore, peracarids (i.e. amphipods, tanaids, mysids) were never found within the forests despite their occurrence on the adjacent

intertidal flats. Because peracarids are a key component of juvenile fish diets, their absence within the forest is likely to limit foraging ground for juvenile nektons. Based on these results, we used the amphipod *Melita* spp. as a case study to test whether the DO fluctuations experienced within the mangrove forests were responsible for the lack of peracarids. *Melita* spp. was highly susceptible to low DO with less than 30% of the tested individuals surviving at 10% DO. Additionally, behavioural stress started to appear at around 20% DO. As the mangroves investigated experience DO lower than 5% saturation, it is plausible that *Melita* and other peracarids cannot use these estuarine *R. stylosa* forests due to the unsuitable DO conditions.

Building on these findings, we tested whether *R. stylosa* forests located in different environmental settings (e.g. non-estuarine) have differences in benthic communities. The comparisons involved two estuarine and two island *R. stylosa* forests located in tropical north Queensland in close proximity to one another. The results highlight distinct differences in benthic communities between the island and the estuarine *R. stylosa* forests. In particular the two forests were dominated by different polychaete families, with Opheliidae and Spionidae characterizing the island forests, and Glyceridae and Capitellidae characterizing the estuarine forests. Additionally, in contrast to the estuarine situation, peracarids were observed throughout the island forests. Such variability in dominant taxonomic groups is likely to affect food-web interactions as well as the function of the mangrove forest within the system. Limited DO sampling indicated overall higher DO levels at island sites compared to the estuarine conditions. These differences could be the result of higher wave energy that limits organic matter deposition within the island forests, leading to reduced DO depletion and different benthic communities. The importance that environmental settings have on determining infauna communities within *R. stylosa* forests could explain some of the variability in mangrove utilization by aquatic fauna between studies conducted in the Caribbean and the Indo-Pacific. Research in the Caribbean is often carried out in island-type mangroves, while in the Indo-Pacific

the majority of studies have focused on estuarine mangroves. The results of this thesis suggest that DO dynamics, together with environmental settings, are important in determining fauna utilization of mangrove forests. Consequently, great caution should be taken when generalizing mangrove forest ecological functions.

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Dedicated

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Chapter 1 - General Introduction

1.1 Dissolved oxygen in relation to marine organisms and trophic interactions

Oxygen is a fundamental element, necessary for animals' metabolic functions. In air oxygen is highly abundant, so it does not generally affect the distribution of air-breathing animals. In contrast, oxygen is much scarcer in aquatic environments, and it can influence the distribution of organisms (Stramma et al. 2008). This is because, for oxygen to be metabolized by aquatic organisms it must not be part of a compound (e.g. water), but it has to be in its free gas state (i.e. O_2). This is referred to as dissolved oxygen (DO), and its concentration in water can be measured using various units. One of the most common and biologically meaningful DO scale is based on percentage saturation, where 100% means water is fully saturated with oxygen, and 0% means there is no oxygen dissolved (anoxic).

DO is constantly fluctuating in the aquatic environment, influenced by a variety of factors, including biological activity (e.g. respiration), physical dynamics (e.g. turbulence) and chemical interactions (e.g. pH)(Kenner & Ahmed 1975, Sammut et al. 1995, Diaz 2001, King et al. 2012). For instance, during the day water generally has higher DO than at night because of photosynthetic activity (Butler & Burrows 2007).

Studies into factors affecting DO dynamics have been conducted since the first half of the last century (Streeter 1931, Odum 1956). This has allowed us to understand the various correlations between DO and other physical and chemical parameters such as: pH, temperature, biological oxygen demand (BOD) and sediment oxygen demand (SOD)(Simonsen & Harremoës 1978). However, the early models focused on large scale (e.g. seasonal) rather than small scale fluctuations (e.g. hours), mainly due to technological limitations that prevented finer temporal scale monitoring in field situations, and did not permit monitoring of DO continuously. Consequently, understanding how small scale changes in DO affected ecological processes was limited. However, nowadays with technological improvements, the importance of DO in regulating ecological functions has been re-evaluated with a rising number of studies implicating DO as a key driver of patterns and processes in aquatic ecosystems (Ingole et al. 2010, Hladyz et al. 2011, Long et al. 2016). In fact, because DO fluctuates constantly, animals have to be able to cope with variations in saturation levels. Evaluating how aquatic animals respond to DO fluctuations is often complicated, because they can be affected in different ways depending on their physiological needs and adaptations (Butler et al. 2007, Levin et al. 2009). Consequently, the level at which oxygen becomes a limiting factor for animals varies among taxa. Many fish can sustain small drops in DO (about 80% to 70% DO saturation) with little consequence, by mechanically increasing water flow through their gills, actively facilitating aeration and DO extraction into the blood stream (Butler et al. 2007). However, if DO falls below 40% many fish species start displaying sub-lethal and lethal consequences. Therefore many fisheries and environmental agencies consider 40% DO to be the threshold below which oxygen saturation is too low (i.e. hypoxic) for fish survival (Breitburg 2002). However, unless DO declines rapidly, fish can easily detect changes in DO, enabling them to move to more oxygen-rich areas before the onset of physiological consequences (Breitburg 1994, Butler et al. 2007).

The situation is different for benthic invertebrates. In fact, 40% DO is generally high enough for many invertebrates to live without any physiological concern, and water is considered hypoxic around 15% DO (Connolly et al. 2004). Nonetheless, despite invertebrates higher tolerances to low DO some invertebrate communities and organisms are highly impacted by DO dynamics. This is because many benthic invertebrates dwell within sediments (i.e. infauna) meaning they live further from atmospheric oxygen. As a result, gas exchange is limited and DO levels are generally lower, and re-oxygenation more restricted, compared to areas higher in the water column (Vaquer-Sunyer & Duarte 2008). Additionally, due to their limited mobility most benthic invertebrates do not have the option to move to more favourable areas when DO changes (Vaquer-Sunyer & Duarte 2008). This results in benthic fauna getting strongly affected when DO declines (Jorgensen 1980, Dauer et al. 1992, Rosenberg et al. 2001). For example, there is an almost complete absence of peracarid crustaceans (i.e. amphipods and isopods) and a lower polychaete diversity in areas that experience seasonal hypoxia compared to areas that do not experience seasonally low DO (Rabalais et al. 2002). Because benthic assemblages are important within coastal food-webs and can affect the distribution of higher trophic groups (Grebmeier et al. 2006, Davis et al. 2014), altered benthic structure will often have repercussions for local fish communities, even after DO as returned to high levels. Consequently, DO has the potential to regulate ecological interactions in the aquatic environment. To date, the majority of studies that investigated animal responses to DO have either experimentally test their physiology (Scholz & Waller 1992, Miller et al. 2002, Butler et al 2007, Mandic et al 2008), or aimed to assess faunal changes following extreme hypoxic events (Diaz 2001, Bell & Eggleston 2005, Gooday et al. 2009, Ludsin et al. 2009, Ingole et al. 2010). In most cases, the final goal of the research was to develop guidelines to improve water quality within costal and riverine systems and reduce the occurrence of mass animal deaths (e.g. fish kills) due to hypoxic events (Breitburg 2002, Butler et al. 2007). However, because DO can be so influential in animal distribution, it is likely that even DO variations too small to cause fish kills, can be influential in structuring both the distributions of organisms and ecological processes in marine and freshwater systems. For instance, at night, lower DO is likely to limit sensitive animals to utilizing specific habitats, actively acting as a refuge against predation for more tolerant organisms (Diaz and Rosenberg 2008).

1.2 Mangrove forests and dissolved oxygen

Although the distribution and dynamics of DO in mangrove ecosystems is poorly understood, it is known that mangrove forests tend to have relatively poor oxygen conditions because mangrove forest sediment becomes anoxic just a few millimetres below the surface (Andersen & Kristensen 1988, Marchand et al. 2004, Hogarth 2015). This is caused by the high rates of decomposition by bacteria in the sediment (Alongi 1994, Holmboe et al. 2001). Consequently, mangrove forests are likely to have a fauna composition that reflects DO dynamics. However, there has been little investigation of how bacterial activities within the sediment affect DO on the overlaying water of mangrove forests.

In fresh water rivers, organic sludge can account for almost half of the total oxygen demand in the water (Streeter 1931, Martin & Bella 1971). A similar situation within mangrove forests would have repercussions for how biota utilize these systems, and could be responsible for determining the outcomes of many ecological interactions. For instance, until recently it was believed that mangrove aquatic fauna could find refuge against desiccation during low tide by utilising remnant pools of water. However, Knight et al. (2013) showed that in basin mangrove forests, the water quickly becomes anoxic once the tide has ebbed and that DO does not rise again until the following tidal connection, leaving the fauna of these pools dominated by mosquito larvae that utilise atmospheric oxygen. This predictable hypoxia implies that both marine nekton and benthos that use mangrove forests at low tide might require special adaptations (behavioural or physiological) in order to live in such an oxygen depleted environment. There is evidences that this might be the case for some taxa: for instance, some of the crabs inhabiting mangrove dominated estuaries (e.g. *Uca* spp., *Sesarme* spp. *Metopograpsus* spp.) are known to have a semi-terrestrial behaviour, capable of "breathing" air during low tide (MacNae 1968, Takeda et al. 1996, Morris 2002). Besides giving them the opportunity to inhabit the intertidal when it is not innundated, this would also allow them to inhabit hypoxic environments such as remnant pools. Similarly, fish of the genus *Periophthalmus* (mudskippers) are known to be able to respire across body surfaces, and to actively transport air into their burrows at low tide to mitigate the hypoxic water conditions (Ishimatsu et al. 1998). However, besides these limited examples, we have little knowledge of how benthic organisms cope with low DO inside mangrove forests. It may be that mangrove benthic communities are composed of highly tolerant species. This appears not to be the case, with the one available study from Australian mangroves (Dittmann 2001) suggesting a paucity of organisms when compared to adjacent intertidal flats. This could be caused by lower DO within mangroves. However, at this stage these are just speculations based on a single DO study.

Benthic invertebrate communities, particularly infauna organisms, are vital conduits for the regulation of primary productivity and nutrients cycling within aquatic systems. The way the invertebrate community shifts can affect system productivity (Covich et al. 1999, Pinnegar et al. 2000, Solan et al. 2004).

In the face of the many important roles that benthic invertebrate fulfil in aquatic systems, our fundamental lack of understanding on both DO dynamics in mangroves and their impact on benthic fauna means it is crucial to develop a knowledge base on how DO affects mangrove benthic composition. This requires a much better understanding of how DO fluctuates within mangrove forests, what factors affect DO dynamics, as well as a deeper understanding of the tolerance of mangrove benthic fauna to DO.

1.3 Mangrove forests importance and knowledge gaps

Mangrove forests are the dominant vegetation of tropical and subtropical shorelines and are an important link between land and sea (Duke et al. 1998, Rönnbäck 1999, Giri et al. 2011). Mangroves provide a variety of environmental and ecological services, including coastal protection from waves, wind and erosion (Costanza et al. 2008), carbon sequestration (McLeod et al. 2011), and facilitate ecological functions (Kathiresan & Bingham 2001). The economic value of mangrove forests has been estimated to approximately 1 million USD per Km^2/Year (Costanza et al. 1997). However just part of the estimated value is based on the direct market price of goods, such as harvesting the forests for molluscs, fuelwood, and timber (Bennett & Reynolds 1993, Rönnbäck 1999), while the majority of mangroves economic asset is attributed to services outside the market (Costanza et al. 1997, Rönnbäck 1999). This includes their role as nursery grounds, which is believed to boost fisheries yield of commercially important fish and invertebrate (Bell et al. 1984, Ley et al. 1994, Meynecke et al. 2008). Nurseries are areas where juvenile nekton can find extra protection against predation, while having the appropriate food sources and nutrition to grow (Sheridan & Hays 2003). It is this service provided by mangroves that is often used to advocate in favour of mangrove conservation (Faunce & Layman 2009). However, the nursery ground function of mangroves is not always evidence-based, and different studies have reported varying degrees of mangrove utilization by nekton (Blaber 2007). For example, in the Caribbean there is strong evidence that supports the view that many coral reef fish rely on mangrove forest during their juvenile phase (Mumby et al. 2004, Serafy et al. 2015). In contrast, in Australia the same correlation has yet to be confirmed (Barnes et al. 2012). This uncertainty does not just relate to their value to coral reef fish, but many coastal, riverine and pelagic species also show different degrees of dependency on mangroves, based on where the study was conducted and the taxa investigated (Faunce & Layman 2009). Some of the variability in results is attributed to the different tidal ranges and hydrological dynamics (Lugendo et al. 2007). This makes sense, because inundation time can vary quite significantly between different coastal areas and this would limit the time available for fish to take advantage of mangrove structural complexity (Baker et al. 2015). As a result, it is likely that species that inhabit different parts of the world might have evolved to rely on mangroves to different extents based on different patterns of mangrove habitat availability (Barnes et al. 2012). Consequently, different taxa might use the forest in different ways and for different purposes. For example, some might use forests because of their higher structural complexity and the enhanced predator avoidance that affords (Acosta & Butler IV 1997, Primavera 1997). Other taxa might enter the forest in order to gain access to specific food sources (Laegdsgaard & Johnson 2001). However, while there is experimental evidence that demonstrate root structure to provides some degree of protection against predation (Laegdsgaard & Johnson 2001, Sheridan & Hays 2003, Verweij et al. 2006), there is not substantiative evidence supporting mangroves provision of food, and indeed, studies have reported contrasting evidences on the reliance of nekton on mangroves for feeding (Tse et al. 2008).

The reason for the contrasting results is largely due to the lack of detailed information on the type of resources available to feed on inside mangrove forests. For instance, one of the major food sources for juvenile nekton in estuaries (including mangrove dominated systems) is small benthic invertebrates (Salini et al. 1990, Baldoa & Drake 2002, Nanjo et al. 2008). However, we lack detailed information on the composition of benthic infauna assemblages within mangrove forests. In fact, although there is a large amount of literature that focuses on crabs and large molluscs (Wells 1983, Lee 1998), smaller benthic epifauna and infauna organisms have received far less attention (Dittmann 1995), despite the fact that smaller invertebrates are the most likely prey for juvenile nekton. Hence, smaller invertebrates are most likely to support the feeding ground value. Differences in sampling techniques and methodologies among the studies that have sampled small benthos within mangrove forests make direct comparisons difficult (Alfaro 2006). When studies that used similar approaches are compared there are considerable differences in results. For instance, Sheridan (1997), reported an extremely high density of benthic invertebrates (averaging between 22 591 to 52 914 individuals per m^2) within a *Rhizophora mangle* forest in Florida, with peracarids crustaceans (i.e. amphipods, tanaids, mysids) the dominant taxa. In contrast, in the Indo-Pacific, animal densities are generally lower (averaging between 200 and 6000 individuals per m^2), with the majority of studies reporting low diversities (Sasekumar & Chong 1998, Dittmann 2001). Additionally, the taxonomic composition within mangrove forests varies among the dominant mangrove species (*Bruguiera* sp., *Avicennia* sp., *Ceriops* sp., *Sonneratia* sp., *Rhizophora* sp.), and with forest age (Schrijvers et al. 1995, Sasekumar & Chong 1998). Because mangrove forests are often considered as discrete habitat units that provide specific services independently from their location and environmental settings, there is a missing link between mangroves and the synergistic effects of other habitats in their proximity (Sheaves 2009, Törnroos et al. 2013). It is plausible that the discordant outcomes could result from differences in the chemo-physical environment of the forest or forest location. Such a disparity in results and the general paucity of studies into the composition of mangrove's benthic fauna has the potential to lead to substantial misinterpretation of the roles of mangroves. This is because benthic invertebrates fulfil many important roles within marine systems, from facilitating sediment oxygenation due to their burrowing activity, to recycling nutrients that support higher trophic levels (Abrantes & Sheaves 2009, Kristensen & Kostka 2013, Queirós et al. 2015), with individual taxa potentially contributing differently to each function. Therefore the benthic community can influence the system functionality depending on its taxonomic composition. For instance, high densities of bioturbating organisms (e.g. sipunculids, crabs, burrowing annelids) is likely to lead to nutrient recycling being the main function of the benthic community (Queirós et al. 2013). Similarly, high densities of peracarids and other infaunal crustacean, might represent a feeding hotspot for higher trophic groups (Sheaves et al. 2016a).

1.4 Implications and thesis objectives

The current knowledge gap regarding some aspects of mangrove ecology can have repercussions in the way we conserve and evaluate the services mangrove forests provide. For instance, investigating the benthic composition within mangrove forests and how it is influenced by DO becomes more important in light of the increased frequency of hypoxic events induced by human activities in coastal areas. Vaquer-Sunyer and Duarte (2008) observed that cases of hypoxia in coastal waters have increased 5.5% annually in the globally published literature over the past decades. The impacts of such events on faunal assemblages are difficult to estimate because of a lack of understanding of faunal distribution and responses under normal conditions. Because mangrove forests occupy coastal regions, which are areas likely to experience human induced hypoxia, understanding the benthic invertebrate-DO link becomes a priority in order to predict the repercussions that hypoxic events might have on mangrove functionality, and develop appropriate management and monitoring plans.

A full understanding of the variability of the mangrove infauna communities based on their environmental settings is also necessary to improve our ecological knowledge and develop better conservation policies. For instance, governmental agencies have policies aimed to offset mangrove loss and preserved mangroves forests function within the system (Fisheries Queensland 2012). However without a strong understanding of the benthic infauna community composition and how the benthic fauna changes based on forest type and environmental settings, conservation efforts might be unsuccessful. In fact, variations in the benthic infauna communities can cause differences in the system functionality (Solan et al 2004), meaning that mangrove functional roles may vary across mangrove forests and perhaps not all forests provide feeding ground for nekton.

Currently, there is still uncertainty in regards to what factors influence the infauna community of mangrove forests, therefore evaluating how aquatic organism use the system can be complex. At a global scale, the largest differences in the degree of mangrove utilization by aquatic fauna are between studies conducted in the Caribbean and the Indo-Pacific. However, the differences might not be solely due to the different geographical regions and tidal ranges. The variation in results could be due to the fact that many studies conducted in the Indo-Pacific have been carried out in estuarine mangrove forests, as opposed to clear water lagoons and islands in the Caribbean. There has not yet been an evaluation of how environmental settings can lead to changes in benthic community and the overall function of the forest within the system. Therefore, understanding these aspects can substantially improve our understanding of mangrove ecology and their functional role.

The overarching aim of this thesis is to assess the extent to which DO and environmental settings influence benthic infauna composition and distribution in tropical *Rhizophora stylosa* mangrove forests. This is accomplished by addressing four main questions:

1) What are the dynamics of DO within *Rhizophora stylosa* forests?
2) What is the structure of benthic invertebrate communities of estuarine *Rhizophora stylosa* forests?
3) To what extent is DO responsible for determining the composition of benthic assemblages of estuarine *Rhizophora stylosa* forests?
4) To what extent do the benthic communities of *Rhizophora stylosa* differ between estuarine and non-estuarine forests?

Chapter 2 - Ecological implications of dissolved oxygen dynamics in tropical mangrove forests

2.1 Introduction

The coastal intertidal zone is a harsh environment where temperatures, wave energy and tidal levels undergo extreme variations over relatively short time frames (e.g. hourly) (Peterson 1991). Despite the harshness of this environment, species richness and biomass are often high, supporting a variety of important ecological processes (Sheaves et al. 2016a). In tropical regions the mid to high intertidal zones are occupied by mangroves, a unique habitat that fulfils a variety of ecological roles for many fish and invertebrates, at various life stages (Dorenbosch et al. 2004, Duke 2006, Aburto-Oropeza et al. 2008). Mangroves also provide many environmental services, including coastal protection from waves, wind and erosion (Costanza et al. 2008), and carbon sequestration (McLeod et al. 2011), making them highly valuable ecosystems (Rönnbäck 1999, Wilson et al. 2005).

Despite the values attributed to mangroves, little research has focused on understanding the biological and physio-chemical properties of these habitats. For instance, due to high rates of decomposition by bacteria (Alongi 1994, Holmboe et al. 2001), soil within mangrove forests becomes anoxic within a few millimetres below the surface (Andersen & Kristensen 1988). This high oxygen demand is likely to result in substantial oxygen depletion of overlying waters, paralleling the situation seen in freshwater environments, where organic sludge can account for over 40% of the total oxygen demand of fresh waters (Streeter 1931, Martin & Bella 1971). However, this has rarely been verified (Knight et al. 2013), nor have the consequences of anoxic water conditions for physical and ecological processes within mangrove forests been thoroughly investigated.

Logic suggests that dissolved oxygen (DO) is likely to be an important determinant of the species that use mangroves, and the patterns and extent of their utilisation. Knight et al. (2013) were the first to investigate DO in mangroves. They observed substantial spatio-temporal variation in DO, and identified two particular patterns. Firstly, there were substantial differences in DO trends between night and day. Secondly, DO in remnant pools in basin forests (forests where water remains after tidal disconnection) fell rapidly to zero after the tide had ebbed. The extent and nature of the DO variations suggests that DO has the potential to affect animal access and use of mangrove forests. In fact, Knight et al. (2013) found that the anoxic conditions were beneficial for mosquitoes that were absent from more oxygenated waters accessible to fish predators. It is likely that the anoxic conditions also influence the behaviours and adaptations developed by animals that inhabit mangrove forests. These are known to be quite varied. For instance, during low tides mangrove forests are occupied by semi-terrestrial crabs (e.g. *Uca* spp., *Sesarme* spp. *Metopograpsus* spp.) capable of “breathing” air (MacNae 1968, Robertson 1986, Takeda et al. 1996, Morris 2002), while, fish of the genus *Periophthalmus* (mudskippers) are known to actively transport air into their burrows at low tide to overcome hypoxic conditions (Ishimatsu et al. 1998) as well as being able to respire across body surfaces. In contrast, many other marine organisms that inhabit mangrove forests, such as benthic infauna and other nekton, do not display obvious ‘air breathing’ behaviours, and so are likely to rely on daily hydro-connections to replenish oxygen levels and allow them to perform crucial metabolic functions.

Detailed understanding of natural DO fluctuations, and the major factors affecting them, is central to expanding our understanding of the ecological processes influencing life in mangrove forests. It is also critical in providing a basis for mitigation plans aimed at protecting the quality and ecosystem values of mangrove systems (Diaz 2001).

In order to begin developing an integrated understanding of DO dynamics in mangrove forests we investigated DO variations in the water flooding a near-pristine tropical Australian meso-tidal mangrove fringe forest across multiple tidal cycles, seasons and tidal heights. Our aim was to create a predictive model to explain the pattern of DO change across the forest and determine the major factors affecting DO trends.

2.2 Materials and Methods

2.2.1 Study location

The study was conducted at Blacksoil Creek, located within Bowling Green Bay National Park in tropical north Queensland, Australia (Figure 2.1). Blacksoil Creek is a bar-built estuary with a tidal range of approximately 4 m above LAT (Lowest Astronomic Tide). The estuary is surrounded by dense mangrove forests, which are mostly composed of *Rhizophora stylosa* around the water's edges, with *Avicennia marina* occurring throughout the forest and *Ceriops* spp. present at landward margins. Because Blacksoil Creek is part of a National Park human disturbance is relatively low with no urban development or agricultural land in its immediate catchment.

Sampling areas were located on the west bank of the estuary (19.29806 S, 147.04083 E), close to its mouth, within a fringing *R. stylosa* forest approximately 600 m long and 60 m wide. The forest is characterized by high canopy cover and thick root webbing, making access to the forest complicated. The landward margins are characterized by the high presence of new recruits, indicating that the forest is still expanding landwards. Forest inundation begins once tides exceeds 2.6 m, meaning the forest remains disconnected at low tide and during neap high tides.. Tidal cycles at Blacksoil Creek are diurnal unequal, with the hotter months (October to March) having diurnal tides larger than the nocturnal tides and vice versa during the cooler months (April to September).

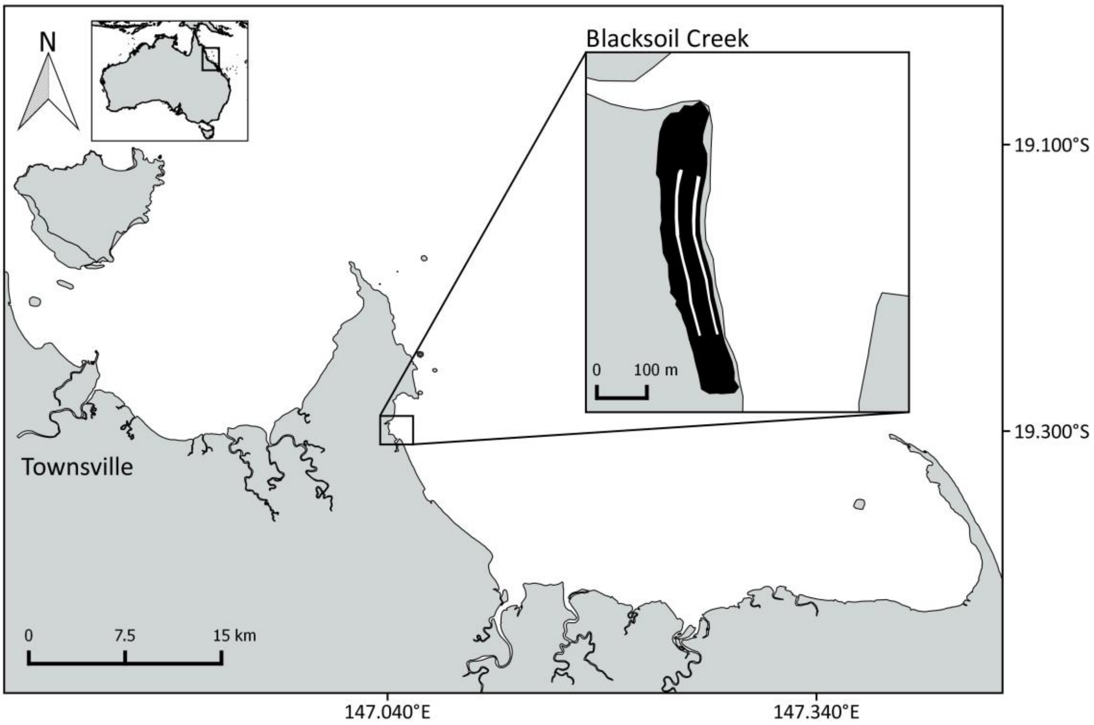

Figure 2.1: Map of the study site, Blacksoil Creek. The blackened area represents the *Rhizophora stylosa* forest used for this study, the white lines are the regions within the forest that were sampled. The seaward site was 10 m from the mangrove edge, while the landward site was at 40 m.

2.2.2 Data Collection

Sampling began in November 2013 and continued at regular intervals throughout the following year (December 2013, April 2014, June 2014, July 2014, October 2014, and November 2014). Dissolved oxygen and temperature measurements were obtained using an YSI ProODO Optical Probe, while water depth was measured using an in-situ Level Troll 100 pressure transducer attached to the oxygen probe. The DO probes were placed within the forest at 10 and 40 m distances perpendicular to the water's edge. At each distance, probes were placed as close as possible to the substrate (but not in depressions that retained water at low tide), in order to provide a measurement as soon as the tide entered the forest. Two probes were placed at each distance within the forest spaced by at least 50 m, in order to overcome possible equipment failure and have a second independent reading at each location. During October and November 2014, a second set of DO probes and pressure transducers were placed at each site and distance. This second set was placed in a depressed area of the substrate that retained water at low tide (i.e. small pools). The depressed-substrate probe provided a measurement of water that remained during low tide, providing potential refuge for animals. The probes recorded temperature (°C), DO (percentage saturation) and depth (cm) at intervals of 5 minutes. A total of 32 tidal cycles were sampled across the range of tidal phases (spring and neap), with the probes left in place for multiple consecutive tidal events.

2.2.3 Data Analysis

Visual inspection of the data indicated that some tidal cycles were incomplete due to equipment failure (e.g. expended batteries), so these were removed from the dataset. The data were represented graphically using LOESS smoothers with a span level of 0.2, in order to highlight the general DO trends. The smoothers' span was chosen to be small (i.e. 0.2) enough to display and highlight the natural DO profile, while reducing the statistical noise that arises due to the intrinsic variability of DO fluctuations. To provide a general understanding of the variability around the fitted line we plotted the 95% confidence intervals estimated for each fitted value. The smoothers were constructed after dividing the dataset into diurnal and nocturnal tides, to highlight any effects of time of the day, and then grouped based on the maximum realized depth (greater than 80cm, between 80 and 40cm and less than 40cm), in order to compare DO profiles for tides of similar amplitudes. Additionally, to highlight any pattern that could arise by the different tidal phases (i.e. flooding, ebbing), each DO profile was synchronized in time, according to the peak of the tide.

The data are displayed for both the 10 m site and the 40 m site. The data for the smaller night tides at 40 m was excluded from this analysis as the probe was underwater for less than 1 hour at each tidal connection, and therefore there where too few points available to provide a valuable representation of the general DO patterns. There were no diurnal tides greater than 80 cm during the study period.

To investigate variations in the rate of DO change during the ebbing phase of the tide, the slope of DO change during the ebbing tide was calculated for each tide profile and plotted against maximum realized depth. This dataset was again divided based on time of the day and distance within the forest. Data were analysed using polynomial regressions for the night tide and linear regressions for diurnal tides, the best fitting model was chosen using a partial F test that compared polynomial and linear regression models.

Data were analysed using Random Forest predictive models, an optimized version of Classification and Regression Trees (Breiman 2001, Segal & Xiao 2011), employed on the entire dataset to determine which predictor variables were best at explaining tidal DO patterns at both the 10 and 40 m sites. Predicting variables used were: Daytime (time of the day), sun classification (i.e. early day= first hour since sunrise, day = until sunset, early night=first hour since sunset, night = until sunrise), depth (cm), tidal velocity (cm/min), tidal phase (i.e. ebb, flooding), temperature (°C), time after sunrise (with minutes of no light after sunset, represented by negative values), season (post-wet, pre-wet, dry). Random Forest Decision Trees were chosen for their ability to handle repeated measures and variables measured on different scales (Zuur et al. 2007, Kane et al. 2014). All tests were carried out using R statistics (RCoreTeam 2015) and the Random Forest Model was implemented using the 'randomForest' Package (Liaw & Wiener 2002).

2.3 Results

There were consistent patterns in DO dynamics among tidal cycles based on the sensors just above the sediment (Figure 2.2). During nocturnal inundation, and regardless of tidal height and duration, the 40 m sites always had lower DO levels than the 10 m sites; generally at least 5% lower. Furthermore the 40 m sites (regardless of time of the day) always had lower DO when the tide first flooded the forest and then rapidly increased to normal levels. Larger tides were generally richer in oxygen, with a smoothed trend that maintained higher DO for longer than during lower tides. In contrast, medium and lower tides showed trends with occasional humps caused by more erratic changes in DO levels during smaller tidal inundations. Additionally, during flooding tide the overall DO trends were fairly constant, with small fluctuations, particularly during the larger tides. However, after the tidal peak, the DO trend displayed an increasingly negative slope indicating that DO was decreasing rapidly. These DO patterns were repeated across all tidal categories, except for medium tides during daylight. In that situation the DO patterns became inverted and, instead of decreasing, the oxygen levels became oversaturated. This pattern of oversaturation was not observed during any smaller diurnal tides.

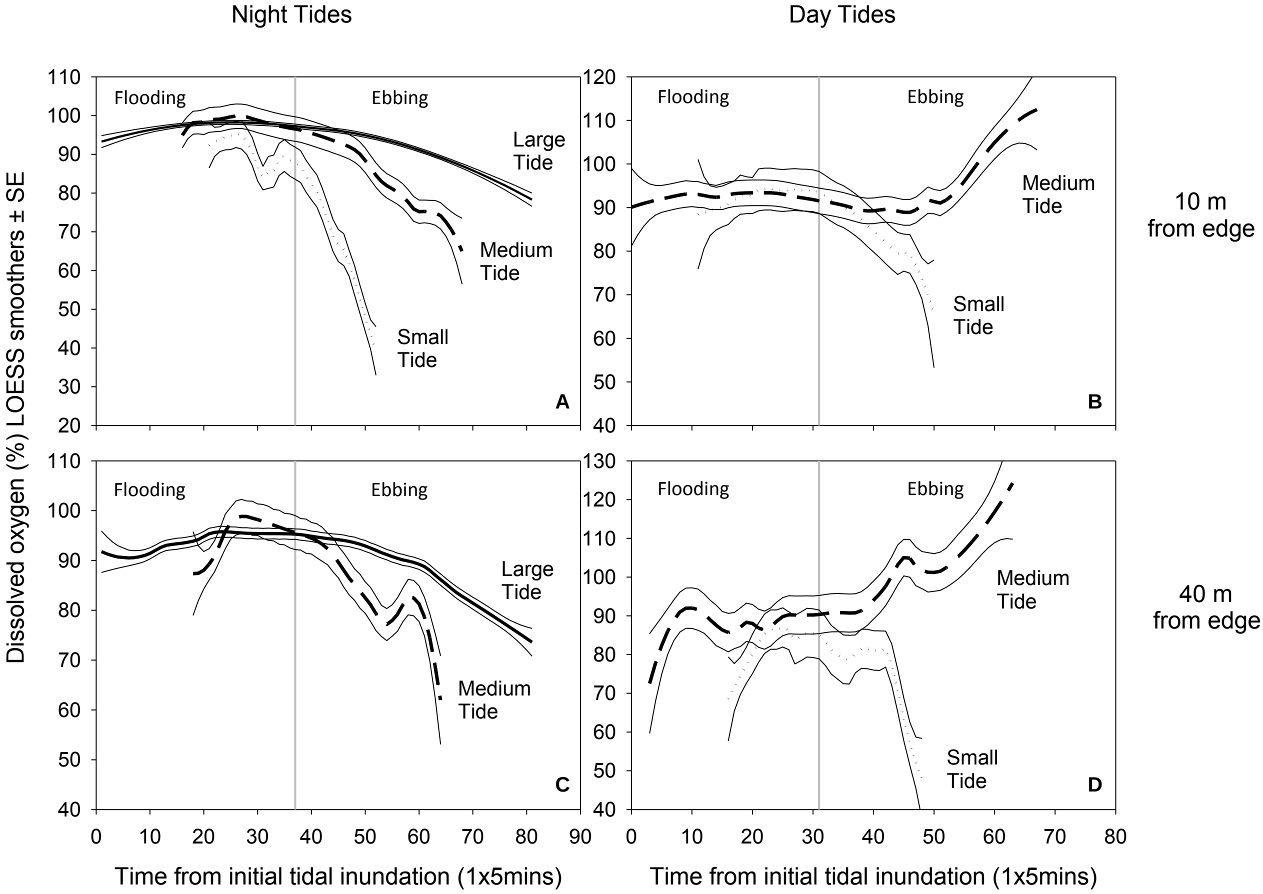

Figure 2.2: Dissolved oxygen (%) trends for the water column, displayed using LOESS Smoothers (span=0.2), the left plots display nocturnal tides (A,C), and right plots diurnal tides (B,D). The plots at the top are the 10 m sites (A,B), while the bottom plots are the 40 m sites (C,D). The different line intensities represent the DO profiles at different realized tidal ranges, each line is surrounded by the 95% confidence intervals estimated for each fitted value (solid line = tides larger than 80 cm, dashed line= medium tides comprised between 40cm and 80cm, dotted line= tides smaller than 40 cm). The vertical line represent the peak of the tide.. The X-axis represent the recorded data points at 5 minute intervals. Note: There were no day tides larger than 80cm. The 40 m sites did not have a DO profile for tides smaller than 40cm because the inundation time was limited and the variance too high to display meaningful smoothers.

The rate of change of ebb tide DO with maximum realised depth of each tide indicates patterns of dependency of DO on tidal height, with contrasting trends between night and day (Figure 2.3). During night tides both the 10 and 40 m sites displayed negative slopes (indicating decreases in DO levels), with the rate of DO decrease declining as maximum depth increased. At both distances, DO trends followed a polynomial distribution ($r^2 = 0.87$, $F_{2,14} = 46.01$, $p<0.001$, and $r^2=0.89$, $F_{2,11}=46.27$, $p<0.001$, for 10 m and 40 m respectively), characterized by large negative slopes at shallow maximum depths. Once the maximum depth reached a critical value (60 cm at the 10 m sites, and 40 cm at the 40 m sites), DO index rate of change become smaller reaching an asymptote, indicating that DO levels were declining at a stable low rate. In contrast, during day tides DO slopes became positive at greater maximum depths and, unlike the night tides, there was no obvious critical value at which the slopes become positive (Figure 2.3), but rather the relationship between DO slope and maximum depth continues to exhibit a more or less linear trend ($r^2=67$ $F_{1,11}=22.79$, $p<0.001$, and $r^2=0.24$, $F_{1,10}=3.16$, $p>0.05$, for 10 m and 40 m respectively).

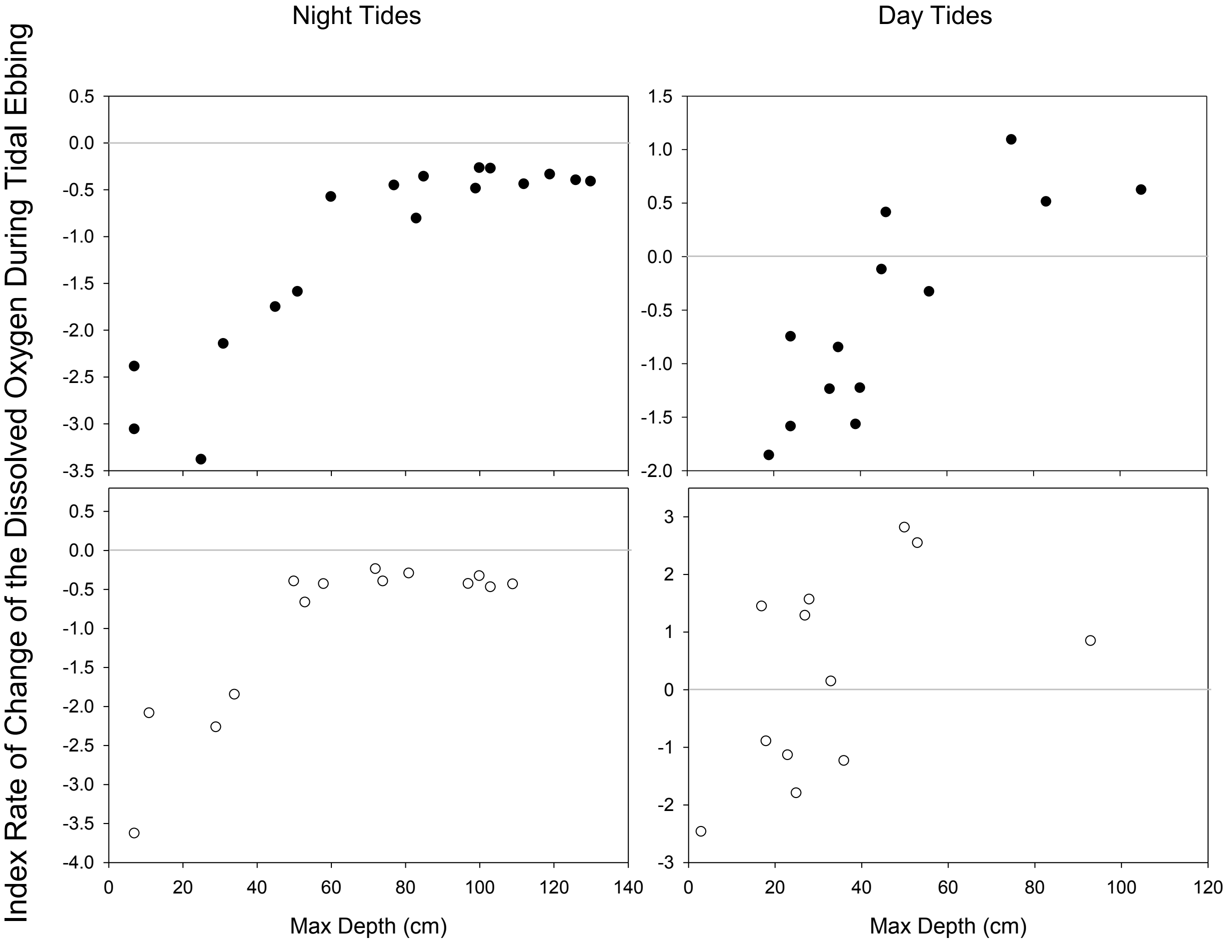

Figure 2.3: Scatterplot of the index rate of change of the dissolved oxygen during tide ebbing versus the maximum tidal depth. Divided by night tides (on the left) and day tides (on the right) the full circles are the sites at 10 m within the forest, while the empty circles are the 40 m sites. The negative value of the slope means that the DO is declining, while the bigger the value the steeper is the slope, with zero being perfectly parallel (not increase nor decrease in slope).

The Random Forest model of dissolved oxygen levels for the 10 m site, constructed using the DO readings of the probes just above the sediment (Figure 2.4a) explained 77.45% of the total variance, indicating that the model had good predictive capability. Depth was the most important variable for predicting dissolved oxygen level at 10 m, with its exclusion from the model increased the mean square error by more than 60% (Figure 2.4a). Time after sunrise was also an important predictor of DO levels at 10 m sites (50% increase in mean square). tidal phase and tidal velocity were of lesser importance but still accounted for 40% and 30% change in mean square respectively.

A Random Forest model of dissolved oxygen levels at 40 m site (Figure 2.4b) explained 62.55% of the total variance, with depth again the most important predictor (56%). All other variables were substantially less important for predicting DO (ranging from 10-30% variable importance), in particular, time after sunrise was not an important predictor in contrast to the 10 m.

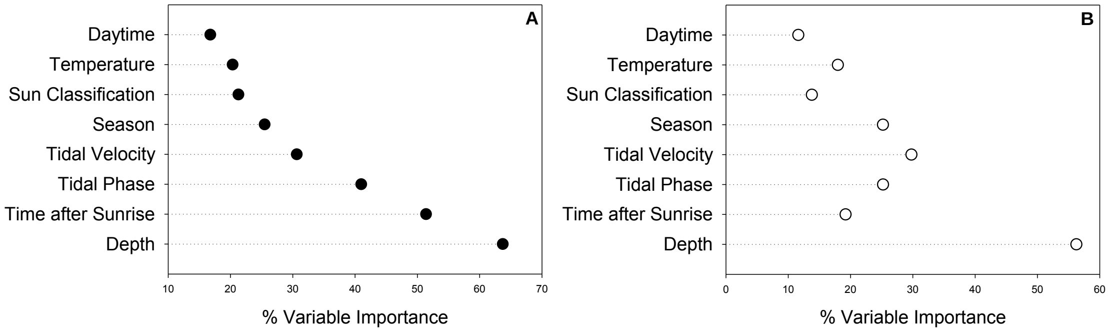

Figure 2.4: Random Forest importance variable plot, describing the importance of each variable in constructing the two models, A) for the 10 m sites, and B) for the 40 m site.

DO dynamics in forest pools (i.e. where water was retained at low tide) (Figure 2.5) varied substantially between spring and neap tides for both 10 and 40 m sites. During spring tides, DO reached levels well above 80% during tidal connections and decreased to almost zero during tidal disconnection (Figures 2.5a,c), with the rate of decrease occurring at a faster rate at the 40 m site. There was a sole anomaly in the pattern, presented by DO staying low during diurnal spring tide at the 10 m site. This could have been caused by limited water mixing occurring within the depression point. However, this pattern does not match what has been seen elsewhere and therefore need to be interpret with caution. At the 10 m sites DO rarely exceeded 60% during neap tides and remained well below 20% during tidal disconnection. These very low DO levels were maintained for almost 24 hours when high tide occurred at night. This was likely due to a combined effect of little water mixing (due to the low water depth) and the high oxygen demand of the sediment (e.g. organic decomposition and aquatic bacteria). DO trends at the 40 m sites were similar, with the exception that during tidal disconnection DO goes well below 10%, indicating a border-line anoxic environment (Figures 2.5b,d).

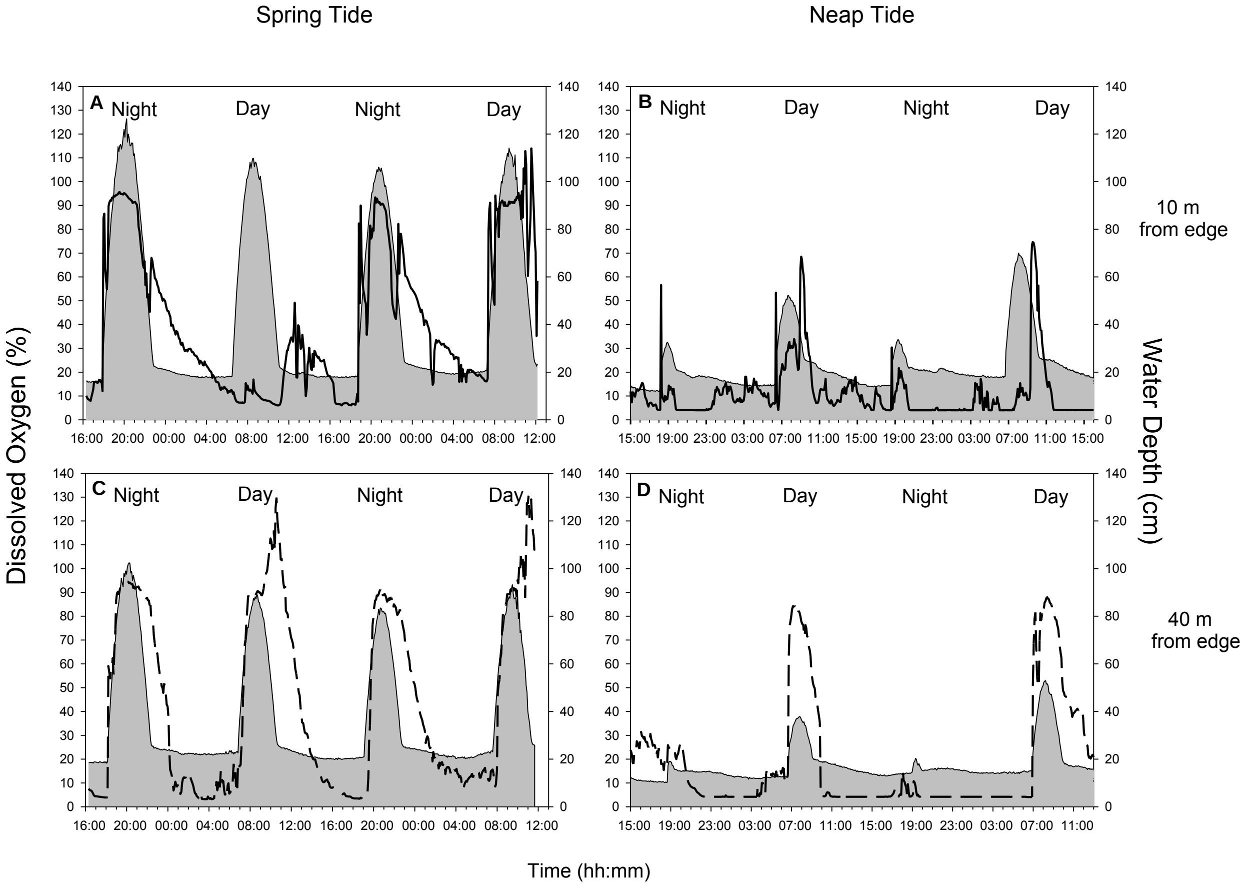

Figure 2.5: Dissolved oxygen and water level trends at 5 minute intervals in small pools within the forest. Left plots show dissolved oxygen trends during spring tide connection, while right plots are during a medium neap tide. The shaded area is the water depth, while the line describes the dissolved oxygen trends. On the top the DO trends are for the 10 m sites (A,B), while the bottom plots are for the 40 m sites (C,D).

2.4 Discussion

2.4.1 Environmental factors affecting dissolved oxygen

DO dynamics in mangrove forests showed clear, mostly repeated patterns, with oxygen levels varying across the whole saturation spectrum, from normoxic to anoxic. Variation in DO was strongly associated with tidal amplitude and diel variability, with diurnal tides showing different trends from the nocturnal ones. This variability in DO levels based on discrete factors, could imply that some of the biological processes occurring inside the forest might be regulated, or at least affected by DO dynamics, during tidal connection. Additionally, DO levels in pools of water remaining during tidal disconnection rapidly became anoxic and remained low in oxygen for extended periods. This extended anoxia suggests that we may need to rethink our current conceptualization of the way animals use mangrove forests at low tide.

Water depth, tidal phase, time of the day (time after sunrise) and tidal velocity had the greatest influences on the DO dynamics during tidal connection at both 10 and 40 m into the mangrove forest. The interactions between these factors led to substantial variation in DO levels**.** For instance, DO dynamics were generally more variable during the ebbing phase of the tide as opposed to the flooding phase, this extra variability was caused by the interactions of factors such as the amount of light (day or night) and tidal amplitude (i.e. neap or spring)(Figure 2.6). Light and tidal height are likely to influence the effect that sediment oxygen demand (SOD) has on the DO within the water column. SOD varies with flow velocity and water volume, therefore a smaller slow moving tide (i.e. neap tide) would enhance the oxygen stripping by SOD leading to lower DO (Mackenthun & Stefan 1998). This effect could also explain why tidal velocity was important in determining DO levels at 40 m, because water had to pass across a greater expanse of sediment to reach the landward site. Enhancing the effect of SOD, particularly during slow flowing tides.

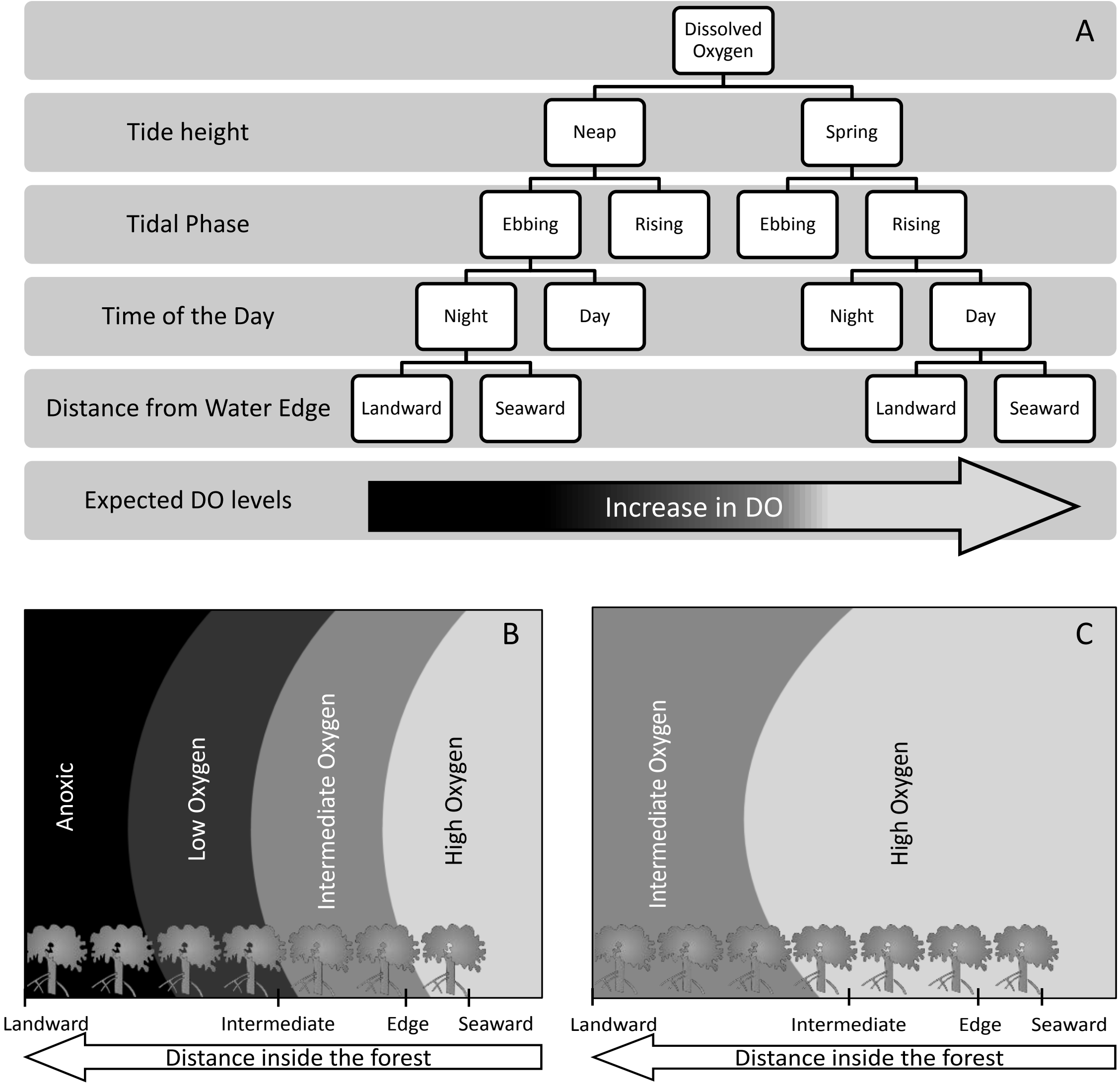

Figure 2.6: Conceptual model and conceptual maps of the generalized trend of dissolved oxygen inside mangrove forests. The model (A) describes low DO levels on the left, and high DO levels on the right. Conceptual maps are a visual representation of the two extreme outcomes described in the model (B= the left path of the model, C = the right path of the model). Intermediate levels of DO have more variable outcomes, but generally lie within the extreme conditions. The factor Time of the Day is a categorized version of time after sunrise.

There were some unexpected results but these can be related to known physical and biological factors. In fact in the present study the daylight larger tides have DO increasing as the tide ebbed at both distances (observable in the smoothed profiles). This pattern was likely due to interaction with cyclic tidal fluctuations. In tropical Queensland larger diurnal tides occur in summer months (October to March), a time when chlorophyll concentration in estuaries have been shown to increase (Alpine & Cloern 1992, Gong et al. 2003). Hence, the higher photosynthetic activities and the longer exposure to light at the end of the tide would cause DO to spike effectively counterbalance the DO stripping by SOD. More generally, there was an interesting pattern at the 40 m site that was not observed at the 10 m site. Usually at the 40 m site DO was initially very low at the beginning of the flood tide rising rapidly shortly after. This may be explained by the channelling of water through crab burrows during the flooding phase of the tide (Ridd 1996, Stieglitz et al. 2000), resulting in the first water reaching internal parts of the forest being anoxic water accumulated in burrow systems during tidal disconnection.

2.4.2 Ecological Inferences

The observed patterns in DO dynamics have implications for the ecological functioning of mangrove forests. The current understanding is that when the tide floods mangrove forests, fish and other nekton move in to take shelter among mangrove roots and/or access food (e.g. benthic invertebrates) (Robertson & Duke 1987, Nagelkerken et al. 2001, Verwey et al. 2006). However, the low DO levels regularly occurring in mangrove forests during certain tides (i.e. ebbing night tide, neap tide, and low tide) would limit the extent to which many organisms are able to utilize mangrove forests. This means that the length and timing of organisms using mangrove forests might not simply be dictated by water level, but substantially affected by dissolved oxygen dynamics. Despite contrasting views in the literature regarding the maximum tolerance of most fish to low DO (Vaquer-Sunyer & Duarte 2008, Butler et al. 2010), it has been proposed that DO levels do not need to reach extremely low saturation to render sites unpleasant for many nektonic organisms. Instead, DO that are just 20% or 30% below saturation can already lead to respiratory stress for most nekton (Butler & Burrows 2007, Levin et al. 2009). This means that DO is likely limiting the time and duration that nekton are able to access mangrove forests, as well as preventing mangrove utilisation by species intolerant to low DO. This could explain the low utilisation of tropical northeastern Australian mangrove forests by nekton (Sheaves et al. 2016b). Moreover, DO levels may act as sensitive cues allowing nekton to determine when to access or leave mangrove forests.

Low oxygen levels also have far-reaching implications for mangrove macro- and micro-benthic animals. In fact marine and estuarine environments that undergo periodic hypoxia (Jorgensen 1980, Rosenberg et al. 2001), have been observed to have a much lower taxonomic diversity with some taxa almost completely absent from the affected region (Rabalais et al. 2002).

There are some studies that have described the mangrove benthic fauna as having lower taxonomic diversity and animal density when compared to adjacent habitats (e.g. seagrass, mud/sand flats)(Wells 1983, Dittmann 2001, Alfaro 2006). This would align with what would be expected of a low oxygen environment, as the DO dynamics observed at Blacksoil Creek showed that remnant pools quickly became anoxic during tidal disconnection, reflecting similar findings by Knight et al (2013). This means that mangrove forest benthic composition could be shaped by DO dynamics, unless they have developed some physiological or behavioural adaptation (Johansson 1997a). This could be the consequence of the high presence of semi-terrestrial organisms, such as crabs and mudskippers inside mangrove forests. However, currently, it is not known whether other benthic organisms have also developed adaptations to cope with a highly anoxic environment. Additionally low oxygen level do not need to be a common occurrence, as even one low oxygen event may render a site unsuitable due to the low motility of most benthic taxa once settled (Lawrie & Raffaelli 1998, Negrello Filho et al. 2006).

If DO levels are indeed a limiting factor for benthic taxa distribution, including key components to fish diets, this would reduce mangrove forest’s value as feeding sites for many species of nekton. It is evident that some mangrove forest processes are still under-investigated and there are substantial gaps in our current understanding. It is likely that DO dynamics regulate some of these processes, however further investigation is needed in order to quantify the response of mangrove fauna to DO. Understanding these processes and how DO affects them, is particularly relevant as human activities in the rivers catchments, together with the current climatic uncertainty, are leading to an increase of hypoxic events in estuarine systems (Diaz 2001). As DO in estuarine mangrove forests already seems to be in precarious balance, it is likely that small disruptions in the natural hydrology and chemistry might have deleterious effects for the system. It is likely that a single low oxygen event could shift the fauna community (King et al. 2012) and impact the ecological function of mangrove ecosystems. For this reason, implementing DO monitoring and management in areas at risk could help preserve mangrove forest functionality or identify high risk areas.

Chapter 3 - Benthic invertebrate community composition within macro-tidal estuarine *Rhizophora stylosa* forests

3.1 Introduction

The importance of benthic invertebrate fauna in regulating environmental processes in marine ecosystems is indisputable. Their functional roles include: influencing structural complexity, nutrient recycling, sediment bioturbation, and providing trophic linkages between primary producers and higher consumers (Underwood 2000, Coen et al. 2007, Queirós et al. 2013, McPhee et al. 2015). Benthic invertebrate communities are often instrumental in regulating the composition and distribution of nekton at higher trophic levels by providing bottom-up control (Grebmeier et al. 2006, Davis et al. 2014). Furthermore, benthic invertebrates are key indicators for the assessment of environmental changes and are used to detect habitat degradation (Dufrene & Legendre 1997, Dauvin 2007). Due to their importance in the ecology of estuarine and coastal systems, many studies have focussed on understanding the factors influencing benthic invertebrate distribution, and determining hotspots of benthic biodiversity (Connel 1972, Bredenhand and Samways 2008, Sheaves et al 2016). In coastal and estuarine systems, intertidal flats are a zone of high benthic density and species diversity, as most of the subtidal population is depleted due to constant availability to predation (Gilinsky 1984, Little 2000, França et al. 2009, Sheaves et al. 2016a). However, in tropical regions the higher intertidal zone is commonly occupied by mangrove forests, thereby spliting the intertidal into two distinct biotopes. Nonetheless the majority of research on benthic communities in tropical intertidal mangroves has focussed on tidal flats, with fewer studies conducted within the mangrove forests themselves (Dittmann 1995, Dittmann & Vargas 2001, Bosire et al. 2004, Gladstone-Gallagher et al. 2014). Furthermore, research within estuarine mangroves forests has mostly focussed on the larger epibenthic taxa, such as crabs and large whelks (Wells 1983, Lee 1998, Koch & Wolff 2002, Macintosh et al. 2002, Bosire et al. 2008, Rajpar & Zakaria 2014), which are easily observed in the field with the naked eye. In contrast, the infauna is underrepresented in the literature, with just a few studies conducted in forests characterised by different attributes. For instance, studies were conducted in forests dominated by different mangrove species, affected by different degrees of human disturbance, and with differences in annual rainfalls and tidal ranges (Sasekumar & Chong 1998, Dittmann 2001, Alfaro 2006). This has led to a substantial gap in knowledge of the benthic invertebrate community of mangrove forests and their role within these systems. Understanding the benthic community composition of mangrove forests can assist our evaluation of some of the ecosystem services fulfilled by mangroves. For instance, there is general consensus that mangrove forests are important feeding grounds for juvenile nekton. However, the scientific evidence to support this paradigm is not convincing, with studies reporting contrasting evidence (Huxham et al. 2004, Tse et al. 2008, Bower 2010). This is because most of the studies focussed on determining the value of mangroves as nekton feeding ground have used one of two approaches; analysing fish feeding studies, or more recently by performing stable isotope analysis (SIA) on nekton (Cocheret De La Morinière et al. 2003, Lugendo 2007, Baker et al. 2014). However, both techniques have flaws that complicate interpretation. While feeding analyses provides detailed information on prey consumed, it does not indicate when and where the prey was ingested (Lugendo et al. 2007). This is an even greater problem with SIA because it integrates over time periods of days to months so there is no way of determining the extent to which a carbon signature reflects feeding inside the mangrove forest (Bouillon et al. 2008). Additionally, to interpret SIA results it is necessary to have detailed information on the isotopic signature of prey resources and the diet-tissue discrimination factors for consumer tissues. However, these data for mangrove forests are still poorly understood and new studies are constantly challenging our understanding (Bui & Lee 2014, Connolly & Waltham 2015). If we understand the benthic community of mangrove forest, in turn we would also improve our understanding of nekton reliance on mangrove resources. This is because by knowing what taxa occur within mangrove forests and their distribution we can infer whether mangroves are indeed key feeding grounds. For instance, using feeding analyses we know that many estuarine juvenile fish tend to heavily feed on small crustaceans, particularly peracarids (i.e. amphipods, tanaids, mysids) (Salini et al. 1990, Baldoa & Drake 2002, Nanjo et al. 2008), therefore if mangrove forests are important feeding grounds for juvenile nekton, we would expect to have high density of the prey types (e.g. peracarids) that feature prominently in diets of estuarine fish. Alternatively, if some key prey taxa do not occur within mangrove forests, but just on the adjacent tidal flats, we could easily infer that food acquisition occurs on the tidal flat for that particular fish species.

This study aims to describe the composition and distribution of benthic taxa along a longitudinal gradient, across multiple macro-tidal tropical estuarine mangrove forests. Additionally this study would provide an insight on the prey availability for juvenile nekton within mangrove forests based on known diet preferences.

3.2 Materials and Methods

3.2.1 Study Location

The study was conducted in two estuaries, Blacksoil Creek (19.29806 S, 147.04083 E) and Cassady Creek (18.74166 S, 146.28916 E), in tropical northeast Queensland, Australia (Figure 3.1). Both estuaries have a tidal range of approximately 4 m above LAT (Lowest Astronomical Tides) and are surrounded by thick mangrove forests dominated by *Rhizophora stylosa*, with sparse occurrences of *Ceriops* spp. and *Avicennia marina*. Urban and agricultural development is not extensive in the immediate vicinity of either study location, therefore direct human impact on the study sites is considered low. Additionally, Blacksoil Creek is located within the Bowling Green Bay National Park, further ensuring minimal impact (Scheltinga et al. 2005)

The Blacksoil Creek sampling area consisted of a small *Rhizophora stylosa* forest approximately 600 m in length and extending landward for approximately 60 m. The sediment within the forest appeared to be mostly firm, composed by mud and rich in organic matter. The Cassady Creek sampling location was approximately 160 m in length and extended approximately 60 m landward. The sediment was also very firm, but composed by a mix of mud and sand, with less organic detritus when compared to Blacksoil Creek. High level of root webbing (between 50% and 80%) and thick canopy cover (more than 80%) was observed at both forests. Both study locations are close to the estuary entrance and have similar elevations with inundation starting when the tide exceeds 2.8 m in height.

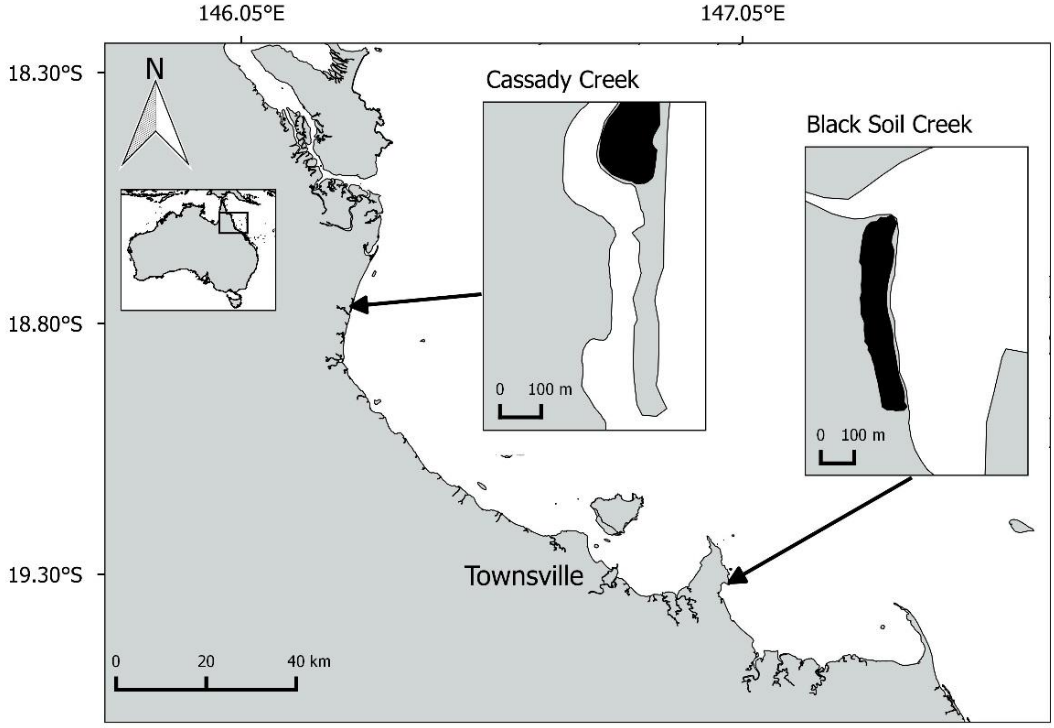

Figure 3.1: Map of the study sites, Blacksoil Creek and Cassady Creek. The black shaded area represents the *Rhizophora* forests that were sampled.

3.2.2 Data Collection

Sampling commenced in February 2014 and was carried out over the following 12 months. Samples were taken at Cassady Creek in February, April, August and December of 2014 and in January 2015. Blacksoil Creek was sampled in July, September and October 2014. The first two sampling events at Cassady Creek were part of the pilot study to evaluate the most appropriate sampling gear and design. As a result of the pilot study Cassady Creek was sampled two more times than Blacksoil Creek as the data collected using the same sampling techniques were retained to increase replication.

Sampling was conducted using a corer of 10 cm diameter and 30 cm depth. Samples were retained when the corer penetrated 20 cm to allow a standardized sampling depth. At each study location, two sites were located 50 m apart, Samples were then taken inside the forest at 10 m from the forest edge and again at 40 m, herein referred to as ‘Edge’ and ‘Deep’ respectively. Three replicate samples were collected at each distance and transported to the laboratory where they were sieved through a 0.5 mm mesh and animal sorted alive when possible. Following preliminary analysis, the tidal flat was also sampled, 1 m from the forest edge (herein refer to as ‘Flat’). The additional sampling distance was introduced at both study locations during the final two sampling trips. In total, 120 sediment samples were collected animals extracted from the samples were identified to the lowest possible taxonomic level. As the benthic infauna of mangroves in north Queensland is not well known, many specimens were only identifiable to Family or Order level.

At each location, distance and site, additional core samples of 5 cm diameter were collected and transported to the laboratory for sediment size analysis and organic matter content. All sediment samples were washed in fresh water to remove salt. Sediment size composition was determined by drying samples at 105°C, weighed and filtered through a series of stacked sieves of varying mesh size (2 mm, 1 mm, 0.5 mm, 250 µm, 125 µm, and 60 µm). The content of each sieve was weighed and the relative proportion of material from each size was calculated. The percentage organic content of the sediment was measured by loss of ignition (Dean Jr 1974) by burning 5 grams of dried sediment in a muffle furnace at 500°C for 8 hours. Subsequently, the sample was weighed and the difference in weigh was the organic content.

3.2.3 Data Analysis

Prior to analysis, sediment grains were grouped into size classes (coarse sand = >1 mm, medium sand = 250 µm – 1 mm, fine sand = 60 µm – 250 µm, and mud = < 60 µm). Sediment size and organic matter content were normalised before a principal component analysis (PCA) was carried out to describe similarities in sediment composition between locations and distances along the forest.

Invertebrate abundance data were standardized per surface area and converted into density (individuals /m^2). A two-way factorial ANOVA was performed to assess if there was a detectable difference in total animal density between the two locations and across the three sampled distances.

When assessing benthic community composition, invertebrate taxa were grouped into major taxonomic groups (i.e. Order, Class, Phylum) and only taxa which occurred in more than 5 % of samples were retained. The use of broad taxonomic groups rather than genus and species was considered acceptable for this study, as little information is lost regarding spatial differences between sites and locations, while most of the information about the functional role of the taxa is maintained (Chapman 1998). Brachyuran crustaceans were excluded from the analysis due to their high mobility, compared to other benthos, and their ability to retreat into deep burrows, causing to be sampled inconsistently (Alfaro 2006, Sheaves et al. 2016a).

A Correspondence Analysis (CA) was conducted on the major taxonomic groups to analyse the frequency distribution between the two forests and position along the forest (Flat, Edge and Deep). The CA was only carried out on data for the trips were the Flat was sampled. Consequently, in order to identify changes in benthic composition between distances within the forests (Edge, Deep), and across all sampling events, a non-metric Multidimensional Scaling Analysis (nMDS) for the ordination of species composition was carried out using a Bray-Curtis similarity matrix. Replicates were pooled together by distances and sites to minimize the effect of benthos' patchiness and every sampling event was included for this analysis. Prior to run the nMDS data were log (x+1) transformed to down-weigh the effect of highly abundant taxa, and then row standardized to emphasise taxonomic composition. In addition, loading vectors of the taxa that correlated higher than 0.5 were super-imposed on the nMDS. Analysis of Similarity (ANOSIM) was performed on the Bray-Curtis similarity matrix to quantitatively compare the differences in benthic assemblages between locations and distances.

The ANOVA and CA were conducted using R open source software (RCoreTeam 2015), employing the "CA" package (Nenadic and Greenacre 2007) for the correspondence analysis. The nMDS, ANOSIM and PCA were carried out using Primer E (Clarke 2006).

3.3 Results

The PCA generated with sediment grain size and organic matter content showed strong differentiation between the two study locations (Figure 3.2). Sediment composition at Blacksoil Creek is relatively homogeneous among replicates, with a high content of fine sand and organic matter. In contrast, samples from Cassady Creek displayed more variability, with a mix of large sand and mud. Overall, neither location displays any substantive variations in sediment composition among the three distances (Flat, Edge, Deep).

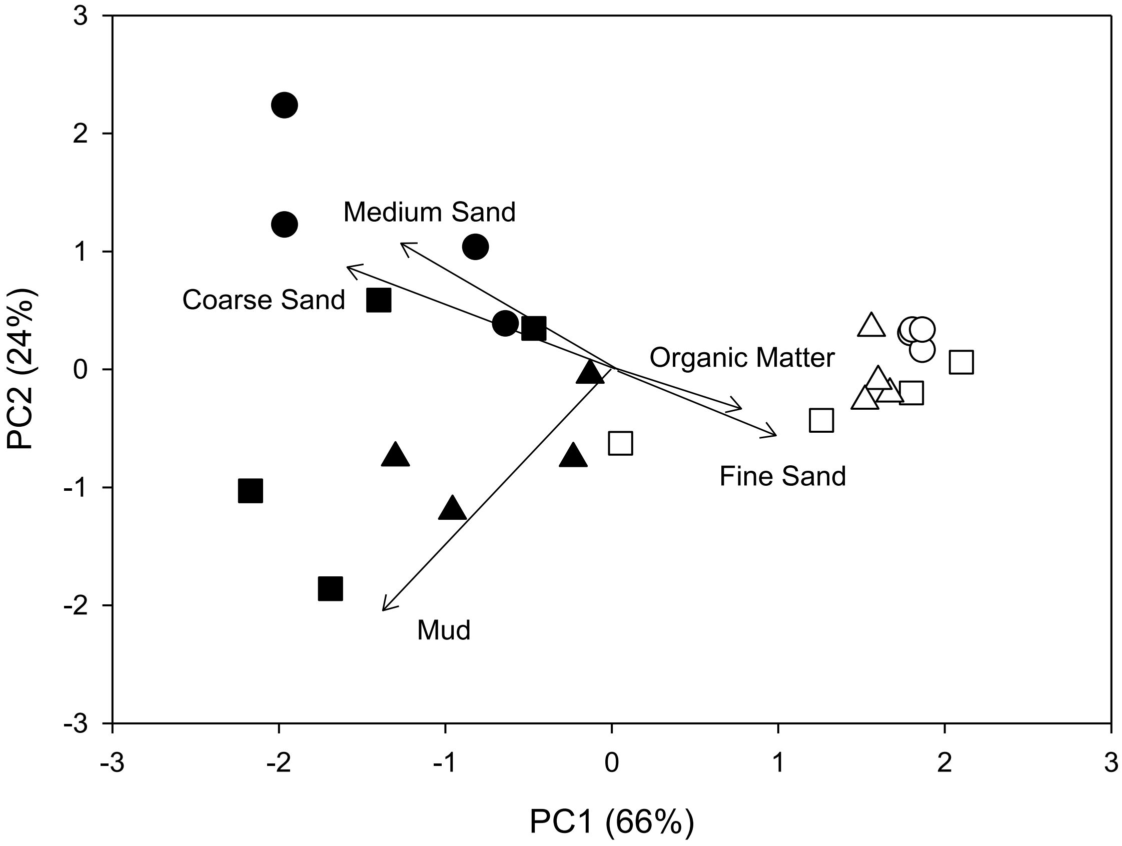

Figure 3.2: Principal Component Analysis for the sediment size and organic matter at the two study locations (Hollow = Blacksoil Creek, Black = Cassady Creek,) and the three distances along the mangrove forest (Circle = Flat, Triangle = Edge, Square = Deep).

The relative density of animals between Blacksoil Creek and Cassady Creek is similar with both locations experiencing a relatively low animal density, averaging approximately 400 ind/m^2 (Figure 3.3). Similarly, the relative density of animals based on their position within the forest (Flat, Edge, Deep) did not reveal any trends, and they all possessed analogous invertebrate density. The only exception to this was observed at the Edge at Blacksoil Creek, which had a slightly higher average of 620 ind./m^2. However, this was not considered as different from the other study sites, and the factorial ANOVA did not detect any differences in density among distances and between locations (Table 3.1).

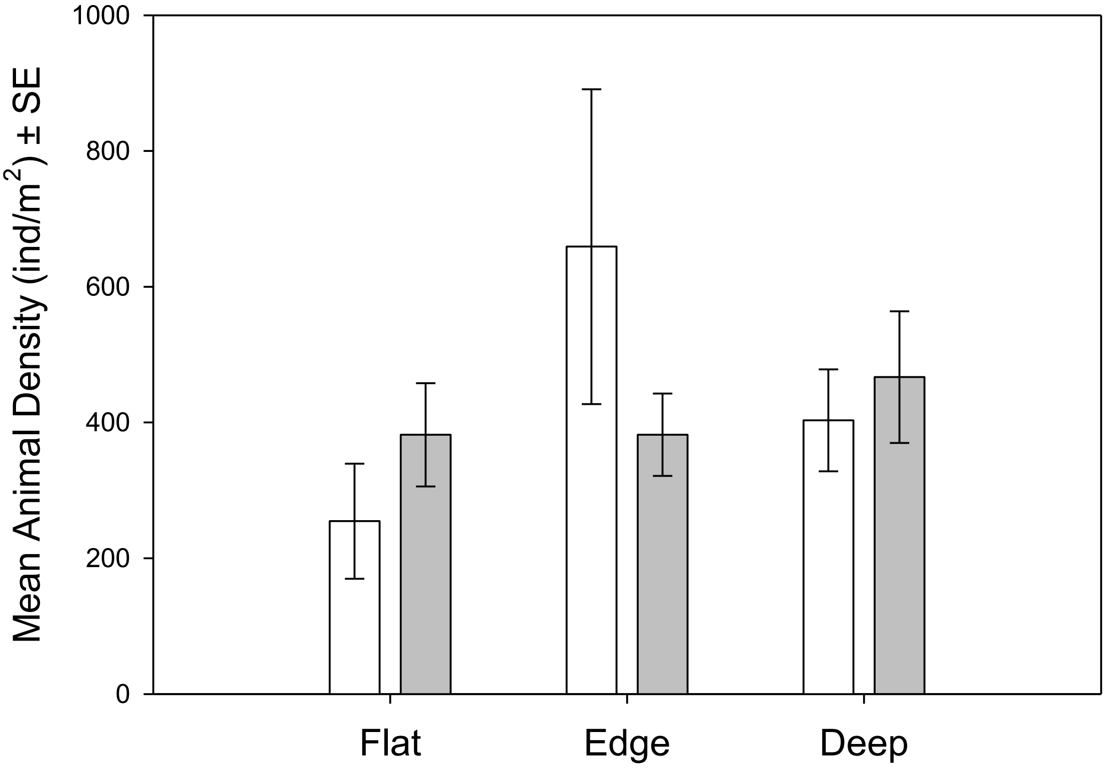

Figure 3.3: Mean animal density plot (± SE) between the three sampling distances within the mangrove forest (Flat=Outside the forest, Edge=10 m within the forest, Deep= 40 m within the forest) at the two locations (White= Blacksoil Creek, Grey= Cassady Creek).

Table 3.1: Two-way factorial ANOVA output between Blacksoil Creek and Cassady Creek, and the three distances across the forest (Flat, Edge, Deep).

	SS	**df**	**MS**	**F**	**p**
Location	14345	1	14345	0.05305	0.818514
Distance	434014	2	217007	0.80250	0.452288
Location * Distance	589001	2	294500	1.08907	0.342163
Error	18929022	70	270415		

Regardless of the similarity in density between locations and distances across the mangrove forest, a pattern was observed when the relative taxa composition per location and distance was compared (Figure 3.4). Despite Peracarida crustaceans representing a large proportion of the taxa community on the flats at Blacksoil Creek and Cassady Creek (40% and 30 % respectively), they were not recorded inside the forest during the study. Insects and Polychaetes were the most frequently occurring taxa at both forests and across the three sampled distances (i.e. Flat, Edge, Deep), with the latter dominating the assemblages within the forests. The only exception was at Blacksoil Creek, where sipunculids were the dominant taxa at the Deep sites (contributing more than 50% to the entire animal composition).

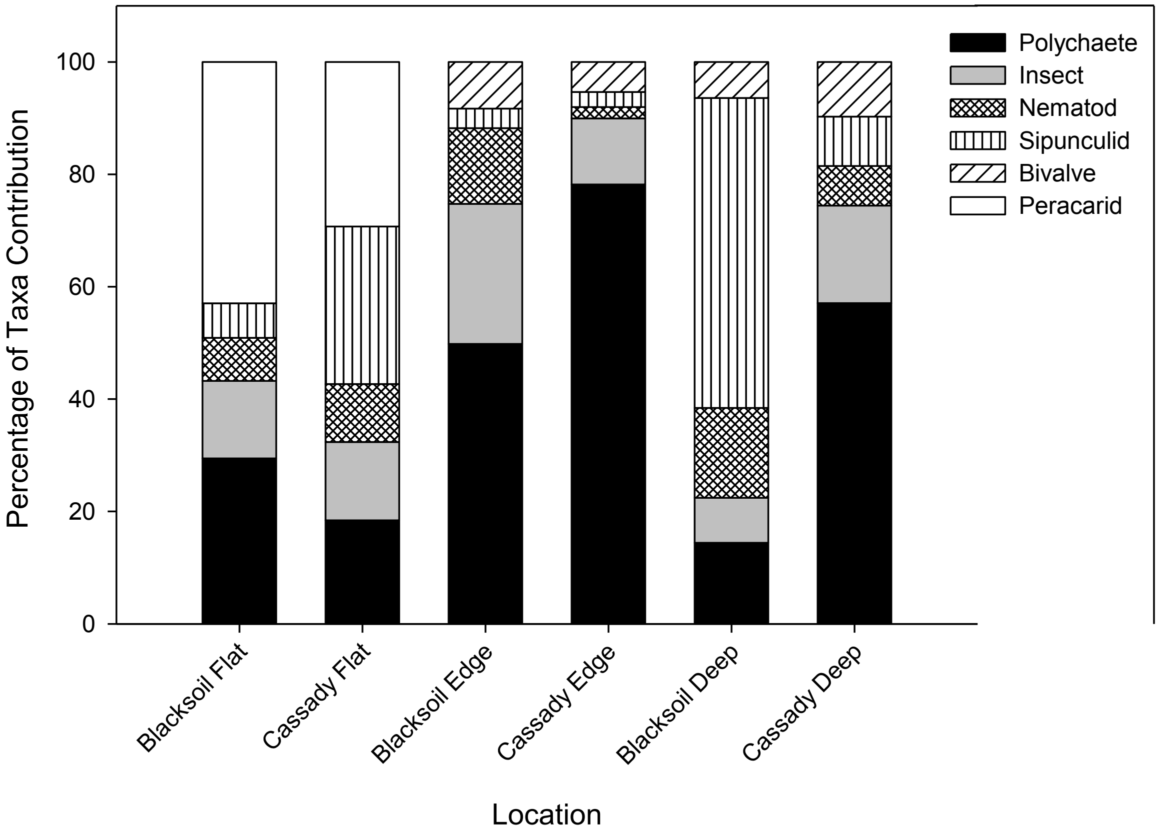

Figure 3.4: Percentage of taxa distribution between the two estuaries at the three distances across the forest (Flat=Outside the forest, Edge=10 m within the forest, Deep= 40 m within the forest).

The Correspondence Analysis highlighted differences in taxa frequency at the two locations and across distances, with the first two dimensions explaining a total of 93.6% of Inertia (Figure 3.5). The first Dimension (Inertia 55.6%) clearly separated the Flats (both Blacksoil Creek and Cassady Creek) from the sites within the forests. The CA found Cassady Creek Edge, Deep, and Blacksoil Edge to have similar taxonomic frequencies. Peracarida crustacean characterised the Flat at both locations, whereas the Edge at Blacksoil Creek and the Edge and Deep at Cassady Creek were characterised by polychaetes and bivalves. The Deep sites at Blacksoil Creek were different from all other sites revealing a higher presence of sipunculids. Overall, all taxa were well represented in the CA with a quality generally higher than 0.8. The only exception was insects with a correlation of 0.2 and to a lesser extent, nematodes (quality of 0.5).

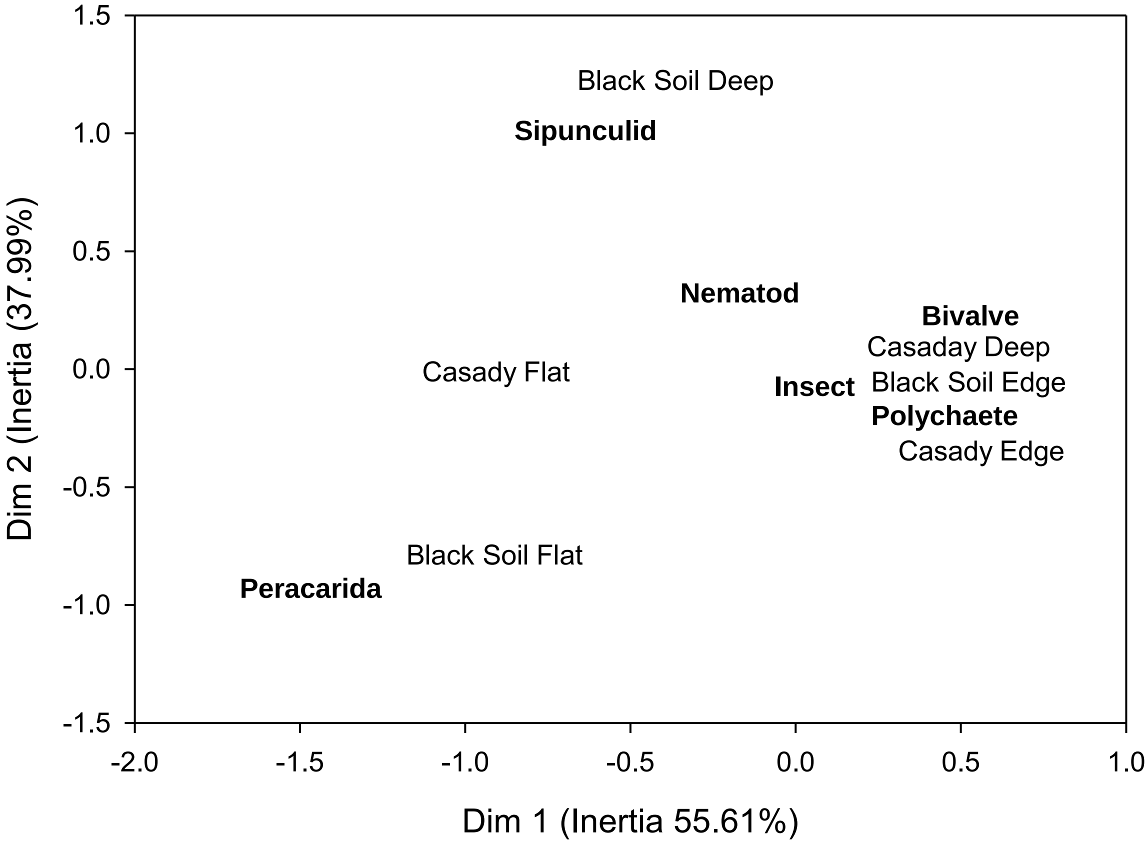

Figure 3.5: Correspondence Analysis (CA) ordination based on the distance across the forest (Flat=Outside the forest, Edge=10 m within the forest, Deep= 40 m within the forest), the Estuary (B= Blacksoil, C=Cassady), and the most common benthic invertebrate taxa.

The nMDS for the ordination of species composition within the two study forests revealed high variability between replicates, however it still described a discrete separation between Blacksoil Creek and Cassady Creek (Figure 3.6). The split in communities, based on location, were further supported by ANOSIM (R= 0.54, p<0.001). Despite an apparent separation between the Deep and Edge at Blacksoil Creek the position within the forest (i.e. Edge or Deep) did not appear to influence the benthic community (ANOSIM R= 0.06, p>0.05). When the loading vectors of the most correlated taxa were super-imposed, it was clear that most of the trend was dictated by two main taxonomic groups, with Polychaetes characterising the benthic fauna at Cassady Creek and Sipunculids at Blacksoil Creek. In addition, insects were strongly correlated with the two dimensional space, yet they were equally distributed across all sites and locations. Sampling events did not display any cluster and therefore this data was not presented.

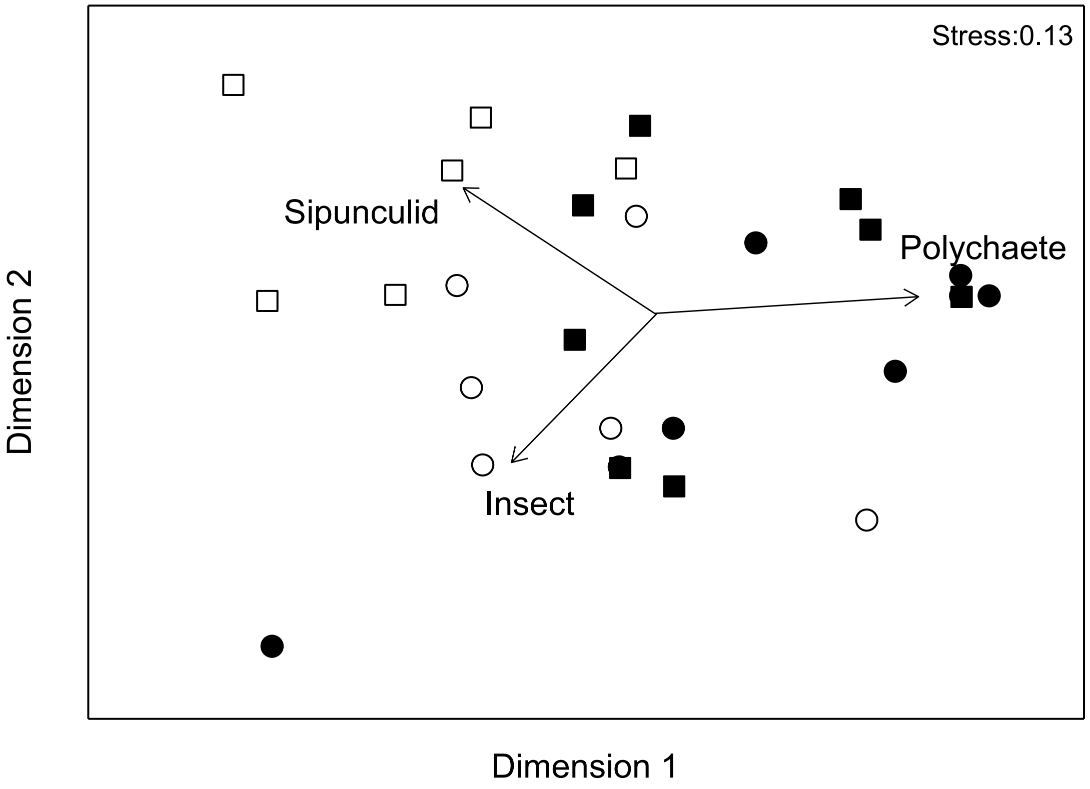

Figure 3.6: nMDS for the ordination of taxa composition, constructed using Bray-Curtis similarity matrix on transformed (log x+1) and row standardized data, between the two estuaries (White= Blacksoil Creek, Black = Cassady Creek) and the two distances within the forest (Circle= 10 m, Square= 40 m). The nMDS display the loading vectors of the taxa that correlated higher than 0.5 with the two dimensional space.

3.4 Discussion

3.4.1 Macro-benthic composition of estuarine mangrove forests

Benthic macrofauna density is relatively constant between the study locations and across the three sampling distances, however the composition differs between the two forests. This is largely, attributed to the higher presence of sipunculids at Blacksoil Creek. Sipunculids are non-selective detritus feeders (Beesley et al. 2000). Their dominance at Blacksoil Creek may be a response to higher levels of organic matter in the sediment, and hence higher food availability, which was evident in the PCA constructed using sediment characteristics.

No large variations in benthic composition were detected within the mangrove forests between the two distances (Edge and Deep). However, a high concentration of peracarids were observed on the Flat, which was not observed within the mangrove forests. These variations between the mud-flat and within the forest cannot be attributed to the sediment characteristics alone, because the PCA constructed for the sediment composition did not reveal any real variations in substrate across the three distances. Furthermore Peracarida are generally found in high abundance on both sandy and muddy substrates (Swennen et al. 1982, Dittmann 2000, Sheaves et al. 2016a). There are contrasting results in the literature with respect to the ability of peracarids to colonise mangrove forests (Schrijvers et al. 1995, Sheridan 1997, Dittmann 2001, Alfaro 2006). This may be attributed to the study sites being dominated by different mangrove species, located at different latitudes, and influenced by different tidal regimes. Logically, this would lead to variations in the factors influencing the distribution and abundance of the mangrove biota.

The absence of peracarids and the high presence of sipunculids within the investigated macro-tidal estuarine *Rhizophora* forest could be attributed to the physio-chemical properties of the mangrove ecosystem. It has recently been identified that in both fringing and basin mangrove forests, dissolved oxygen (DO) dropped to low levels (hypoxic) during tidal disconnection (Knight et al. 2013, Chapter 2). DO is likely to be a limiting factor for many biota, and exposure to very low oxygen for prolonged periods of time is likely to impact the fauna composition. For example, sipunculids are capable of oxyconforming, meaning that they can adapt their metabolic rate according to DO availability (Lombardo et al. 2014). Additionally, many species (e.g. *Dendrostomum cymodoceae*, *Phascolosoma arcuatum*) can switch to anaerobic metabolism and survive several days in anoxic conditions (Edmonds 1957, Ip et al. 1992). These adaptations would allow them to sustain the periodic low DO of mangrove forests. In contrast, peracarids appear to be more susceptible to low DO, as field observations in estuaries that are affected by seasonal hypoxia found Peracarida crustaceans to disappear from the environment (Rabalais et al. 2002).

Because of the paucity of benthic organism within mangrove forests, perhaps there is a necessity to reconsider how benthic organisms take advantage of macro-tidal mangrove forests. If a forest provides shelters from predation (e.g. birds and fish) and shade from the tropical heat (attractants), its low DO content could inhibit some taxa from colonising the forest, leading to a benthic community characterized by highly DO tolerant species. This is evident also in other taxa that were not quantified in the present study, such as mangrove crabs. In fact many of the Grapsidae and Ocypodidae crabs that characterize mangrove forests (Koch & Wolff 2002) display a semi-terrestrial behaviour (Takeda et al. 1996). This may be the result of adaptation to low DO during tidal disconnection. However, further investigation is necessary to test the susceptibility of mangrove benthic fauna to variations in DO.

3.4.2 Ecological implications

Understanding the distribution of benthic organisms across estuarine mangrove forests is critical to evaluating the roles fulfilled by mangroves within the estuarine system. Mangrove forests are considered valuable feeding grounds for juvenile nekton. Although we did not directly sample nekton during this study, it is plausible to speculate that the low density of benthic organisms (within mangrove forests) and the absence of some key taxa for juveniles diets could mean that feeding may be limited within the forest, compared to adjacent habitats (e.g. intertidal flats). This has implications for revaluing the ‘mangrove forests as feeding ground’ paradigm. As peracarids dominate the diets of juvenile fish caught in mangrove-dominated estuaries (Thayer et al. 1987, Baker & Sheaves 2005, Nanjo et al. 2008), the absence of peracarids within the forest would suggests that many juvenile nekton do not enter the forest for feeding and instead acquire food elsewhere (e.g. the intertidal flat). However, mangrove forests could still be important for specialist species. For example, species capable of consuming crabs (e.g. *Lutjanus argentimaulatus, Acanthopagrus* spp.) and insect feeders (e.g. *Toxotes* spp.) would benefit from feeding inside the forest, as these taxonomic groups are abundant within mangrove forests (Koch & Wolff 2002, Burrows 2003). Interestingly, Sheaves et al (2016) found that breams (genus *Acanthopagrus*) were the taxa most commonly found within mangroves in Australia. Additionally other studies in Australia studies have revealed low level of mangrove forest utilization by fish, most of which were concentrated within the first few metres of the forest (Vance et al. 1996, Smith & Hindell 2005). This corroborates with our expectations based on the benthic infauna community of mangrove forests.

Moreover, nekton distribution tends to aggregate on mangroves edges, suggesting that nekton may take advantage of the structural complexity of mangroves for protection against predation, while still gaining access to the intertidal flat benthic community for feeding (Sheaves et al. 2016 a,b).

Perhaps the support that mangrove forests provide to juvenile fish diets does not rely on nekton accessing the forest, but rather relies on the ability of mangroves to improve estuarine conditions. Mangrove forests are known to improve physio-chemical parameters within the estuary by: filtering harmful pollutants, acting as hydrological buffers, trapping sediment and providing carbon and nutrients to adjacent habitats (Alongi et al. 1992, Wolanski et al. 1992, Heminga et al. 1994, Robertson & Phillips 1995). Consequently, these functions would benefit the ecosystem as a whole, thus enhancing estuarine productivity.

This study has highlighted some of the gaps in our current understanding of mangrove forest ecosystem functions. The high variability in benthic composition across habitat types inside and outside the mangrove forest, and between estuaries, suggests further research is necessary to understand how higher trophic groups use these systems, and which factors influence the benthic community. We focused on *Rhizophora* forests in this study, however a study of different mangrove species would likely reveal further variations in benthic communities. Understanding this variability could be of great importance for the protection and management of mangrove ecosystems.

Chapter 4 - Natural dissolved oxygen dynamics in tropical mangrove forests limit the spatial distribution of the amphipod *Melita*

4.1 Introduction

In estuarine and coastal systems it is well documented that benthic invertebrates are an important food source for nekton (Salini et al. 1990, Tomczak et al. 2009). In particular, peracarid crustaceans (i.e. Amphipoda, Tanaidacea, Mysida) are known to be heavily preyed upon by juvenile fish and invertebrates (Edgar & Shaw 1995, Nanjo et al. 2008). Because of the importance of benthic invertebrates as food sources, infauna communities can influence the presence of higher trophic groups within a system (Grebmeier et al. 2006, Davis et al. 2014). Consequently, regions with high infauna diversity and biomass are considered important regions of nutritional up-take. This leads to considerable effort placed on identifying key habitats that provide foraging ground, particularly for juvenile nekton (Šiaulys et al. 2012). In many tropical and subtropical systems, it is believed mangrove forests provide feeding habitat for juvenile nekton. This idea is supported by studies conducted in northern America and the Caribbean, which found a high density and diversity of benthic organisms within mangrove forests (Sheridan 1997). However, evidence in support of the feeding ground function provided by mangroves is lacking in the Indo-Pacific. In fact, key taxa of normal benthic communities are missing within mangrove forests of the region (Sasekumar & Chong 1998, Chapter 2), while they are highly abundant on intertidal flats adjacent to the mangrove fringe (Alfaro 2006, Sheaves et al. 2016a). Biotic and abiotic factors which drive benthic invertebrate assemblages within mangroves forests are not fully understood, but are needed to understand the distribution of resources for organisms at higher trophic levels. In Chapter 2, we suggested dissolved oxygen (DO) saturation may be an important factor influencing benthic invertebrate assemblage in mangroves.

It has been described that drops in DO in coastal systems can alter the local benthic community structure, and function (Levin et al 2009, Kodama & Horiguchi 2011, Rakocinski 2012), and these changes are linked to the intensity, duration, and frequency of the hypoxic events (Kodama & Horiguchi 2011, Kodama et al. 2012). Therefore it is hard to predict the community response to low DO without detailed understanding of the DO dynamics and the biological threshold of the individual taxa (Stachowitsch et al. 2007). Additionally this relationships are often location specific, meaning that predictions cannot easily be applied to different areas without prior testing. Nonetheless, the majority of studies seems to show that crustaceans are among the most DO sensitive species and the first to die during an extensive hypoxic event (Chan et al. 2008, Vaquer-Sunyer & Duarte 2008).

Recent studies found that macro-tidal mangrove forests periodically experience low DO during tidal disconnection (Knight et al. 2013, Chapter 1). As a result, it seems likely that the absence of amphipods and other peracarids inside tropical mangrove forests is a consequence of an observed depression in DO following tidal disconnection (see Chapter 1). Studies which investigated amphipod species tolerance in controlled settings found a wide spectrum of responses, from highly susceptible to highly tolerant (Davis 1975, Sandberg 1997, Modig & Ólafsson 1998). The range in tolerances has often been linked to the environmental properties of where an animal naturally occurs. For example, areas that experience high wave energy and high DO generally harbour the most DO sensitive species (Wu & Or 2005), while more tolerant species are found in areas commonly exposed to poor oxygen conditions (Bauermeister et al. 2013). These location-specific faunal compositions make comparisons of amphipod tolerance between mangrove dominated estuaries and other environmental settings impractical.

This study aims to assess the hypothesis that naturally occurring low DO in tropical mangrove forests limits the distribution of available amphipod by excluding the DO sensitive species. Because amphipods are almost completely absent from mangrove forests in northeastern Australia, the study focused on the genus *Melita* because they are common on the sand and mud flats directly seaward of mangrove forests in northern Australia estuaries.

4.2 Materials and Methods

4.2.1 Animal Collection

Amphipod collection occurred between November 2015 and February 2016 along the estuarine section of Ross River in Townsville, north Queensland, Australia (19.3150° S, 146.7908° E)**.** Collection was conducted during low tide on the mud and sand flats adjacent to *Rhizophora stylosa* mangrove forests. A mix of sampling methods were used, including benthic sled and hand collection. Specimens were stored in temporary seawater tanks and transported to the laboratory at James Cook University, Townsville, where they were identified. Only organisms belonging to the genus *Melita* were retained for this study. Specimens were held in holding tanks for at least one week before experimental treatment to allow them to acclimate. Food and oxygen were not limited during the acclimation period.

4.2.2 Survival Experiment and Behavioural Change

A total of 45 individual amphipods were subjected to DO reduction treatment while another 10 comprised the control group. Experimental tanks consisted of Winkler-type bottles of 80 ml volume, with magnetic stirrer fleas placed at the bottom to ensure homogeneous water mixing. To minimize disturbance and current generated by the flea, each magnetic plate was set to the lowest speed.

A single amphipod was placed in each tank and allowed to acclimatize for three hours before starting the experiment. Consequently, each experimental tank was sealed by placing an YSI ProODO probe in the Winker-type bottle (see Butler et al. 2007). Dissolved oxygen was reduced using the animal natural respiration (Butler et al. 2007). Control tanks were left unsealed and their DO levels were checked at regular intervals to ensure DO did not drop.

Once the experiment had started the amphipods were checked at regular intervals of 30 minutes until stress was observed. The animal was considered stressed once it started to display reduced swimming activity following external stimuli, which consisted of a gentle tap on the tank. Swimming was considered impaired when the amphipod failed to swim in any direction and remained at the horizontal mid-point of the water column. After amphipods displayed reduced mobility, DO was recorded to relate to animal activity changes and the organisms were checked every 10 minutes until mortality.

The same disturbance (tapping the tank) was carried out on both treatment tanks and control tanks to confirm that slower response was not due to acclimation to the disturbance. The experiment ended when it was confirmed the animals were no longer moving for no less than 10 minutes. To ensure the animals were not simply in stasis, 'dead' animals were moved into a highly oxygenated water tank and observed for 30 minutes. Recovery was not detected in any individuals. A constant temperature of 27°C ± 2°C was retained throughout the experiment, which is within the range of temperature observed in the species' natural environment.

4.2.3 Tolerance to Different Dissolved Oxygen Drawdown Rates

The survival experiment was carried out over a long period of time (more than 38 hours), meaning that the survival threshold could have been influenced by a cumulative effect of prolonged exposure to low DO. Additionally, the long exposure to low DO and the slow DO decline failed to reflect the natural DO patterns observed in tropical mangrove forests (Chapter 2). In order to evaluate whether natural DO fluctuations could influence amphipods ability to acclimatize and survive hypoxia, mortality was tested based on different drawdown rates. DO drawdown rate in mangrove forests ranges between 25 % DO loss per hour and 7 % DO loss per hour (Chapter 2). Therefore, two drawdown rates were chosen: one that was within the observed range (12 % DO loss per hour) and one that was slower than the natural DO decline observed in mangrove forests (3 % DO loss per hour).The DO reduction procedure mirrored the one used to assess survival rate. However, to increase oxygen consumption, five individuals were placed in each tank. To test the two specific drawdown rates (3 % and 12 % DO loss per hour) two tanks of different sizes (80 ml and 25 ml) were used to reduce the water volume and hence the total oxygen dissolved in water at 100 % saturation. The experimental design included a total of 10 experimental tanks (5 for each DO drawdown rate) and 5 control tanks, and experiments were considered complete once all animals became unresponsive.

4.2.4 Data Analysis

The cumulative probability of animal's survival was estimated using the Keplan-Meier non-parametric survival analysis. DO was used as the estimator instead of time, as the two factors were correlated and DO provided a more meaningful representation of the survival probability.

The Bayesian estimator supersede t-tests (BEST) analysis were used to assess differences in distributions between drawdown treatments (Kruschke 2013). Due to the number of available replicates and the data distribution, the Bayesian approach was deemed more robust than traditional frequentist linear models (Kruschke 2013). The BEST analysis also provided visual representation of the data density distribution following model fitting.

All tests were carried out using R statistics (RCoreTeam 2015), the survival analysis was performed using the "survival" pachakge (Therneau 2015), while the BEST analysis was performed using the "BEST" Package (Meredith & Kruschke 2015).

4.3 Results

All experimental treatments resulted in amphipod mortality within 36 hours of experiment commencement, and mortality was not detected in the control tanks. *Melita* spp. generally showed a high tolerance to low DO, remaining active at DO levels as low as 20 %. However, as DO declined, mortality occurred rapidly and over a narrow range (Figure 4.1). Specimen mortality began when DO declined to 17 % saturation. Fewer than half of all specimens (45%) survived once DO reached 11 %. No amphipods survived at less than 7% DO saturation.

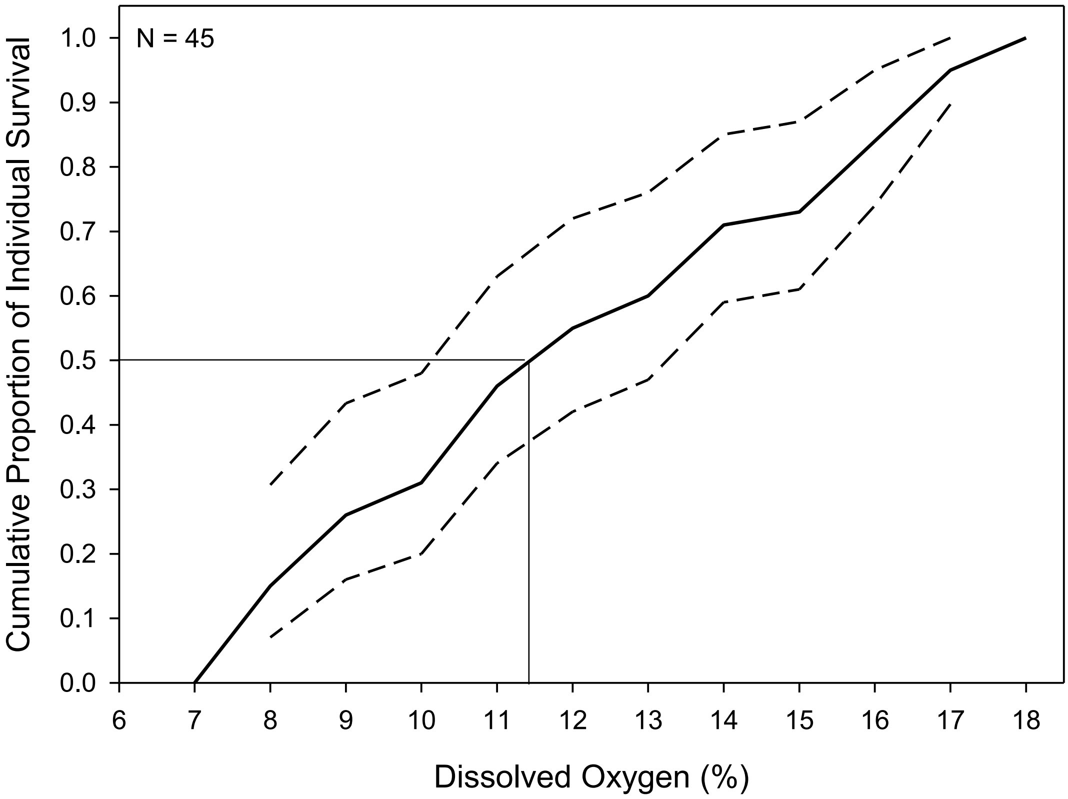

Figure 4.1: Survival analysis showing the cumulative probability of survival for *Melita* spp. at different dissolved oxygen saturations, the dashed lines represent the upper and lower 95% confidence intervals.

Qualitative behavioural changes were detected in 39 out of the 45 experimental tanks while, of the remaining 6 tanks, mortality occurred before behavioural change could be detected. A decline in amphipod response to external stimuli was observed across a broad range of DO levels, from 26 % DO for highly susceptible individuals to 12% DO for the most resistant individuals (Figure 4.2). Half of all specimens displayed a limited response to external stimuli at 17% DO

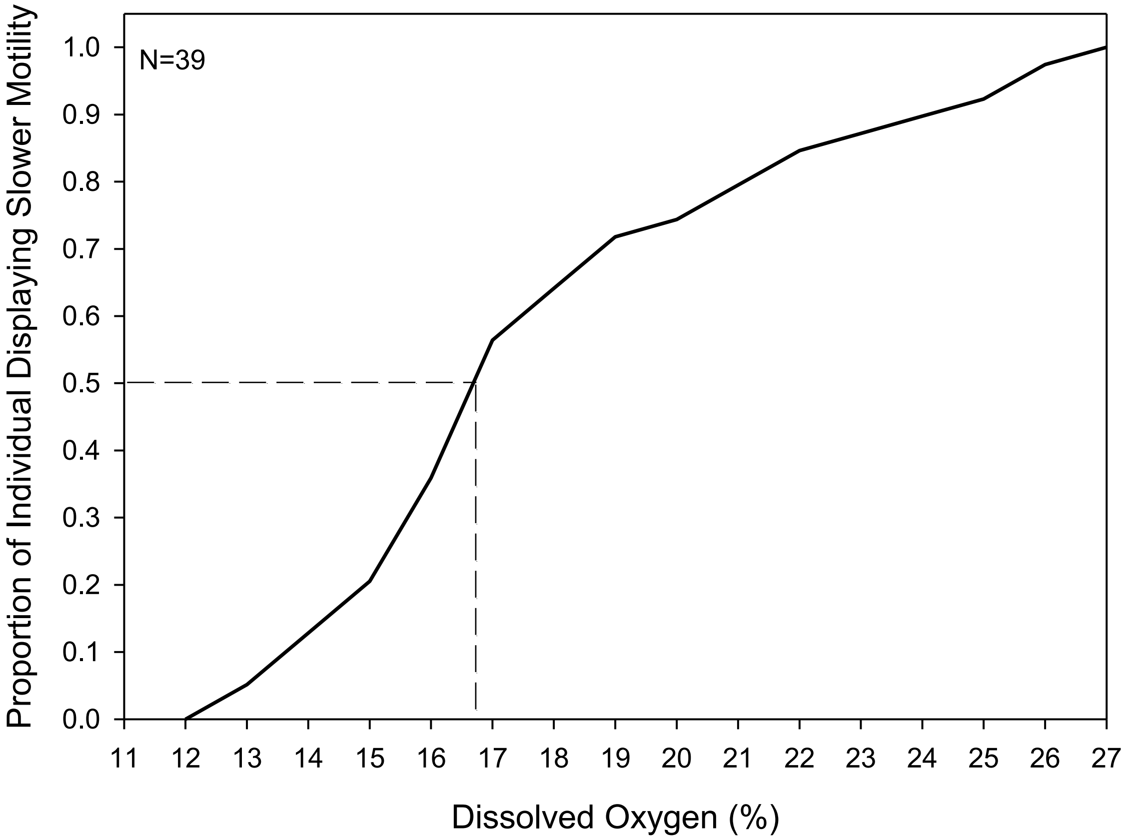

Figure 4.2: Probability of *Melita* spp. individuals having a slower response to external stimuli as dissolved oxygen levels decreased.

Mortality rates occurred over a narrow range (11-12 % DO) in tanks with slow (3% DO loss per hour) drawdown rate treatments (Figure 4.3). The tanks with faster (12% DO loss per hour) drawdown produced a wider range of responses, with visibly larger within tanks variability and mean mortality generally occurring at DO levels (8-9 %) slightly lower than the one observed for the slow drawdown rate. There was just one exception in the trend, with one of the fast drawdown tanks having the same average mortality that occurred at the highest DO (13%). However, despite the apparent variations, the two treatments do not seem to credibly vary between them, as the BEST test described an overlap in the 95% high density interval (Appendix A). This indicates that the variability in trends could be a result of natural variability of individual's tolerance to DO**.** Mortality was not observed in the control tanks.

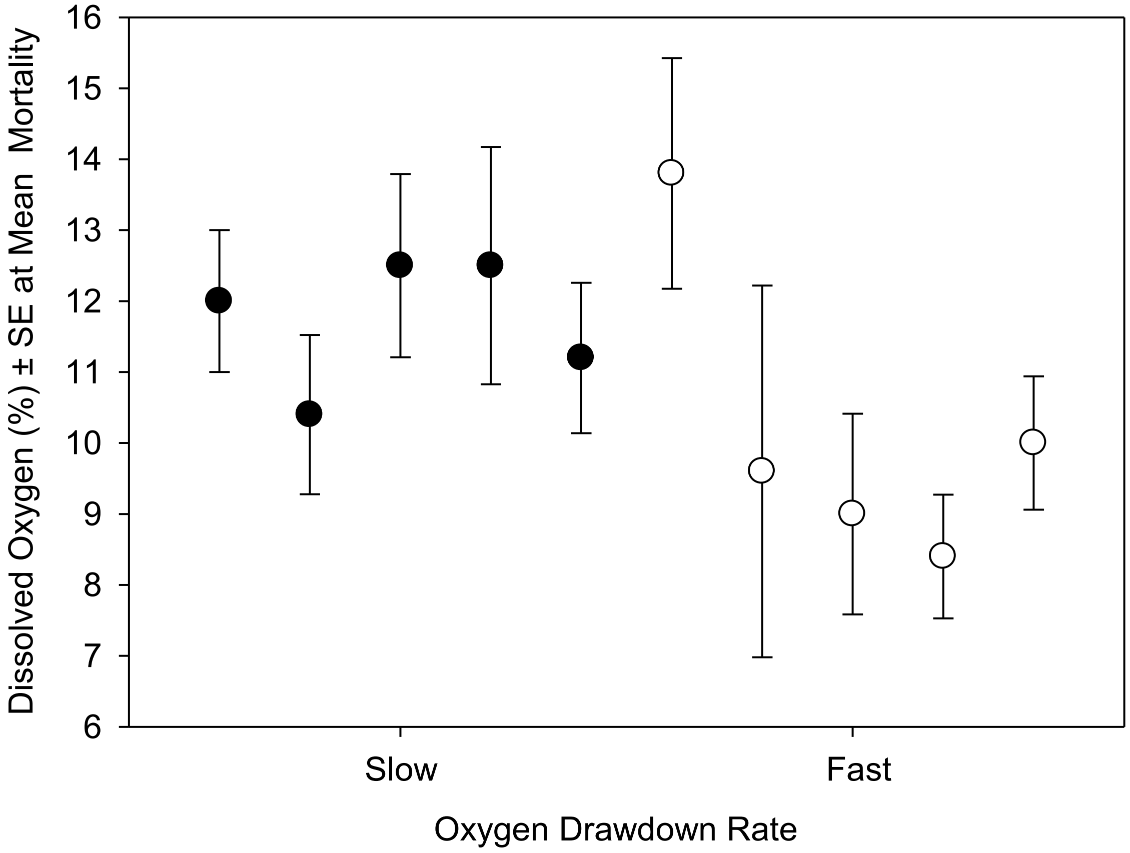

Figure 4.3: Dissolved oxygen saturation at which mean mortality occurred per experimental tank at the different oxygen drawdown rates (Slow= 3%DO loss per hour, Fast=12%DO loss per hour). Each data point represents the mean mortality of one experimental tank, while the standard error represents variability in DO when mortality occurred for each individual amphipod within the tank.

The time elapsed between the first and last individual to be declared unresponsive in each tank seems larger in treatments with the slow drawdown rates (averaging 140 minutes) compared to treatments with fast drawdown rates (averaging 75 minutes) (Figure 4.4). However most of this variability seems to be caused by two outliers, one for each treatment (Fig. 4.4). If we remove the outliers the general trend remains, suggesting that the tanks exposed to a faster drawdown rate require less time to demonstrate full tank mortality (between 30 and 140 minutes) as opposed to the slow drawdown tanks that have a larger elapsed time over a wider range (between 70 and 200 minutes). This trend is likely due to the fact that at the faster drawdown rate even the most tolerant organisms will pass their mortality threshold quicker than at the slower rate. Despite this apparent trend, the BEST analysis detected that the two means are not credibly different based on their 95% high density intervals (Appendix A).

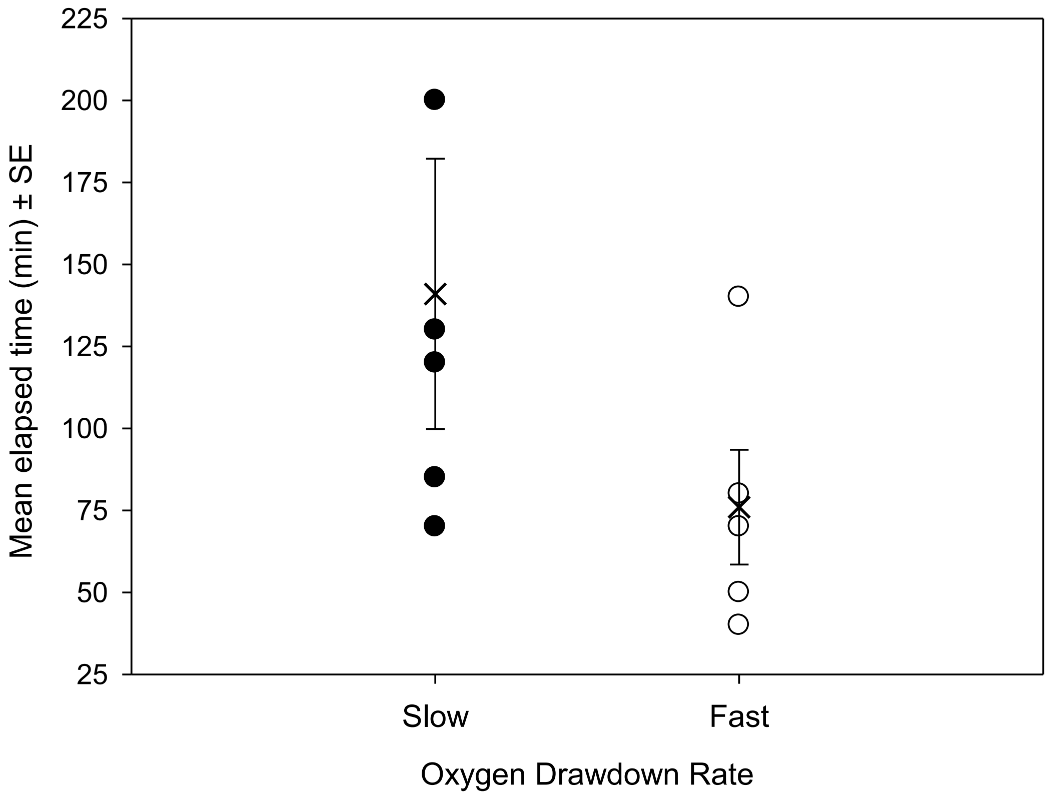

Figure 4.4: Mean elapsed time between the occurrence of the first and the last individual mortality per experimental tank at the two drawdown rates (Slow= 3%DO loss per hour, Fast=12%DO loss per hour). The circles represent the mean elapsed time per individual tank, while the crosses indicate the mean elapsed time (± SE) per drawdown rate.

4.4 Discussion

Amphipods demonstrated relatively high tolerance to reduced DO and mortality was not observed until DO fell below 20 % saturation. However, below this level, mortality rose quickly, with few animals surviving below 10 % DO. Observed *Melita* spp. tolerance was relatively high compared to other studies (Davis 1975, Hoback & Barnhart 1996, Modig & Ólafsson 1998), including one study that investigated amphipods of the same genus (Wu & Or 2005). Despite their ability to withstand hypoxic conditions, *Melita* spp. were unable to survive the severe hypoxic levels commonly observed inside mangrove forests (see Chapter 1). This is supported by the experimental exposure of *Melita* spp. to the same drawdown rate as observed within the mangrove forest. Due to the limited mobility of amphipods (Hay et al. 1988), even if hypoxic conditions occur only during neap tides (e.g. a few days every two weeks) this would be enough to inhibit colonization and survival. Moreover, mortality is the final consequence of an animal's inability to survive certain conditions, however sub-lethal effects are still likely to limit organism distribution (Poulton & Pascoe 1990, Neill et al. 1994, Johansson 1997a). For example, although the Baltic isopod (*Saduria entomon*) is highly tolerant of hypoxia (approximately 5 % saturation, or 0.2 mg/l) (Hagerman & Szaniawska 1988), it stops feeding at oxygen levels as high as 35% saturation (approximately 4 mg/l) (Sandberg 1997). Additionally, Wu and Or (2005) found that the copulation success of the amphipod *Melita longidactyla* was substantially impaired at DO lower than 35 % saturation (approximately 4 mg/l). In line with this, during the experiment amphipod response to external stimuli slowed as DO decreased, with little movement observed at 18% DO saturation and below. Therefore, even if individuals could survive low DO inside mangrove forests, secondary sub-lethal impacts are likely to inhibit their use of mangroves (Johansson 1997b). The avoidance of areas of low oxygen by marine animals, both vertebrate and invertebrate, has been often described in the published literature (Levin 2009, Mandic et al. 2014). Additionally it has been identified that DO is one of the major factors influencing benthic density and diversity, even when other sources of disturbance , such as salinity and temperature, are taken into account (Johansson

1997b, Flemer et al. 1999). The effect of DO is particularly noticeable in the intertidal areas were tidal influences can cause DO to drop more regularly than in subtidal areas (Mandic et al. 2009).

Therefore the results of this research support the hypothesis that low oxygen conditions found in macro-tidal *Rhizophora* mangroves have the potential to limit the distribution of common estuarine amphipods, and possibly other peracarids. Thus, it is likely that the general paucity of benthic organisms found within mangrove forests is at least partially the result of the periodic DO depression that occurs there. However, generalising these results will necessitate testing the tolerance of different taxonomic groups and expanding the investigation of DO dynamics and benthos to different areas and different mangrove forest types. For instance, it seems probable that differences in mangrove forest benthic assemblages between the North America (Sheridan 1997) and the Indo-Pacific (Dittmann 2001) may be the result of differences in DO outcomes under different tidal regimes. Dissolved oxygen has been observed to be at its lowest during tidal disconnections (Knight et al. 2013, Chapter 1), therefore the adverse effects of low DO should be limited in areas where mangrove forests remain inundated for extended periods (e.g. North America and Caribbean) compared to regions with regular connection-disconnection cycles (e.g. Indo-Pacific) (Accad & Pekeris 1978). Additionally, areas with high wave energy and different sediment types could further alter DO dynamics (Martin & Bella 1971, Butler et al. 2007).

This study enhances our understanding of the ability of amphipods to colonize mangrove forests, and offers an important insight into the repercussions of short hypoxic events on estuarine benthic communities. Hypoxic events in coastal and estuarine systems have increased in the last few decades, exacerbated by human activities in coastal areas and river catchments, and pose a serious threat for aquatic organisms and ecosystem function (Vaquer-Sunyer & Duarte 2008). As a result, there has been an increase in research focused on improving our understanding of benthic organism responses to hypoxic conditions for the development of management policies and to avoid functionality loss in the system (Riedel et al. 2012, Kerr et al. 2013). This has rarely been investigated in tropical systems and the present study suggests part of the benthic community of mangrove dominated estuaries would already be affected if DO fell below 12 %, with clear repercussions on ecosystem function. Further investigation on this topic is required to enhance our understanding of the susceptibility of other tropical estuarine taxa to low DO. However the results of this study highlight the susceptibility of tropical estuarine amphipods to sudden changes in DO.

Chapter 5 - Mangrove forests are not all the same: environmental settings determine faunal composition and function

5.1 Introduction

Mangrove forests support a broad spectrum of environmental functions and ecosystem services. Their economic value is estimated at US$700-37,000 per hectare, depending on the country and industries they support (Rönnbäck 1999, Aburto-Oropeza et al. 2008). The support that mangroves provide for fisheries is arguably one of their most important roles (Turner 1977, Meynecke et al. 2008, López-Angarita et al. 2016). However, many studies investigating the causal link between mangroves and fisheries productivity were generally conducted at broad spatial scales encompassing large expanses of coastline (100-1,000 km) (Manson et al. 2005, Meynecke et al. 2008). These large areas include many different types of habitats, so it is unclear whether mangrove habitat is solely responsible for enhancing fisheries productivity. It is likely that the complex interactions between mangroves and the other coastal habitats (e.g. sandflat, seagrass) contribute to maintaining ecosystem functionality for fish and invertebrates (Sheaves 2009, Törnroos et al. 2013, Nagelkerken et al. 2015). Nonetheless, mangrove forests receive special attention in relation to fisheries production because they are thought to provide nursery grounds for juvenile nekton (Robertson & Duke 1987, Ley et al. 1994, Nagelkerken et al. 2001). However, the extent of this is unclear due to a lack of knowledge of how fish and mobile invertebrates (such as prawns and crabs) use mangroves. For a habitat such as mangroves to be considered a nursery ground it must provide one of two key ecosystem services: 1) protection against predators and hence higher survivorship, and 2) high food densities and availability of appropriate forms suitable for the developmental stages of relevant species (Beck et al. 2001). While there is some experimental evidence that mangroves provide protection from predators (Sheridan 2003), the hypothesis that accessing mangrove habitats provides extra food for juvenile nekton has not been confirmed (Tse et al. 2008). Consequently, it remains unclear exactly how much nutrition juvenile nekton extract while feeding within mangrove forests. Much of our knowledge of fish feeding inside mangrove forests stems from analyses of gut contents of fish collected on the forest edge rather than within the forest (Giarrizzo et al. 2010). As a result there is no guarantee the food was obtained within the mangrove forest, leading to the possible misrepresentation of the extent of dietary support from mangroves (See Chapter 3 for details).

Many of the juvenile fish that inhabit mangrove-dominated estuaries are known to feed on benthic invertebrates (Salini et al. 1990, Ley et al. 1994), therefore, by looking at the way invertebrates are distributed within mangrove systems, we can understand the complex role of these systems as juvenile nekton feeding grounds. Moreover, benthic fauna composition can affect fish distribution and utilization of particular habitats (Grebmeier et al. 2006, Davis et al. 2014), and higher availability of important prey can boost foraging success and nekton biomass. As a result, more precise knowledge of the distribution of benthic prey can improve our understanding of the importance of mangroves in supporting fisheries yield.

Despite the obvious importance of benthic invertebrates, few studies have investigated the benthic fauna of mangrove forests. Those that have investigated benthic fauna have often adopted divergent methodologies, thus preventing direct comparison of results (Alfaro 2006).

The few previous studies of comparable methodologies in the Indo-Pacific have reported low invertebrate diversities and densities (Sasekumar & Chong 1998, Dittmann 2000, 2001). Additionally, many of the benthic taxa commonly found in the diet of fish were not observed within the forest. These findings seem to provide an explanation for the observed distribution of fish in Australian mangroves, which see them generally concentrated in channels adjacent to mangroves or only penetrating into the outer edge of mangrove forests (Smith & Hindell 2005, Sheaves et al. 2016b). This contrasts research from the Caribbean and North America, where high densities of benthic invertebrates and fish occur inside mangrove forests (Sheridan 1997, Verweij et al. 2006a).

This disparity in the use of mangroves habitats between Australia, the Caribbean and North America is likely to be the result of differences in environmental settings within which the investigated mangrove forests are situated. In the scientific literature there is a tendency to considering mangroves as equivalent units, dismissing other factors (e.g. tidal range, proximity to other habitat, seascape) that might influence the functional role of this habitat. Consequently, the results of mangrove studies tend to be generalized between different regions, and even different parts of the world, despite often marked differences in environmental settings. For instance, mangroves in riverine settings (where much of Australia's research has been conducted) are affected by sedimentation, freshwater fluxes and land run-off (Wolanski 2014). In contrast, Caribbean studies have focused on mangroves in coastal-island, non-estuarine contexts that are closely associated with other habitats such as coral reefs (Nagelkerken et al. 2001, Verweij et al. 2006b). Such variation in environmental settings is likely to influence the distribution and composition of benthic fauna due to differences in the physical environment, water quality and the availability of recruits. In addition, differences in tidal ranges can affect the duration and frequency of mangrove forest inundation, thus influencing their availability to aquatic organisms (Baker et al. 2015). Nonetheless, the influence of environmental setting on the role and function of mangrove forests has not yet been investigated. This research aimed to address this knowledge gap and improve our current understanding of mangrove forest function by examining whether there are differences in the benthic community composition between mangrove forests in contrasting environmental settings by comparing the benthic community of *Rhizophora stylosa* forests located in estuarine and non-estuarine settings.

5.2 Materials and Methods

5.2.1 Study Locations

The study was conducted in four individual *Rhizophora stylosa* mangrove forests in north-eastern Australia (Figure 5.1). Two field sites were located in estuarine contexts: i) Blacksoil Creek (19.29806 S, 147.04083 E), ii) Cassady Creek (18.74166 S, 146.28916 E), and two along island shorelines settings: iii) Goold Island (18.1667 S, 146.1708 E), and iv) Orpheus Island (18.6112 S, 146.4919 E). All the forests were situated within a radius of 75 km to ensure tidal ranges were similar; a macro-tidal regime extending about 4 m above LAT (Lowest Astronomical Tide) Datum.

The four mangrove forest study locations are in zones that receive limited human impacts, mostly caused by recreational fishers and tourists. Blacksoil Creek, Orpheus Island and Goold Island are situated within National Parks, thus direct human disturbance on the forest and the estuary are minimal. Cassady Creek lies outside of a National Park, however, urban and agricultural development is not extensive in the immediate vicinity, and therefore human impacts are still at low levels.

Both islands sites are in close proximity to coral reefs. Coral influence was evident within the mangrove forest with the presence of coral rubble mixed through the sediment. The Orpheus Island forest is located at the southern end of Pioneer Bay. It is approximately 400 m in length and extends 60 m landwards. The forest at Goold Island is located on the southern side and is approximately 600 m in length, extending landward for approximately 140 m. However, the inner section of the forest, is more than 60 m from the seaward edge and visibly cyclone damaged with many dead trees.

Both mangrove forests at the estuarine locations were located in close proximity to the mouth of their respective estuary. The Blacksoil Creek mangrove forest is approximately 600 m in length and extends landward approximately 60 m. The Cassady Creek mangrove forest sampling location is approximately 160m in length and extends some 60 m landward.

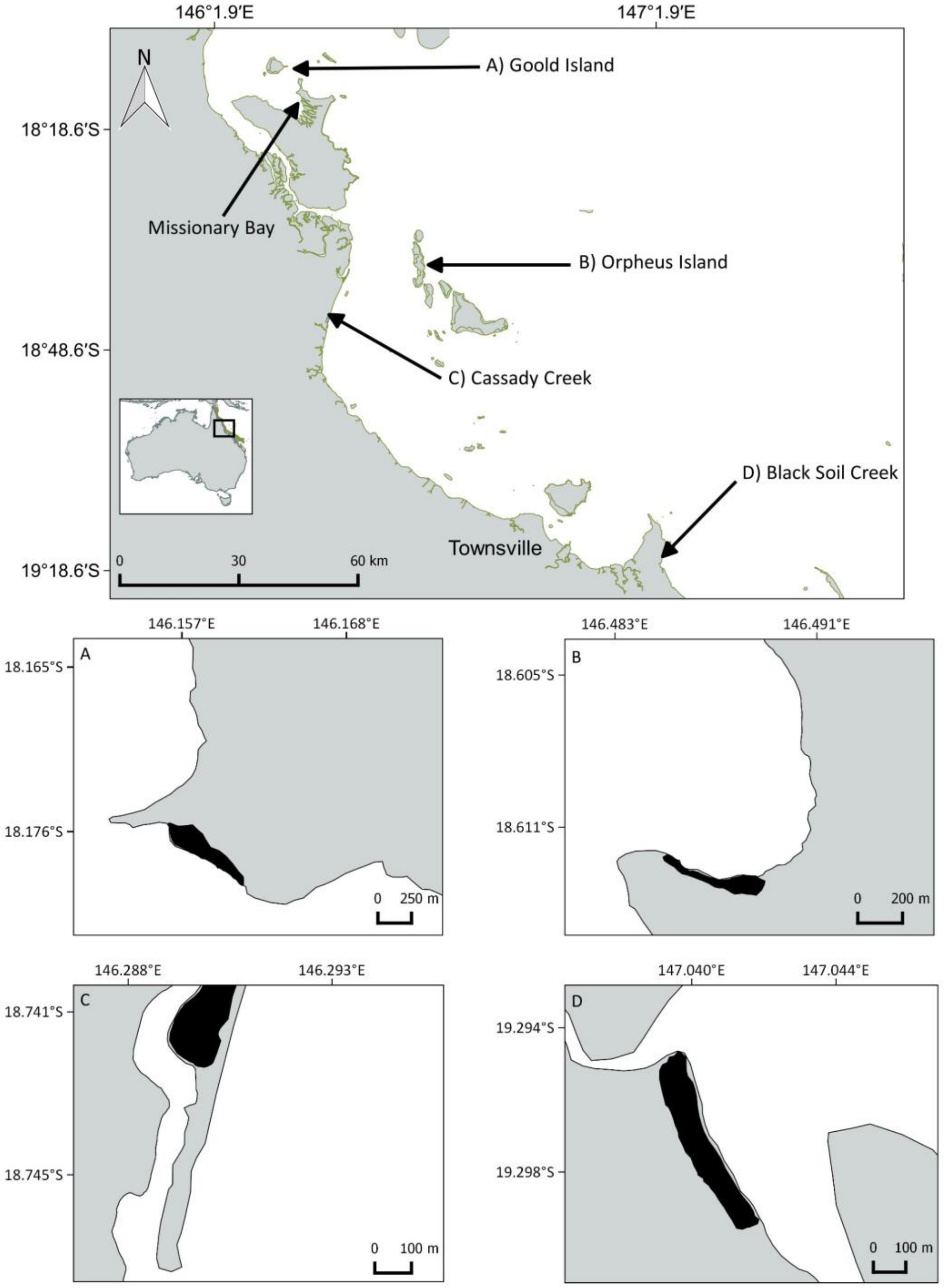

Figure 5.1: Map of the study locations in north-eastern Australia: A) Goold Island, B) Orpheus Island, C) Cassady Creek, and D) Blacksoil Creek. Areas shaded black represent *Rhizophora* sp. forests where sampling occurred.

5.2.2 Data Collection

Sampling was conducted prior to the start of wet season precipitation; Cassady Creek in December 2014 and January 2015, and Blacksoil Creek in September and October 2014 (see Chapter 2 for details). The two island sites were sampled once in September 2015 to determine if estuarine mangroves had different benthic compositions to island mangroves.

At each location, sampling occurred on the tidal flat, 1 m before the edge of the mangrove forest ('Flat'), and at two distances within the forest: 10 m ('Edge') and 40 m ('Deep'). Two sites were defined in each forest approximately 50 m apart. A 10 cm diameter corer was used to sample sediment to approximately 20cm depth. Three core were collected per sampling distance. All samples were sieved though 0.5 mm mesh and animals were identified to the lowest taxonomic level possible, usually Family or Order (see Chapter 2 for details). During each sampling event an additional 5cm diameter core sample was collected for sediment size and organic matter analysis (see Chapter 2 for details). Sediment size was analysed by passing dried sediment through a stack of sieves of 6 different mesh sizes (2 mm, 1 mm, 0.5 mm, 250 µm, 120 µm, 60 µm) and each fraction weighed. Organic content was estimated by loss through ignition (see Chapter 2 for details).

Additional physical measurements including salinity (ppt) and temperature (°C) were recorded during each trip, however, these variables showed no substantive variations between locations.

5.2.3 Data Analysis

A principal component analysis (PCA) was conducted to determine similarities in sediment composition between locations and distances along the mangrove forests studied. The variables used were the proportion of sediment grain size grouped into size classes (coarse sand = >1 mm, medium sand = 250 µm – 1 mm, fine sand = 60 µm – 250 µm, and mud = < 60 µm) and the proportion of organic matter content in the sediment. The variables were normalised before the PCA was conducted.

Invertebrate abundance data were initially standardized by surface area and converted to density (individual per square m = ind/m^2). A non-metric Multidimensional Scaling Analysis (nMDS) for the ordination of species composition was carried out using a Bray-Curtis dissimilarity matrix on log (x+1) transformed and row standardized data. Prior to analysis the organisms were pooled into major taxonomic groups, and only taxa occurring in more than 5% of sample were included in analyses. Bubble plots were superimposed on the nMDS ordination for the taxa with high correlations ($r>0.5$) within the two dimensional space.

5.3 Results

The sediment grain size and organic matter PCA revealed strong differentiation among the four sampling locations (Figure 5.2). Coarse and medium sand characterised the Orpheus Island and Goold Island sites, while finer sand and mud characterized the two estuarine mangrove locations (Blacksoil Creek and Cassady Creek)(Figure 5.2). Goold Island was the only study location that displayed variability in sediment composition among the three distances, with the majority of its 40 m sediment samples characterized by high organic matter content. This pattern likely reflects the high level of dead and damaged mangroves deeper in the forest at Goold Island.

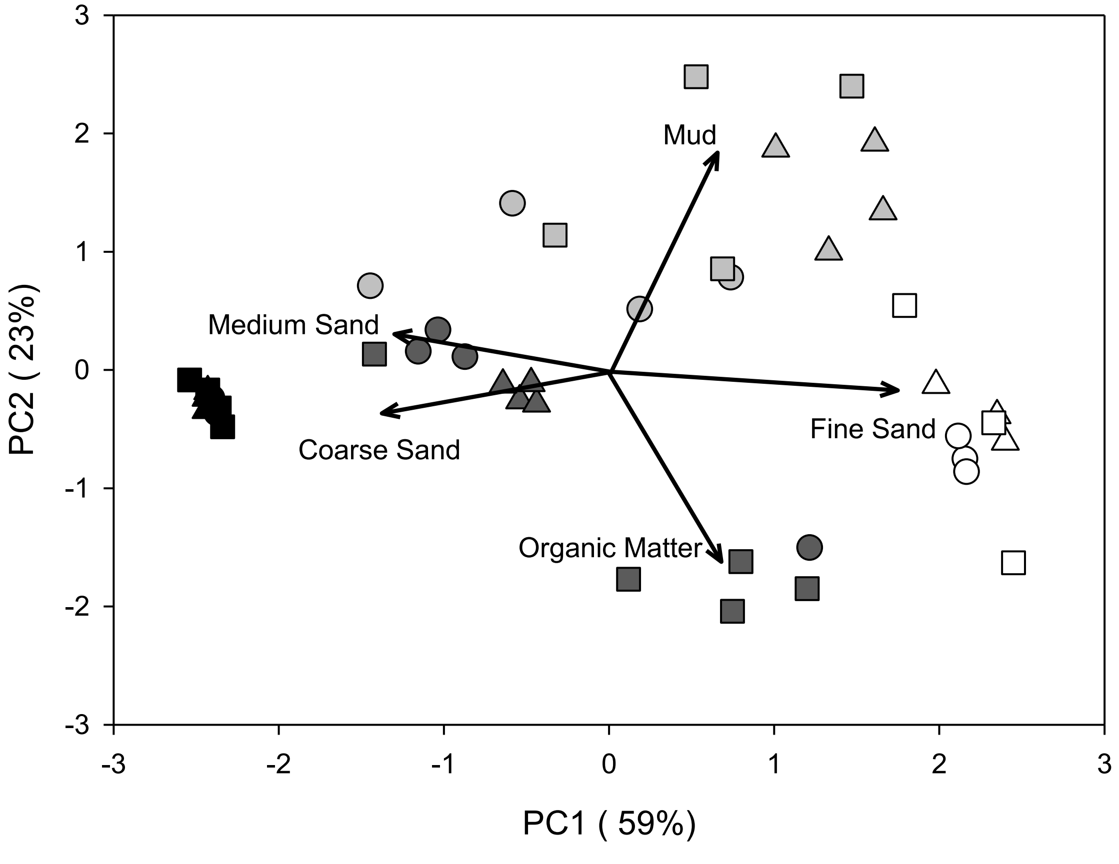

Figure 5.2: Principal Component Analysis for sediment percent size and organic matter at the four study locations (Hollow = Blacksoil Creek, Light Grey = Cassady Creek, Dark Grey = Goold Island, Black = Orpheus Island) and three distances along the mangrove forest (Circle = Flat, Triangle = Edge, Square = Deep). The loading vectors of the sediment size (coarse sand = >1 mm, medium sand = 250 µm – 1 mm, fine sand = 60 µm – 250 µm, and mud = < 60 µm) and organic matter were superimposed on the ordination.

Orpheus Island and Goold Island flats had a mean animal density averaging 2462 and 1847 ind./m^2 respectively, which was substantially higher than inside the forest which averaged 420 ind./m^2 at Orpheus Island and 588.5 ind./m^2 at Goold Island (Figure 5.3). The two estuarine forests had similar animal density within and outside the forest (averaging around 404.5 ind./m^2). Despite the islands having much higher animal density on the Flats than the estuarine forests, once inside the forest, island and estuarine sites had approximately the same mean animal density.

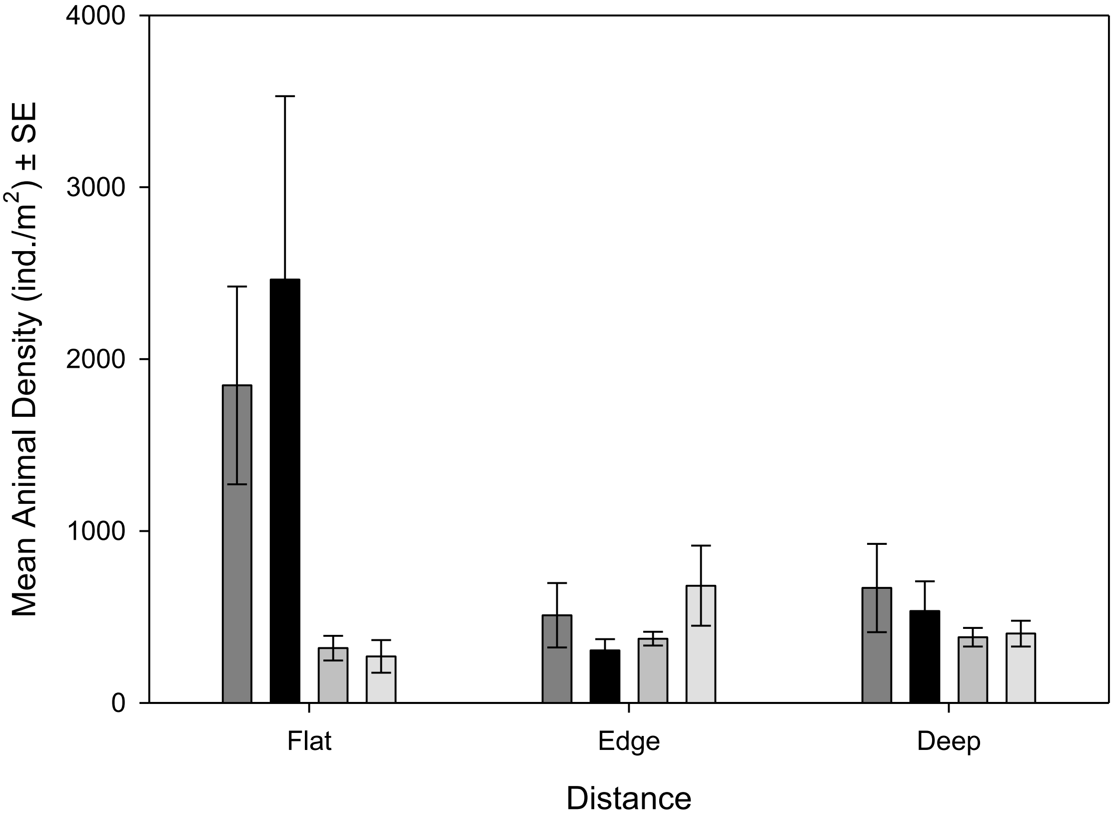

Figure 5.3: Mean animal density along the forest gradient (Flat, Edge, and Deep) at the four study locations (White = Blacksoil Creek, Light Grey = Cassady Creek, Dark Grey = Goold Island, Black = Orpheus Island).

The nMDS described two clear clusters composed by the island mangroves on one side (Orpheus Island and Goold Island) and the estuarine mangroves on the other (Cassady Creek and Blacksoil Creek) (Figure 5.4 A). The super-imposed bubble plots indicated that most of the variability among the two clusters was due to the high presence of sipunculids and insects at the estuarine sites; taxa not observed at the two island sites (Figure 5.4 B, D). Additionally, Peracarida crustaceans were observed throughout the forests at Orpheus Island and Goold Island, but were only observed outside the forest at the two estuarine forests (Figure 5.4 C). Polychaetes were distributed throughout all distances and locations, however their frequency was greater at the island sites than the estuarine sites (Figure 5.4 E).

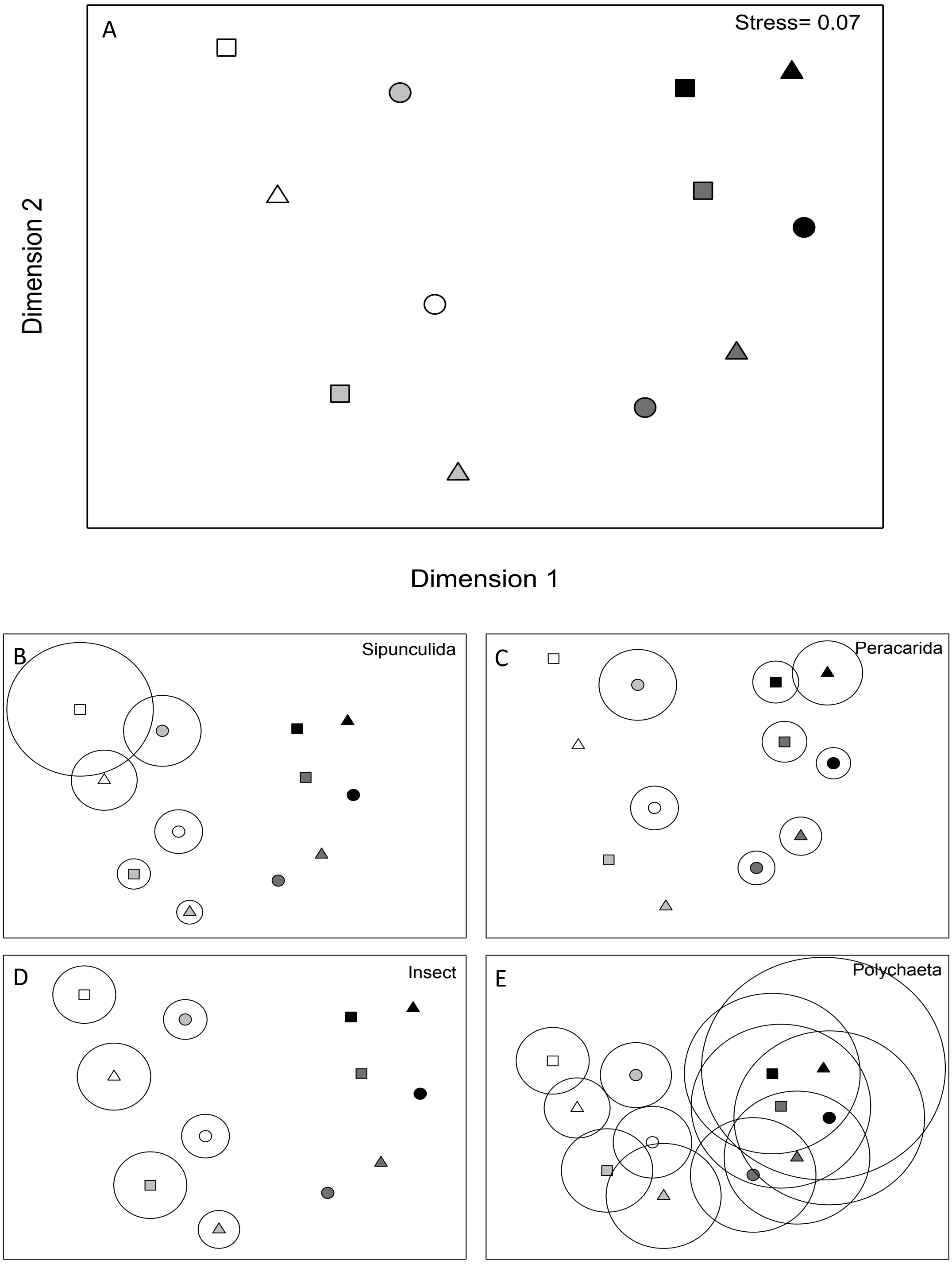

Figure 5.4: nMDS ordination of benthic invertebrate species composition, constructed using a Bray-Curtis similarity matrix on Log (X+1) transformed and raw standardized data, at the four sampling sites (White = Blacksoil Creek, Light Grey = Cassady Creek, Dark Grey = Goold Island, Black = Orpheus Island) across the three distances (Circle = Flat, Triangle = Edge, Square = Deep). Bubble plots of the most correlated species (B= Sipuncula, C= Peracarida, D= Insect, E= Polychaeta) were super-imposed the ordination.

Polychaete composition at the four sampling locations was characterized by mainly five families of polychaetes: Nereididae, Capitellidae, Glyceridae, Opheliidae and Spionidae (Figure 5.5**)**. Opheliidae only occurred at the two islands and were particularly abundant at Orpheus Island, representing more than 50% of all polychaetes identified at that location. Alternatively, at Goold Island the most abundant Family was Nereididae (58 %). The two estuarine locations had the highest abundance of Capitellidae, with Cassady Creek almost exclusively composed of this Family (84%). Blacksoil Creek also had a very high presence of Nereididae and Glyceridae, (38% and 18%, respectively).

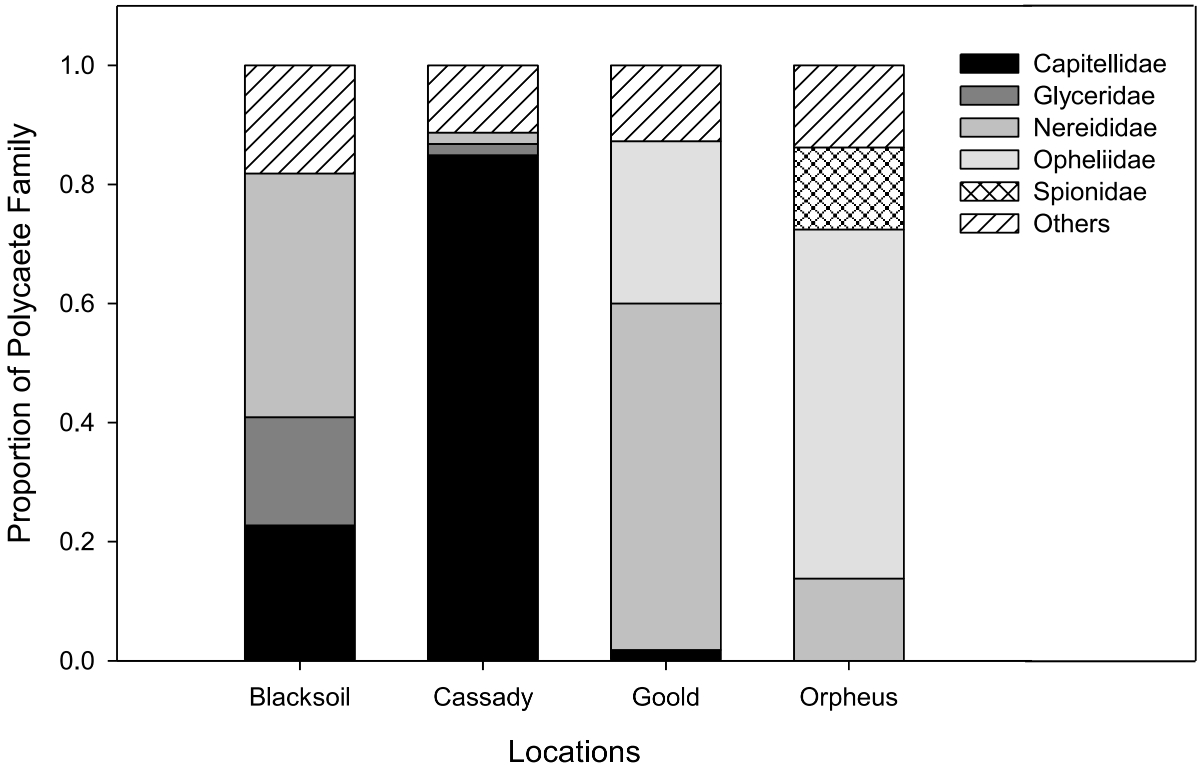

Figure 5.5: Proportion of Polychaete Families abundance per location

5.4 Discussion

5.4.1 The influence of environmental settings on mangrove benthic fauna

The composition of the benthic fauna of *Rhizophora* sp. forests differed according to the environmental settings in which they were located. In fact, estuarine and island mangrove forests were characterized by different taxonomic groups, while no distinction was observed between forests with the same environmental settings. Estuarine mangroves were characterized by insects and sipunculids, which were completely absent at the two island locations. Furthermore, peracarids were absent within the estuarine forests, which contrasts with their distribution at the two island mangroves where peracarids were found throughout the mangrove forests.

Despite different taxonomic compositions between the two forests settings, overall animal density within the mangroves were similar. However, a clear drop in animal density was observed at the island locations between the tidal flat and inside the forest. Sediment structure could be responsible for some of the difference in benthos between the two environmental settings. In fact, estuarine mangroves had finer sediment composition compared with coarser sand at the two island mangroves. The differences in sediment probably stem from the different hydrological dynamics within the two systems. For example, island sites are exposed to high wave energy, which prevents fine sediment from settling and results in coarser sand (Wright & Short 1984). In contrast, estuarine sites are generally exposed to lower wave energy, leading to higher sedimentation rates and finer particles getting trapped within the mangroves roots (Furukawa et al. 1997). Higher sedimentation rates could hinder benthic invertebrate settlement, and could explain the different taxa composition between the two mangrove settings (Alongi & Christoffersen 1992). However, sediment alone cannot fully explain differences in biota within the estuarine mangroves. In fact, the PCA did not identify variations in sediment characteristics between outside the forest (i.e. Flat) and inside (i.e. Edge and Deep), yet peracarids were only observed outside and never inside the estuarine forests.

In Chapters 2 and 3, we suggested that dissolved oxygen (DO) fluctuations could influence benthic fauna distribution in mangrove forests. It is possible that DO dynamics of island mangroves are different from estuarine forests due to the different settings. For example, sediment coarseness can influence porosity, allowing oxygenated water to penetrate deeper (Wu & Wang 2006). Less compact and coarse sediment would also enhance gas exchange, actively allowing more air to penetrate during tidal disconnection and reducing the repercussions of oxygen stripping activity performed by microbes. Additionally, the lower organic matter at the two island mangroves may further limit the impact of aquatic bacteria on DO (Streeter 1931, Martin & Bella 1971).

These variations may explain why DO sensitive organisms, such as peracarids, were observed in island mangroves and not in estuarine mangroves. In fact, although not extensive enough to be definitive, a single DO sampling event over a sequence of 4 tides at both Island mangroves indicated DO saturations only declining to approximately 20%, supporting this possibility.

On the other hand, highly tolerant taxa to low DO are strongly represented in the estuarine mangroves. For instance, Capitellidae, the dominant polychaete Family inside estuarine mangroves, have been known to survive low oxygen conditions by increasing oxygen percolation through their burrows (Davis 1975, Rouse & Pleijel 2001). Similarly sipunculids are known to live within the sediment anoxic layer, indicating that they have adapted to live in low oxygen conditions (Edmonds 1957, Ip et al. 1992).

In addition, variations of other physical properties can also lead to differences in the distribution and composition of benthic taxa. For instance, islands are subject to relatively stable salinity levels, while estuaries have higher freshwater inputs that alter salt concentration. Salinity could explain why Opheliidae were not detected at estuarine sites, as they prefer marine environments (Rouse & Pleijel 2001). During this study no variations were detected in salinity, however, the study was conducted in the dry season when stable salinities are expected. Further research is required to fully understand the influence of salinity and other physio-chemical properties on the benthic fauna of mangrove forests, perhaps using long term monitoring by remote sensing to identify shifts in DO.

The importance of environmental settings goes beyond the contrast between estuary and island mangroves. For instance, Dittmann (2001) investigated the benthic fauna of mangrove forests at Missionary Bay (Hinchinbrook Island) adjacent to Goold Island (see Figure 5.1), and reported a benthic faunal composition more similar to that at the estuarine mangroves sites from the current study than to Goold Island. However, the environmental context of Missionary Bay closely resembles that of estuarine systems (i.e. Blacksoil Creek and Cassady Creek), with relatively high sedimentation and fresh water, once again supporting the hypothesis that environmental settings plays a crucial role in determining the composition of mangrove benthic invertebrate assemblages and, by implication, their ecological roles. The results of this study seem to suggest that some of the observed differences regarding the degree of forest utilization by aquatic organisms between studies conducted in the Caribbean and Indo-Pacific could be partly due to the different environmental settings.

5.4.2 Ecological importance and implications for management

Benthic invertebrate composition is known to affect the distribution of higher trophic groups (e.g. fish) (Grebmeier et al. 2006), therefore the substantially different benthic assemblages found in the two environmental settings are likely to influence their ecological role and how nekton interact with the mangrove forest. For instance, fish that are insect or detritus feeders are more likely to benefit by accessing estuarine mangroves, while fish that are benthic crustaceans feeders would be expected to use island mangroves. Unfortunately, we currently lack detailed information on the type of fish using the different mangrove settings. However, recent studies have established that far less fish utilise estuarine forests than previously believed (Sheaves et al. 2016b). Moreover, the majority of fish species are concentrated in mangrove edge areas (Smith & Hindell 2005, Sheaves et al. 2016b), which is consistent with expectations based on the benthic composition, because utilisation of forest edges would lead to the most favourable trade-off between protection from predation and food acquisition. Clearly, increasing our knowledge of how different environmental settings effect mangrove benthic invertebrate communities is necessary to improve our understanding of how higher trophic groups gain from utilisation of mangrove forests.

Understanding how benthic fauna distribution and composition varies among different coastal contexts will also help to determine key areas to prioritize for conservation. For instance, in recent years, mangrove restoration and replantation projects have been implemented to offset the effects of human development and mangrove land reclamation (Ellison 2000). However, restoration success is often simply based on vegetation characteristics (Lewis III 2005, Ruiz-Jaen & Mitchell Aide 2005), without consideration of the impact on benthic fauna or the functional role of the forest. This means that, despite replication of floral characteristics, replanted forests might not function in the expected way due to the contextual differences in which they are situated. Therefore it is extremely important to improve our current understanding of how environmental settings influence the mangroves system functionality.

Chapter 6 - General Discussion

6.1 Thesis Summary

To address some key knowledge gaps in mangrove benthic ecology and how aquatic organisms utilize mangrove forests, we investigated zoobenthic fauna of *Rhizophora* forests and assessed whether dissolved oxygen (DO) and environmental settings could influence their composition and role within the system. In Chapter 2 we found that DO has highly complex dynamics influenced by a variety of factors, including: tidal range, time of the day and distance within the forest. As a result we hypothesised that the high variability in DO would influence how nekton and benthos use estuarine mangrove forests. The benthic infauna communities of estuarine mangrove forests (Chapter 3), had a similar taxonomic composition to those described from coastal areas experiencing low oxygen conditions. Of particular interest was the complete absence of peracarids from the forests, as they are a key component of the diet of the juvenile nekton that use mangrove systems as nurseries. To investigate the causal link between DO and benthic infauna composition within estuarine mangroves, we used the amphipod *Melita* spp. as a case study, and investigated their susceptibility to the DO levels found within mangrove forests (Chapter 4). In line with our hypothesis, *Melita* spp. were unable to survive at the low DO found within mangroves. However, it would be misleading to generalize the role of estuarine benthic infauna to mangrove forests situated in different environmental settings. Therefore, in the final stage of the project, we assessed whether the infauna community of mangroves located in coastal forests of barrier reef islands, a contrasting setting, were similar despite their differences in surrounding environmental context. We found that island *R. stylosa* forests had functionally different benthic communities to estuarine *R. stylosa* forests, with the differences likely to result from the chemo-physical properties of the two environmental settings. The variability in dominant taxonomic groups associated with different environmental settings can affect the role of mangrove forests within the system. The findings of this thesis have repercussions for understanding mangrove forest function and how best to conserve these habitats.

6.2 Importance of dissolved oxygen in mangrove ecology

High variability in the duration of mangrove inundation limits the time nekton can use the forest for foraging and protection (Lugendo et al. 2007, Baker et al. 2015). However, in this thesis we have demonstrated that dissolved oxygen (DO) also plays a crucial role in determining the accessibility of mangroves. In Chapter 2, DO was found to constantly fluctuate and range from normoxic (100% saturation) to hypoxic (<10% saturation) within the same tidal cycle. However, DO is often only considered low when it reaches lethal or sub-lethal levels, with many organisms displaying respiratory stress at levels as high as 60 % saturation (Butler & Burrows 2007) and actively avoiding areas with DO concentrations that are not optimal (Kramer 1987, Burleson et al. 2001). This suggests that less tolerant organisms might avoid using the forest altogether to avoid the rapid decline of DO concentrations during smaller tides. Alternatively, because DO profiles followed a distinct pattern during the tidal phase (Chapter 2), it is plausible that nekton could use DO concentrations as an additional cue to enter or leave the forest. This cue would allow nekton to leave the forest and avoid being stranded before the water depth becomes too low. This is particularly important because the remnant pools within the forest displayed extremely hypoxic conditions following tidal disconnection, so nekton would not be able to use those pools as a low tide refuge (Knight et al. 2013, Chapter 2).

Although tidal height plays the most important role in determining DO concentrations in mangrove systems, there was also a strong effect of time of the day identified in Chapter 2. In fact, DO was the lowest inside the forest during nocturnal tides, likely when photosynthetic organisms respire. This diurnal fluctuation of DO concentration compounded with those changes associated with tidal phase and height suggests that fish utilization and fish diversity of mangroves at night cannot be very extensive (Nagelkerken et al. 2000a). Furthermore, the difference, albeit minor, in DO between the seaward site (10 m from the forest edge) and the landward site (40 m from the forest edge) may be a result of canopy cover and sediment oxygen demand (SOD). This is because as the water moves inland through a larger surface area, the effect of SOD is likely to be enhanced because photosynthetic activity is hindered from shade provided by the mangrove canopy. Considering that some forests extend inland for kilometres, if the pattern of decreasing DO continued, it is likely that very few nektonic organisms would be able to utilise large forests. Currently, most evidence suggests that few nekton actually penetrate mangrove forests any deeper than the first few m (Vance et al. 1996, Sheaves et al. 2016b). This, together with the poor oxygen conditions identified in the current study, suggests that low DO levels may limit the use of mangroves by nekton. Alternatively, the low levels of key invertebrate food found within mangrove could mean there is little benefit to penetrating deep into mangrove forests. In fact, in comparison to the effects of nekton, DO concentrations are likely even more influential to the distribution of benthic organisms within the forest. In Chapter 3, it was demonstrated that there is a paucity of infauna organisms within estuarine forests compared to the intertidal flats, something that has also been reported by previous studies of Indo-Pacific mangrove benthos (Dittmann 2001, Alfaro 2006). These studies attributed the low diversity of organisms within the forest to the differences in physical characteristics between the forest and mud flats, such as sediment composition. However, during the present study, no differences were detected on the sediment properties between the mud flats and within the forest to justify a marked change in benthic taxa. It seems more likely that low DO during tidal disconnection (< 10% saturation) would provide a more fundamental limit on utilisation by benthos. Similarities in benthic taxa between the present and previous studies of estuarine areas that experience hypoxic events (a lack of small crustacean species, low animal density and few polychaete families)(Rabalais et al. 2002, Levin et al. 2009) supports these ideas. The relationship between invertebrate distribution and abundance was supported by the results of Chapter 4 where we exposed the amphipod *Melita* spp. to the DO levels observed within the mangrove forests examined in Chapter 2, and concluded that this common mangrove taxon was unable to survive. DO concentrations have been used in the past as an ancillary variable when assessing mangrove benthic fauna (Alfaro 2006), however, DO concentrations based on a single temporal observation do not provide enough resolution to determine DO patterns and determine the influence of DO on the benthic composition. Because of the limited motility of many invertebrates (Hay et al. 1988, Swearer et al. 2002), even sporadic declines in DO concentrations below tolerance levels would cause the loss of these taxa from that particular environment (i.e. mangrove forests). Therefore, the influence of DO on nekton and the benthos has multiple implications for mangrove ecosystem functioning and on the way these systems are managed now and in the future.

6.3 Estuarine mangrove benthic infauna communities and their ecological implications

Mangrove forests are generally considered valuable nursery and feeding grounds for juvenile nekton (Robertson & Duke 1987, Nagelkerken et al. 2000b, Laegdsgaard & Johnson 2001). However, there is much evidence to suggest that this paradigm may not necessarily be true in all situations (Sheridan & Hays 2003, Faunce & Layman 2009). The definition of nurseries can vary slightly in the literature, based on the author writing the review (Beck et al. 2001, Able 2005), however for nurseries to work they must provide at least one of two basic services: 1) higher survivorship, due to protection from predators, and 2) higher food density, which increase nekton biomass (Beck et al. 2001, Sheridan & Hays 2003). While there is some evidence supporting the former for some systems (Primavera 1997, Sheridan & Hays 2003), the low diversity and density of benthic organisms, other than crabs, within many estuarine forests (Sasekumar & Chong 1998, Alfaro 2006, Koch and Wolff 2008) suggests that appropriate food is probably scarce in many situations. This is supported by a number of dietary studies conducted in mangrove dominated estuaries that have reported high abundances of species that were not detected within mangroves (Salini et al. 1990, Baker & Sheaves 2005, Nanjo et al. 2008). Indeed, the higher diversity and density of benthic organisms found on intertidal flats (Alfaro 2006, França et al. 2009, Sheaves et al. 2016a) suggests that estuarine fish are likely to gain more nutrition from these areas than from mangrove forests. Therefore, juvenile nekton could follow the rising tide and find refuge in the shallow water while feeding on the flats (Paterson & Whitfield 2000). As water levels on the flats continue to rise, the juvenile nekton are no longer afforded the protection from larger predatory fish provided by shallow water, but could find protection within the mangrove forest edges and wait for the tide to ebb in order to feed again (Giarrizzo et al. 2010, Sheaves et al. 2016a).

Therefore, in many cases, the only part of the forest likely to be used by nekton is the seaward edge, where there is an acceptable compromise between refuge from predation, foraging opportunity and favourable dissolve oxygen conditions. This suggests that inner parts of large inland forests might not be as important for nekton as small fringing areas

Although few fish are likely to gain substantial additional nutrition by entering mangrove forests, there are particular fish that could. For example, Sheaves et al (2016) found that one of the most prominent taxa observed using mangrove forest was the sparid genus *Achantropagrus*, which are capable of feeding on crabs that are known to be abundant inside mangrove forests (Lee 1998, Skov et al. 2002). Additionally, the paucity of benthic infauna could lead to shifts in dietary preferences once fish access the forest. However because of the lack of studies that sampled the diet of fish caught within the forest, the importance of this other resources could be under-reported in the literature. For instance Tse et al. (2008) have observed that some nekton switch to a detritus dominated diet when feeding in the forest. The presence of detritus in juvenile fish has been detected in mangroves dominated estuaries (Nanjo et al. 2008). However, high presence of detritus in the diet does not necessarily mean that fish depend substantially on this as a food source. On the contrary, from an energetic efficiency point of view, they would need to ingest larger volumes of detrital matter in order to gain the same nutritional value as other more nutritional resources**.** This lack of nutritional quality could also explain why detritus is more commonly observed in the diets of smaller juveniles, and as the fish get larger the ingestion of detritus starts declining and get substituted by larger more nutritional preys (e.g. crustaceans, polychaetes)(Horinouchi et al. 1996, Nanjo et al. 2008).

6.4 Environmental Settings

In ecology there is the tendency to consider areas characterized by similar features as a specific habitat type. The properties and functions of a particular habitat are often generalized to fit specific trends. However, different environmental settings (e.g. proximity to other habitats, physical dynamics) can lead to a shift in the biotic and abiotic properties of the habitat and affect their functional role within the system, but this is rarely investigated (Sheaves 2009, Törnroos et al. 2013). This knowledge gap can lead to misinterpretations of the services provided by the habitat. This is particularly evident in the literature regarding mangrove forests and their function. In fact the ecological functions of mangroves are often generalized, despite variation in the characteristics of the investigated forests by location (e.g. riverine or coastal), tidal range (e.g. micro-tidal or macro-tidal), and dominant mangrove species (e.g. *Rhizophora* spp. or *Avicennia* spp.). Mangrove trees have a high tolerance to a wide range of physical factors (e.g. salinity, temperature, and wave energy) and are found over a diverse range of coastal and riverine systems (Duke 2006, Webber et al. 2016), therefore it is naive to think that mangrove ecology works in the same way across all environmental settings. In chapter 5, we demonstrated that the environmental settings can have a significant influence on the composition of mangrove infauna communities. This in turn is likely to alter how nutrients move within the food-web. The variability in benthic infauna between mangrove forests is not only observable when environmental settings are largely different as in the present study (i.e. estuarine vs. Island), but significant shifts in benthic communities can arise from other sources of variability. For instance, the age of the forest, the amount of fresh water run-off and the degree of nutrient enrichment can all alter the composition of mangrove benthos (Sasekumar & Chong 1998, Rakocinski 2012). At Missionary Bay, Hinchinbrook Island, Dittmann (2001) found a benthic composition that strongly differed from the one we found at Goold Island, despite their close proximity to each other. This variability in benthic composition is likely due to the different environmental factors influencing the two forests, with Missionary Bay having properties that closely resemble an estuarine system. The differences in benthic composition between the two forests are likely to affect their functionality. This is because benthic community composition affects how higher trophic groups use aquatic systems (Grebmeier et al. 2006, Davis et al. 2014). For example, in island-type mangroves, it would be expected to find less detritus feeders and more organisms that feed on small crustaceans. Therefore, the nekton that depend on mangrove forests would vary between the two systems. This suggests that it is necessary to improve the current understanding of how organisms interact with mangrove forests by taking into account the different properties of the system in which they are found.

6.5 Management Implications of dissolved oxygen and environmental settings

Different benthic communities can affect mangrove systems in different ways, and therefore understanding how they change can lead to the development of better management policies. Because mangrove forests are a highly endangered environment with an annual loss of 1.5% cover per year (Farnsworth & Ellison 1998), understanding how DO and environmental settings affect the benthos and their biological interactions can substantially improve our preservation of mangrove forest functionality. For example, there has been an increase in the number of policies aimed at conservation, rehabilitation and restoration of degraded mangrove systems (Ellison 2000). In particular, many governmental agencies are trying to preserve mangrove habitat under the umbrella of fisheries protection, trying to limit the biodiversity loss at an impacted site and keep the ecological function of the system (Maron et al. 2012). However, for many systems not enough is known about the ecological function to guarantee a successful outcome. Because mangrove systems are accepted as important nurseries and feeding grounds for juvenile nekton, fisheries departments tend to devise policies in order to preserve such services (Palmer & Filoso 2009, Fisheries Queensland 2012). However, management policies are often developed with the assumption that mangroves are all the same, with little consideration as to whether they are coastal or estuarine, wide or narrow, or composed by many mangrove species or a single species (Ellison 2000, Bosire et al. 2008). In this thesis, we demonstrated that benthic fauna changed based on the surrounding context, highlighting the complexity in conserving mangroves functionality. For instance, there is no guarantee that preserving coastal mangroves in order to offset development in nearby estuaries will preserve functionality because the two mangroves forests are likely to differ in both benthic communities and chemo-physical properties, leading to different functional roles within the broader seascape. In fact, in many situations mangrove replantation could lead to loss rather than preservation of functionality if planting mangroves in intertidal areas, results in changes in community structure or declines in benthos biomass. Another aspect that needs investigation if we want to improve management of mangrove systems is the effect that DO dynamics have on structuring the benthic fauna. In fact mangrove forests are clearly already DO stressed (Marchand et al. 2004, Kinght et al. 2013, Chapter 2). This means that even small changes on DO dynamics by human interferences could have repercussions on the infauna of mangrove forests and the adjacent habitats.

The occurrence and intensity of short hypoxic events has increased in the last few decades, predominantly due to anthropogenic influences within rivers catchments (Vaquer-Sunyer & Duarte 2008, Melzner et al. 2013). The consequences of such events have been thoroughly investigated and monitoring plans to mitigate the effects have been suggested (Rabalais et al. 2002, Kerr et al. 2013, Tweedley et al. 2015). However, from the results presented in this thesis, it is evident that measuring DO concentrations at single time periods is insufficient and instead needs to be monitored over prolonged periods in order to gain sufficient information on DO patterns. Consequently, this can be related to benthic fauna composition and management practices. The ability to measure DO concentrations over longer periods has recently become more practical due to technological advances in DO probes and remote sensing. Perhaps well-developed monitoring techniques could detect changes in DO patterns before the onset of hypoxic condition, allowing enough time to respond and mitigate the drop in DO by increase water flow, or reducing discharges in the river.

6.6 Robustness of Results and Future Directions

Throughout this thesis we evaluated the benthic composition of mangrove forests using commonly used techniques (Sasekumar & Chong 1998, Dittmann 2000, Alfaro 2006). Therefore, we focused on the macro-benthic infauna (organisms retain within a 0.5 mm sieve), ignoring the smaller meso- and meio- fauna. Further research on the smaller infauna would lead to a more comprehensive understanding of benthic ecology. There is some evidence suggesting that meso-fauna utilization of estuarine-type mangroves is similar to the one of macro-infauna presented in this study (Dittmann 2001). However, to further develop our understanding of mangrove benthic ecology, it is necessary to conduct further research before generalization can occur. Additionally, during this study the focus was mostly directed towards *R. stylosa* forests in order to optimize comparisons and highlight trends, however, there is a multitude of other mangrove genera that build large forests (e.g. *Bruguiera* spp., *Ceriops* spp., *Avicennia* spp.) that could alter some of the dynamics described in this thesis. For instance, *Avicennia* spp. have less root structure and reduced canopy cover than *R*. stylosa . These variations could affect light penetration and water movements, possibly resulting in higher oxygen saturation within *Avicennia* spp. Forests that could have flow-on effects on the benthic fauna composition. Additionally, different mangrove species prefer different positions along the tidal gradients (Duke 2006), which could further modify our understanding of the various associated ecological processes. This is because benthic distribution is strongly associated with tidal gradient, and this relationship has been thoroughly investigated in non-vegetated tidal flats (Peterson 1991, França et al. 2009, Sheaves et al. 2016a). In this thesis, we attempted to minimise the effect of tidal gradient by examining mangrove forests that have similar slopes and inundation time, however, we would expect that forests located higher on the intertidal would host different benthic organisms.

An additional aspect that needs further investigation is to quantify how nekton utilization of mangrove forests is limited by DO dynamics. In Chapter 2, we hypothesised that DO dynamics could limit nekton utilization of the forest during certain tidal phases, however this aspect remains to be tested. In the literature it is evident that nekton can avoid areas with low DO (Ludsin et al. 2009), although this is very species and size dependent (Burleson et al. 2001, Bell & Eggleston 2005). Therefore, it is likely that some nektonic organisms are still capable of using the forest when DO concentrations are low. Perhaps some organisms could use low DO as a 'chemical' refuge against more DO susceptible predatory fish. Further studies could develop investigations that monitor DO while simultaneously assessing nekton utilization of the forests. Based on the present study, it is likely that fewer taxa would be able to access *R. stylosa* at neap tide because of the lower DO.

The relationships between DO and benthic fauna also need further research to strengthen causality. In this thesis, we provided initial evidence suggesting that some infauna taxa might be limited in their utilization of mangroves due to low DO. However, this is based on two fundamental assumptions: 1) that the DO in the pools formed during tidal disconnections are a proxy for sediment DO and 2) that DO saturation is not higher at the interface between the water and the atmosphere. Indeed, it is plausible that benthic organisms do not need high DO throughout the water/sediment to survive and may be able to persist until the following tidal connection using the water-atmosphere interface. Unfortunately, current technology does not allow for the reliable measurement of small scale variations in the DO, but it would be interesting to evaluate these dynamics in the future. Perhaps a different approach that could be used to better understand whether DO limits the benthic distribution could be the translocation of organisms from tidal flats to mangrove forests using cages and assessing their survival rates.

A final consideration is that the chemo-physical and ecological aspects that regulate mangrove functionality, and how they interact with their surroundings, are poorly understood. This means that there are plenty of opportunities and directions to explore and improve our ecological knowledge of mangroves ecosystems.

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Appendix A - Chapter 4

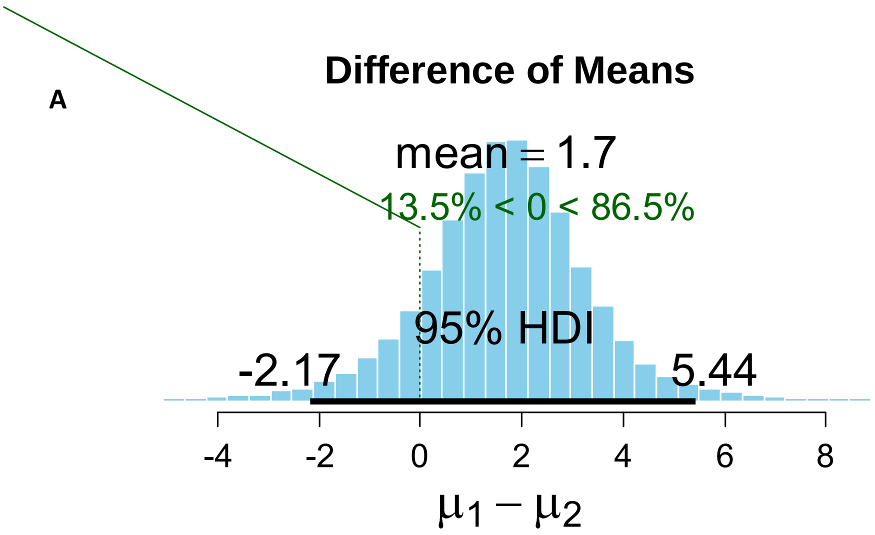

Figure A: BEST possibilities distribution for the mean mortality between the two different drawdown rates (~3% DO loss per hour and ~12% DO loss per hour). The histogram represent the difference between means based on their posterior distributions, and the 95% high density interval is shown.

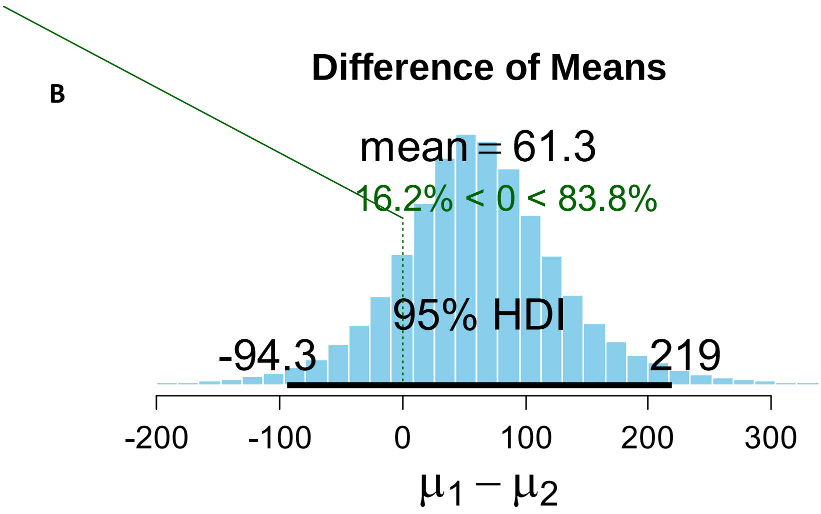

Figure B: BEST possibilities distribution for the mean elapsed time between the time mortality was first observed in the tank and the last individual to become unresponsive in the tank between the two different drawdown rates (~3% DO loss per hour and ~12% DO loss per hour). The histogram represent the difference between means based on their posterior distributions, and the 95% high density interval is shown