

**Ecological studies of the mangrove-associated meiofauna in
Southeast Queensland, Australia**

Maizah Mohd Abdullah

B.Sc. (Marine Biology)

M.Sc. (Ecology)

Griffith School of Environment

Griffith University

A thesis submitted in fulfilment of the requirements of the degree of Doctor of
Philosophy

June 2016

ABSTRACT

Meiofauna are ubiquitous but poorly-studied components of soft-bottom marine communities around the world, including mangroves. However, information on the ecological role of the meiofauna in subtropical intertidal habitats is scarce compared to knowledge of the benthic macrofauna. The dynamic environmental conditions and heterogeneous sediments of mangroves present challenges to understanding the structure of mangrove meiofaunal assemblages at various spatial and temporal scales. This study was designed to elucidate the ecological role of the meiofauna in mangroves by studying their three main ecological elements: 1) assemblages structure; 2) top-down interaction with macrofauna; and 3) bottom-up interaction in terms of nutrient utilisation. Firstly, how meiofaunal assemblage respond to estuarine sediment conditions was described by analysing the assemblages associated with different mangrove species (*Avicennia marina*, *Rhizophora stylosa* and *Aegiceras corniculatum*) at three locations in sub-tropical Southeast Queensland, Australia. Secondly, the significance and nature of top-down control on the density of meiofauna based on their interactions with deposit-feeding crabs was investigated in a mangrove and the adjoining sandflat. Field manipulative experiments were conducted within the aggregation zones of soldier crabs (*Mictyris longicarpus*) and fiddler crabs (*Uca vomeris*) in a mangrove-lined creek, specifically to determine whether the interaction is primarily physical or trophic. Thirdly, trophic ecology of the meiofauna was studied to examine their role in the organic matter utilization using stable isotope analysis, and divided into two separate studies. Trophodynamics of the meiofauna and macro-invertebrate consumers from connected sandflat (SF), mangrove (MG) and saltmarsh (SM) habitats was compared using natural abundance stable isotopes of ^{13}C and ^{15}N . These habitats are located along a gradient at low, mid and high intertidal positions, respectively. Meanwhile, a separate dual-stable isotope enrichment experiment was conducted, where ^{13}C and ^{15}N enriched compounds

were used in a pulse-chase experiment to evaluate the importance of microalgae as a food source to the mangrove associated meiofauna.

Different meiofaunal assemblages were found between and within sites, along with significant associations with the environmental variables studied. In general, high availability of food proxies (phaeopigments, Chl a or total organic carbon), moderate tannin content and components of habitat structure (sediment particle size, belowground root biomass and/ or moisture content) promoted meiofauna density.

The inclusion and exclusion experiment with manipulation of crab's feeding activities suggests that the top-down control by soldier crabs on the meiofauna is fundamentally trophic, i.e. predation. Fiddler crabs significantly impact the meiofauna through their physical activities such as sediment turnover and burrowing, but their trophic activities did not significantly reduce meiofaunal density.

Application of stable isotope studies using natural abundance and labelling of dual elements ^{13}C and ^{15}N helped to elucidate the trophodynamics of the mangrove-associated meiofauna. In the natural abundance study, partitioning in resource utilization exists between different but connected habitats. Where habitat connectivity resulted in the availability of multiple carbon sources, the consumers exploited all available food sources, but with clear and consistent differences in utilization patterns between different taxa or species. In the labelling experiment, it was shown that nematodes and harpacticoids utilized different food sources, and carbon and nitrogen were also utilized in different ways. However, stable isotopes analysis in both studies show that there are "cryptic" or unknown sources contributing to the diet of the animals studied.

Overall, this study has provided additional understanding and knowledge of the ecological roles of the meiofauna in sub-tropical mangroves ecosystem. The role of the meiofauna, as a ubiquitous and abundant component of soft-sediment marine habitats, is complex and requires investigations to be conducted at the relevant spatial and temporal scales. Despite their small body size, this study has shown that meiofauna

could be efficiently used to answer ecological questions and also can, and should be included in trophic studies employing stable isotope analysis of soft-sediment habitats.

TABLE OF CONTENTS

Abstract.....	ii
List of figures.....	viii
List of tables.....	x
Acknowledgements.....	xii
Statement of Originality.....	xiii
Chapter 1 General introduction.....	1
1.1 Definition of the meiofauna.....	1
1.2 Abundance of the meiofauna in mangroves.....	1
1.3 Ecological roles of the meiofauna.....	2
1.4 Meiofauna as consumers in benthic food webs.....	5
1.5 Stable isotope analysis.....	6
1.6 Research questions of this study.....	8
1.7 Thesis structure.....	10
Chapter 2 Structure of mangrove meiofaunal assemblages respond to local sediment conditions in subtropical eastern Australia.....	11
2.1 Introduction.....	11
2.2 Materials and methods.....	14
2.2.1 Study Area.....	14
2.2.2 Field sampling.....	15
2.2.3 Laboratory analysis.....	16
2.2.4 Data analysis.....	18
2.3 Results.....	19
2.3.1 Environmental variables.....	19
2.3.2 Meiofaunal density and assemblage structure.....	24
2.3.3 Correlations of meiofauna and the environmental variables across spatial and temporal variatio.....	28
2.4 Discussion.....	29
Chapter 3 Meiofauna and crabs in mangroves and adjoining sandflats: Is the interaction physical or trophic?	33
3.1 Introduction.....	33
3.2 Materials and methods.....	35
3.2.1 Study area.....	35
3.2.2 Quantification of natural crab density.....	36

3.2.3 General experimental design.....	37
3.2.4 Soldier crabs: experimental design.....	40
3.2.5 Fiddler crabs: experimental design.....	40
3.2.6 Data analysis.....	41
3.3 Results.....	41
3.3.1 Crab density and sediment conditions.....	41
3.3.2 Chl a, LOI and Irradiance.....	42
3.3.3 Meiofaunal density.....	44
3.4 Discussion.....	45
3.4.1 Physical vs. trophic interactions.....	45
3.4.2 Experimental design.....	48
Chapter 4 Partitioning organic matter utilization by the meiofauna and macrofauna in connected subtropical intertidal habitat.....	51
4.1 Introduction.....	51
4.2 Materials and methods.....	53
4.2.1 Study area.....	53
4.2.2 Sample collection and preparation.....	54
4.2.3 Stable isotope analysis.....	54
4.2.4 Establishment of the trophic resources from the consumer data.....	56
4.2.5 IsoError mixing model.....	58
4.2.6 Data analysis.....	58
4.3 Results.....	59
4.3.1 Sediment organic matter (SOM)	59
4.3.2 Primary producers.....	60
4.3.3 Meiofauna.....	61
4.3.4 Macrofauna.....	61
4.3.5 IsoError modelling results.....	66
4.4 Discussion.....	67
4.4.1 Differences in SOM food sources between habitats.....	67
4.4.2 Resource utilization by the consumers.....	69
Chapter 5 Application of dual stable isotope additions of ¹³C and ¹⁵N to investigate the importance of microalgae as a food source to the meiofauna in a mangrove.....	73
5.1 Introduction.....	73
5.2 Materials and methods.....	75
5.2.1 Study site.....	75
5.2.2 Experimental design.....	76

5.2.3 Sample preparation for stable isotope analysis.....	77
5.2.4 Mass spectrometr.....	78
5.2.5 Data analysis.....	79
5.3 Results.....	79
5.3.1 Natural abundance stable isotope values (Day 0, before label addition)..<	79
5.3.2 Dual isotope labelling ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ average enrichment).....	79
5.3.3 ^{13}C and ^{15}N label uptake by SOM and MPB.....	81
5.3.4 ^{13}C and ^{15}N label uptake by the meiofauna.....	82
5.4 Discussion.....	85
Chapter 6 General conclusion.....	87
6.1 Summary of findings.....	87
6.2 Significance of this research and implication for future studies.....	89
Appendices.....	94
APPENDIX A.....	94
APPENDIX B.....	96
APPENDIX C.....	97
APPENDIX D.....	98
APPENDIX E.....	99
References.....	100

LIST OF FIGURES

Figure 2.1: Map showing the study locations: Tallebudgera, Currumbin and Terranora mangroves located in subtropical eastern Australia.....	14
Figure 2.2: Principal Component Analysis plot describing the divergence of environmental variables, to explain the spatial variations at Tallebudgera (black-filled), Currumbin (unfilled) and Terranora (grey-filled) sites based on mangrove species. Circles = <i>A. marina</i> , triangle = <i>R. stylosa</i> , and square = <i>A. corniculatum</i> . All samples were collected in one season (autumn). Variance explained by PC1 is 61.7 % and PC2, 19.9 %.....	20
Figure 2.3: Principal Component Analysis plot describing the temporal variation of the environmental variables at the Tallebudgera site based on mangrove species. Autumn (black-filled), winter (unfilled), and summer (grey-filled). Circles = <i>A. marina</i> , triangle = <i>R. stylosa</i> , and square = <i>A. corniculatum</i> . Variance explained by PC1 is 50 % and PC2, 21 %.....	23
Figure 2.4: 2D Non-Metric MDS ordination showing the spatial variation of the meiofaunal assemblages collected from Tallebudgera (black-filled), Currumbin (unfilled) and Terranora (grey-filled) locations. Circles = <i>A. marina</i> , triangle = <i>R. stylosa</i> , and square = <i>A. corniculatum</i> . The solid, dashed and dash-dotted ellipses indicate the main groups that were significantly different ($P < 0.05$) according to pairwise test on Tallebudgera, Currumbin and Terranora data, respectively. No clear delineation is discernible for <i>A. marina</i> from Currumbin because of the wide variation.....	25
Figure 2.5: 2D Non-Metric MDS ordination for the seasonal variation of the meiofaunal assemblages in autumn (black), winter (grey) and summer (unfilled) for the samples collected from (A) <i>A. marina</i> , (B) <i>R. stylosa</i> and (C) <i>A. corniculatum</i> forests in Tallebudgera (n=9).....	27
Figure 3.1: Schematic diagram of (A) Complete cage and (B) Half-cage designs of the experimental cages.....	38
Figure 3.2: A) Chl <i>a</i> concentration (mg g ⁻¹ sediment), B) LOI (%) and C) Irradiance (μmol m ⁻² s ⁻¹) in the Ambient (black bars) samples within <i>U. vomeris</i> and <i>M. longicarpus</i> activity areas compared to the Exclusion cages (white bars). All data are mean ± SE.....	43
Figure 3.3: Meiofaunal density (mean ± SE, n=9) in the five experimental treatments for (A) <i>M. longicarpus</i> and (B) <i>U. vomeris</i> . Treatments with different letters are significantly different from each other ($p < 0.05$).....	45
Figure 4.1: Map showing the sites where all the samples from saltmarsh (SM), mangrove (MG) and sandflat (SF) were collected at Tallebudgera creek (specific sampling site indicated as black circles).....	53
Figure 4.2: Mean values of the LOI (n = 5) and Chl <i>a</i> (n = 6) contents for the saltmarsh (SM), mangrove (MG) and sandflat (SF) habitats. Letters indicate samples are significantly different at $p < 0.001$. Error bars indicate standard error.....	59
Figure 4.3: Results for the regression analysis between SOM and the meiofaunal δ ¹³ C and δ ¹⁵ N isotopic values (n = 9). (A) Relationship between the δ ¹³ C values of the nematodes and SOM ($y = 1.15x + 7.02$, $R^2 = 0.49$, $p < 0.05$), (B) Relationship between	

the $\delta^{15}\text{N}$ values of the nematodes and SOM ($y = 0.69x + 3.71$, $R^2 = 0.69$, $p < 0.01$), (C) No significant correlation found between the $\delta^{13}\text{C}$ values of the harpacticoids and SOM ($p > 0.05$), and (D) Relationship between the $\delta^{15}\text{N}$ values of the harpacticoids and SOM ($y = 1.08x - 0.84$, $R^2 = 0.86$, $p < 0.001$).....60

Figure 4.4: The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots of the meiofaunal nematodes (triangle) and harpacticoids (square), and also the macrofauna (circle) consumer data for the saltmarsh (unfilled), mangrove (black-filled), and sandflat (grey-filled) habitats. Data are mean \pm SE. Values are corrected for trophic fractionation. Triangle vertices (S_1 , S_2 , S_3) indicate the estimated (virtual) end-members inferred based on consumer data from the three habitats. a = amphipod, c = crabs, g = gastropod, h = harpacticoids, n = nematodes, o = ostracod, p = polychaetes, s = juvenile shrimp.....64

Figure 4.5: The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots of the meiofaunal nematodes (triangle) and harpacticoids (square), and also the macrofauna (circle) consumer data for the saltmarsh (SM), mangrove (MG), and sandflat (SF) habitats. Vertices from the solid line triangle (S_1 to S_3) indicate the estimated (virtual) end-members inferred based on consumer data. Data are values of replicate samples, corrected for trophic fractionation. Dashed line triangles were plotted based on the actual end-members data collected from the local habitats for detritus (MG/ SM/ SG), SPOM and MPB resources. PP = phytoplankton, SM = saltmarsh, MG = mangrove and SG = seagrass.....65

Figure 5.1: (A) Map of the study area in southern Moreton Bay, Queensland, Australia. The black circle indicates the area on Kangaroo Island. (B) and (C) are detailed photographs of site where the experiment was conducted.....76

Figure 5.2: (A) Dual natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) of the nematodes (NEM), harpacticoids (HAR), MPB, SOM and mangrove leaves (MG), prior to isotope addition (Day 0). (B) The enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for each component, averaged over the sampling period after labelling. Values of the mangroves in both plots are obtained from the same site before label application ($n=3$). Values are mean \pm SE, and plotted without correction for trophic fractionation.....80

Figure 5.3: Carbon (A) and nitrogen (B) uptakes (δ^E) following dual ^{13}C and ^{15}N addition for the SOM (-----) and MPB (— —) over the 12-day sampling period. Values are mean \pm SE.....81

Figure 5.4: Carbon and nitrogen uptakes (δ^E , solid lines) for the nematodes and harpacticoids over the 12-day dual ^{13}C and ^{15}N addition. Average carbon and nitrogen label uptakes for the SOM (-----) and MPB (— —) are also indicated. The best fitting curves for the meiofauna are plotted with the respective polynomial functions and R^2 values are given. Values are mean \pm SE, and plotted without correction for trophic fractionation.....83

LIST OF TABLES

Table 2.1: Variation of the environmental variables (A) and meiofaunal density (B) at the mangrove forests in Tallebudgera, Currumbin and Terranora, and temporal variations at the Tallebudgera site. AM = <i>A. marina</i> , RS = <i>R. stylosa</i> and AC = <i>A. corniculatum</i> . Samples for the particle size descriptions were only collected during autumn, '-' symbol denotes data not available. Values are mean \pm SE (n=9).....	22
Table 2.2: Spearman's rank correlation coefficients (<i>r</i>) between the single, pair and group of three environmental variables that best correlated with the meiofaunal similarity matrix across (A) spatial variation and (B) temporal variations (n = 27). All the environmental variables were included for spatial variation (A), but the sediment particle and sorting were excluded in temporal variation as no data is available for winter and summer (sediment particle size analysis was not repeated in the consecutive seasons). Bold type indicates the overall optimum that gives the highest rank correlations at each location and season.....	28
Table 3.1: Summary of the type of effects expected to be present for each experimental treatment, namely "Physical" "Trophic" or "Cage" effect (n=9). Symbols signify the presence (+) or absence (-) of each effect.....	39
Table 3.2: Description of the substrate particle sizes at the two habitats based on the Wenworth classification. Values are mean \pm SE (n=3).....	42
Table 3.3: Meiofaunal density (n=9) for the <i>M. longicarpus</i> and <i>U. vomeris</i> experimental treatments. All data are mean \pm SE.....	44
Table 4.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SE) of the food sources and consumers collected from the Tallebudgera mangrove ecosystem. Values are not corrected for fractionation.....	63
Table 4.2: IsoError modelling results proportional contribution of each potential food sources for consumers in saltmarsh, mangrove and sandflat habitat, as computed by the isotope mixing model IsoError (values are mean \pm SE). Bold values indicate > 50% contributions from a single resource. Isotopic values (‰) were adjusted for fractionation prior to mixing model analysis.....	66
Table 5.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) of the natural abundance (Day 0), the post-labelling values and also the label uptake (δ^{E}) from Day 0.5 until Day 12. Values are mean \pm SE from three sample replicates (n = 3) unless for the harpacticoid samples on Day 6 (n = 1) and Day 12 (n=2).....	84
Table A.1: Results from the univariate PERMANOVA analysis of each PC scores that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B) based on 2-way crossed design. "Perm" indicates number of unique permutations available. Bold values are significant ($\alpha = 0.05$).....	94
Table A.2: Results from the pairwise comparison for the univariate PERMANOVA analysis of each PC scores that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B)	

based on 2-way crossed design. “Perm” indicates number of unique permutations available. Bold values are significant (alpha = 0.05).....95

Table B.1: Results from the univariate PERMANOVA analysis (Table C) and pairwise comparison of the meiofaunal density that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B) based on 2-way crossed design. “Perm” indicates number of unique permutations available. Bold values are significant (alpha = 0.05).....96

Table C.1: Carbon (C) and nitrogen (N) content (mean ± SE) of harpacticoids and nematodes and the resultant number of individual animals required for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. 5 µg is the minimum amount of nitrogen required for the stable isotope analysis in this study.....97

ACKNOWLEDGEMENTS

I would like to acknowledge my sincere appreciation and gratitude to my principal supervisors: Prof. Joe Lee and Prof. Brian Fry for their guidance through this journey. A special thank-you also to Prof. Rod Connolly for the opportunities and trust provided.

To all the Australian Rivers Institute (ARI) staff and postgraduate members, thank you for the assistance and help, and knowledge shared. To members of the School of Environment (ENV), your support and sweet treats for morning tea will always be remembered.

To Belinda Hachem and Daniel Tonzing, thank you so much for everything. To friends Hanh Bui, Patricia, Jason van de Merwe, Shafagh Kamal, Majid, Christopher Henderson, Yisheng Peng, Nadeeka and Daniel Ouyang, thanks for everything and I wish you all good luck and all the best for your future.

To the loves of my life, my husband Uzair Rusli, our son Irfan Muqri and the little newborn Aisya Sofea, nothing on earth beats your unconditional love and support through the whole journey. I am strong when I am on your shoulders. To my mother Rahmah Yussoff, and late father Mohd Abdullah who passed away at the beginning of this journey, this is for you. Thank you for everything.

The work was conducted under Self-assessable code MP05 of Queensland Department of Fisheries and Scientific Collection permit P10/0004-2.0 issued by the New South Wales Department of Industry and Investment.

STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university.

To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)_____

MAIZAH MOHD ABDULLAH

CHAPTER 1

GENERAL INTRODUCTION

1.1 Definition of the meiofauna

According to Giere (2009), the term 'meiofauna' refers to the microscopically small, motile aquatic animals living mostly in and on soft substrates at all depths in the marine and freshwater realm. The term 'meiofauna' is usually used synonymously with 'meiobenthos'. The formal size boundaries of the meiofauna are operationally defined based on the standardized mesh width of sieves from 1000 μm to 44 μm . In the context of this study, the size boundaries of the mesh sizes used are 500 μm and 63 μm as upper and lower limits, respectively, in order to align with the size used by most of the research and publications on the meiofauna.

The meiofauna may fulfil the size criterion either for the whole period of the life cycle (permanent meiofauna) or only for the first juvenile stages, the latter group is then known as temporary meiofauna (Wołowicz et al., 2011). Meiofauna are usually higher in abundance than macrofauna and generally have metabolism up to five times higher due to their smaller body sizes (Gerlach, 1971).

1.2 Abundance of the meiofauna in mangroves

Meiofauna are ubiquitous in soft-bottom habitats around the world, such as estuaries (Hodda & Nicholas, 1985; Coull, 1999), lake ecosystems (Kurashov, 2002; Dye, 2005) and deep sea sediments (Danovaro et al., 2008; Gaever et al., 2009; Nascimento et al., 2012). Descriptive studies on the distribution and abundance of the meiofauna in mangrove habitats have been published from different parts of the world (Dye, 1983; Nicholas et al., 1991; Sasekumar, 1994; Ólafsson, 1995; Ólafsson et al., 2000;

Chinnadurai & Fernando, 2006; Chinnadurai & Fernando, 2007; Torres-Pratts & Schizas, 2007; Zhou et al., 2015). However, information regarding the ecology of the meiofauna associated with mangroves are comparatively lacking despite the fact that mangroves cover much of the soft-sediment shores especially on tropical and subtropical coasts (Nicholas et al., 1991; Bouillon et al., 2002).

In fine sediments, the majority of the meiofauna are concentrated in the upper first centimetres of surface sediments but they can go deeper in coarse-grained sediments and on sandy beaches (Wołowicz et al., 2011). Besides, they are also found on the walls of burrows of macrobenthic animals such as crabs (Dittmann, 1996). In fine sands with a high silt content, nematodes are numerically dominant, up to 98 % (Moens & Vincx, 1996) of total meiofaunal abundance, typically followed by harpacticoid copepods, oligochaetes and the other groups (McIntyre, 1969). A detailed record on the occurrence and abundance of the meiofauna from various locations in coastal waters including mangroves has been reported by Wołowicz et al. (2011).

1.3 Ecological roles of the meiofauna

Biological interactions between the metazoans and the microbial community are important in structuring food webs in aquatic sediments (Nascimento et al., 2012). Despite their diverse feeding habits, e.g. detritivores, algal feeders or carnivores (Wieser and Kanwisher, 1961; Findlay and Tenore, 1982; Pinckney et al., 2003; Wołowicz et al., 2011), meiofaunal taxa such as nematodes, harpacticoids, and ostracods are important grazers of bacteria (Rieper, 1978; Montagna, 1984; Carman and Thistle, 1985). In marine systems, most of the data available focused on the interactions between microbes and the macrofauna (Andersen & Kristensen, 1992; Banta et al., 1999), and less is known on the roles played by the meiofauna despite the fact that they are typically more abundant than macrofauna in most benthic habitats (Nascimento et al., 2012). Meiofauna are able to consume their body weight equivalent in microbes each day, and

this grazing pressure could exert a significant stimulatory effect on the microbial community (Montagna, 1984). Through grazing, meiofauna stimulate bacterial populations and maintain their growth in exponential phase, produce extracellular polysaccharides to cultivate bacteria and their mechanical activities to breakdown detrital particles cause them to be more accessible and susceptible to bacterial degradation (Wołowicz et al., 2011). In addition, by having short generation times (weeks to months), meiofauna are able to return nutrients into the sediments efficiently and increase their availability to bacteria rapidly (Coull, 1999).

In addition, bioturbation activities including burrowing activities and construction of tubes and burrows are major modulators of microbial activities and biogeochemical processes in benthic habitats (Mermillod-Blondin, 2011). Bioturbation activities by the meiofauna haven been described in detailed by Cullen (1973). Nematodes have been observed to rapidly establish an intricate, closely spaced network of thread-like intergranular burrows within the surface layer of freshly emplaced sediment, through which they could be observed gliding at relatively high speeds, estimated at 2-3 mm s⁻¹ (Cullen 1973). Harpacticoid copepods, a major group that inhabit in muddy, estuarine sediments, have been observed to build and inhabit elongate, mucous tubes, which may extend to a depth of 3.9 mm into the sediment (Chandler & Fleeger, 1984). Furthermore, locomotory activities of the meiofauna also enhance oxygen and carbon dioxide diffusion in the interstitial spaces and contribute to pH regulation (Wołowicz et al., 2011).

The meiofauna also play a significant trophic role in soft-sediment habitats. According to Findlay and Tenore (1982), nematodes increased carbon mineralization of *Gracilaria* detritus up to 300%, and 50% for more refractory (high in cellulosic components) detritus of *Spartina*. In an attempt to investigate the importance of the meiofauna on the benthic decomposition of a labelled diatom bloom, Nascimento et al. (2012) found an increment of nearly 50% in cumulative production of ¹⁴CO₂ after 17 days in sediment with high meiofaunal abundance, and also a strong correlation between the abundance and

biomass of the meiofauna with the amount of diatoms mineralized. Unfortunately, there is no solid information available regarding the role of meiofauna in the decomposition of mangrove litter except for a report by Zhou (2001). In this study, the bacterivorous nematode *Diplolaimella* sp. bloomed in the cores treated with mangrove litter addition, demonstrating the role of the meiofauna in the decomposition of mangrove litter.

Initially, the meiofauna have been considered as a sort of a trophic dead end, receiving energetic inputs from the lower trophic levels but not being consumed by higher trophic level consumers (McIntyre, 1969). However, in more recent studies, meiofauna especially the harpacticoid copepods and nematodes have been demonstrated to be an important food resource for the higher trophic levels, e.g., fish, prawns, crabs, polychaetes (Bell and Coull, 1978; Reise, 1979; Bell, 1980; Leh and Sasekumar, 1980; Chong and Sasekumar, 1981; Wołowicz et al., 2011). In subtropical Australian mangroves, harpacticoid copepods were found to be the dominant prey items in the guts of various juveniles of various fish families, such as Sillaginidae, Gobiidae, Theraponidae and Leognathidae, with their mean dominance as prey by number ranged between 41 % to more than 80% (Coull et al., 1995). There is also available, though limited, evidence, for top-down impact on the meiofauna by benthic invertebrates (Bell & Coull, 1978; Kennedy, 1993, 1994; Feller, 2006), and even by larger animals such as shore and migratory birds (Gaston, 1992; Sutherland et al., 2000). In a detritus-based ecosystem of a small lagoon in the central Gulf of Mexico, meiofauna have been found to be the principal link to higher trophic levels when they consumed most of the detrital organic carbon in surface sediments, and constituted the main food supply to the local consumers such as fish and crustaceans (Rosado-Solórzano & Guzmán del Prío, 1998).

1.4 Meiofauna as consumers in benthic food webs

Given their ubiquitous abundance, meiofauna are potentially important consumers in benthic food webs but their role has been less extensively studied compared to the larger

epibenthos such as crabs and molluscs (Scharler, 2011). As a group, the meiofauna consume a wide variety of food sources including detritus, bacteria, diatoms and other small photoautotrophs, cyanophytes, ciliates and other meiofauna (Moens & Vincx, 1996). Due to their small size, high metabolic and life-cycle turnover rates, and diverse feeding patterns, meiofauna are expected to respond rapidly to changes in food availability (Danovaro, 1996). However, our understanding on their trophic dynamics is still fragmentary due to their small sizes and the diversity of food sources available in benthic habitats (Couch, 1989; Leduc et al., 2009).

Feeding-type classifications for nematodes were traditionally based on the morphology of their buccal cavity (Jensen, 1987; Moens & Vincx, 1997). Jensen (1987) reported four feeding guilds of free living aquatic nematodes, namely: 1) deposit feeders; 2) epistrate feeders; 3) scavengers; and 4) predators. However, more recent observations by Moens and Vincx (1997) reported a new scheme with six major feeding guilds: 1) microvores; 2) ciliate feeders; 3) deposit feeders; 4) epigrowth feeders; 5) facultative predators; and 6) predators. Nevertheless, nematodes are often opportunistic feeders, which may change their feeding habits in response to available food (Moens & Vincx, 1997) and therefore it is complex and difficult to understand their feeding interactions (Moens & Vincx, 1996). Whether their selection of a specific food is due to its morphology, energetic value, nutritional quality, and/ or availability remains a subject of much research effort (Wołowicz et al., 2011).

A close trophic interaction among the meiofauna, microbes and microalgae in a saltmarsh has been reported by Montagna (1984); when meiofauna of the high marsh were found to remove approximately 3% of the bacteria and 1% of the diatoms standing stock per hour. The polychaetes dominated ingestion of bacteria by up to 95%, while traditional meiofaunal taxa such as nematodes, copepods, ostracods have selected diatoms 8 times more frequently than bacteria. Harpacticoid copepods are important grazers of microalgal primary production (Coull, 1990), but they are also known to feed

on a wide variety of food sources (Hicks & Coull, 1983). Studies on copepods feeding strategies have mainly focused on pelagic systems and whether harpacticoid copepods feed at random, ingesting as food particles are encountered. Whether they select specific food items based on the morphology or sizes of the foods is unknown (Wyckmans et al., 2007).

The microphytobenthos has been highlighted in some studies as an important nutrient source to the harpacticoid copepods (Montagna et al., 1995; Wyckmans et al., 2007). Though Leduc et al. (2009) found similar preferences, they also found higher variability of harpacticoid copepod isotopic signatures at densely vegetated sites, which indicated that a greater variety of food sources was ingested when they were available. Meanwhile, Couch (1989) discovered a close correspondence between meiofaunal and detrital *Spartina* isotopic signatures in the North Inlet Estuary, USA, and concluded that the bulk of carbon assimilated by meiofaunal populations may be derived from *Spartina* detritus. Nevertheless, some other studies showed preferential ingestion or assimilation of bacteria (Rieper, 1978; Rieper, 1982; Carman & Thistle, 1985). Therefore, the preliminary data may suggest that the importance of different food sources to harpacticoid copepods may depend largely on availability (Leduc et al., 2009).

1.5 Stable isotope analysis

The actual food sources for meiofauna have been a matter of speculation, and the advent of stable isotope analysis (SIA) has provided a powerful tool for clarifying their food sources and their position in the trophic chain (Couch, 1989; Leduc et al., 2009; Wilson & Luczkovich, 2011). The application of these methods is based on the assumption that potential basal sources have distinct signatures and the source signature is predictively transmitted from the food to the consumer (Bec et al., 2011). In spite of high abundance of the meiofauna in mangroves, stable isotope studies of food webs involving the meiofauna are very limited, and detailed knowledge of their trophic position, resource

partitioning, and feeding ecology in mangroves is lacking (Demopoulos et al., 2007). The relative scarcity of stable-isotope studies on the meiofauna is primarily because meiofauna have low biomass and therefore large numbers of them must be collected and isolated to obtain sufficient materials for analysis (Carman & Fry, 2002).

Natural abundance of the stable isotopes of carbon and nitrogen (expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) are the two most commonly used indicators in ecological SIA (Layman et al., 2011). $\delta^{13}\text{C}$ is useful for determining the diet of the organisms because the difference in $\delta^{13}\text{C}$ signatures between the consumers and food sources, i.e. trophic discrimination, is on average small ($< 1\text{‰}$ enrichment) (McCutchan et al., 2003). The range of reported values is, however, still substantial. Meanwhile $\delta^{15}\text{N}$ is a useful tool to identify the food resources and trophic level of the organisms because the $\delta^{15}\text{N}$ signature of a consumer is on averaged enriched relative to their diet by about 3‰ (Post, 2002; Layman et al., 2011). Again, a wide range of values has been reported, depending on the specific consumer-food pairs and the nature of the food such as N content. However, the use of the dual-isotope approach to resolve the dietary relationship becomes limited when the consumers utilized several food sources, or when the food sources have similar isotope values. In this case, the use of additional elements e.g. $\delta^{34}\text{S}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$ is useful to help resolving the problem (Connolly et al., 2004; Soto et al., 2013; Vander Zanden et al., 2016).

While the use of multiple natural abundance stable isotopes has helped to further our understanding of resource use in complex systems that have multiple primary producers with similar or highly variable isotope values, e.g. mangrove habitats or estuaries, this approach still has limitations (Cloern et al., 2002; Galvan et al., 2011). Data analysis becomes difficult when the number of food sources is greater than one plus the number of elements used, as no definite solution on contribution will be possible. Recent advances in Bayesian approaches to analysing stable isotope data may alleviate the quantitative, but not necessary the biological, interpretation of SIA data. One way to

increase the power of stable isotope analysis is to use natural abundance stable isotopes in combination with isotope additions (Hughes et al., 2000; Middelburg et al., 2000; Carman and Fry, 2002; Levin et al., 2006; Maddi et al., 2006).

In the isotope addition (labelling) approach, natural differences in primary producer isotope values are enhanced by creating a distinct isotope label in specific or targeted primary producers, where the label can then be followed through the food web via consumption of a labelled primary producer by the consumers (Galvan et al., 2011). The labelling process takes advantage of the difference in rate of label uptake by primary producers of different tissue turnover times to differentially enrich certain producers. The MPB, for example, would respond more rapidly to labelling compared to mangrove trees because of the former group's shorter tissue turnover time. A combination of natural-abundance isotope surveys and isotope-addition experiments appears to be a powerful approach for investigating both average patterns and interspecific variability in resource exploitation (Carman and Fry, 2002; Galvan et al., 2008).

1.6 Research questions of this study

Despite the wide distribution and high abundance of the meiofauna in mangrove habitats and preliminary evidence suggesting a significant ecological role in marine ecosystems, there is a distinct lack of knowledge of the meiofauna in mangroves. This paucity of information might be due to the small sizes of the meiofauna and also the complex structure and temporal dynamics of mangrove forests, making collecting and studying small meiofauna tedious and time consuming.

The central theme of this study is to fill this gap in knowledge by addressing three main research questions (RQs):

- I. How do dynamics of the environmental conditions and the spatial and temporal heterogeneity of mangrove sediments affect meiofaunal abundance and assemblage structure?

- II. How may key macrofaunal species such as brachyuran crabs impact the abundance and assemblage structure of the meiofauna on tropical mangrove shores? Are the interactions fundamentally trophic or physical in nature?
- III. What are the roles of the meiofauna as consumers, and where are they trophically positioned along the macrofaunal consumers in subtropical mangrove ecosystems?

1.7 Thesis structure

This thesis is structured as a series of data chapters, each designed to be published as a standalone research paper (Chapters 2-5), bookended by a general introduction chapter (Chapter 1) and a general conclusion (Chapter 6). As such, there is some repetition in general themes in the introduction and discussion sections of some chapters, and also the methods sections. The co-authors of the published/draft papers contributed scientific advice and editorial guidance on the manuscripts while all field and laboratory data collection was performed by me.

RQ	Chapter	Objective	Publication status
	1	Introduction	
I	2	To examine the variability of the sediment environments within each of three subtropical mangrove species, and also to see how meiofaunal assemblages would respond to changes in environmental conditions in their habitats	<i>In revision.</i> This chapter has been submitted to the journal <i>Estuarine, Coastal and Shelf Science</i> and is undergoing editorial and formatting revision for a resubmission. All the comments and reviews received from two reviewers have been addressed and presented and incorporated into this thesis.
II	3	To investigate the significance and nature of top-down control on the density of mangrove meiofauna based on their interactions with deposit-feeding crabs; specifically, whether the interaction is fundamentally physical or trophic	<i>Published.</i> Abdullah, M.M. and Lee, S.Y. 2016. Meiofauna and crabs in mangroves and joining sandflats: Is the interaction physical or trophic? <i>Journal of Experimental Marine Biology and Ecology</i> 479: 69-75 (Appendix D).
III	4	To evaluate the meiofaunal and macrofaunal food webs associated with three connected intertidal habitats, namely saltmarsh, mangrove and sandflat, along a tidal gradient using natural abundance stable isotope analysis of carbon and nitrogen	In preparation for submission.
	5	To measure the importance of the utilization of MPB resource by harpacticoid copepods and nematodes through a combined dual-isotope natural abundance-labelling approach in a subtropical mangrove	In preparation for submission.
	6	General conclusion	

CHAPTER 2

STRUCTURE OF MANGROVE MEIOFAUNAL ASSEMBLAGES RESPOND TO LOCAL SEDIMENT CONDITIONS IN SUBTROPICAL EASTERN AUSTRALIA

2.1 Introduction

Meiofauna are ubiquitous in soft-sediment marine environments and contribute significantly to ecosystem functioning (Montagna, 1984; Coull, 1999; Wolowicz et al., 2011; Nascimento et al., 2012). Despite their abundance and ubiquity, detailed knowledge of the taxonomy, biology and interactions of the meiofauna, and their role in the functioning of mangrove ecosystems, is lacking (Nagelkerken et al., 2008). The close association of meiofauna with the sediment matrix in their habitat means that any changes in interstitial chemistry are expected to result in fast response in the meiofaunal assemblage. In addition, meiofauna spend their whole lifetime within these habitats and have limited motility, suggesting significant potential of the use of meiofauna in assessing anthropogenic impacts (Kennedy and Jacoby, 1999). Another major contributing factor is the short life-span of meiofauna taxa, which means that population, and thus assemblage, fluctuations can be temporally significant, with very fast response towards change in local environmental conditions. The meiofauna may therefore act as good temporal indicators of sediment conditions in soft-sediment habitats such as estuaries.

Estuarine sediments are highly diverse in their physico-chemical properties as well as temporally dynamic. One of the important features characterising tropical estuarine sediments is the soluble and condensed tannins leached from mangrove roots and litter, produced by the trees for chemical defence against herbivores, which impregnate the sediment (Alongi, 2009). Tannins have long been proposed to have a negative effect on

the meiofauna (Alongi, 1987a, b). However, the variability of the tannin content in mangrove habitats, and its implications for the meiofauna is largely unknown. Besides, the aboveground structures of different mangrove species shape the heterogeneity or complexity of the habitat, with implications for both physical (e.g. degree of shading) and biotic (e.g. predator abundance) conditions. While this heterogeneity and the resulting conditions are poorly known (Kamal et al. 2014), they probably influence the structure of the animal assemblage including the meiofauna. Such feedback between mangrove species and their associated fauna has been demonstrated for sesarmid crabs (Lee and Kwok 2002).

While the belowground roots also contribute to the heterogeneity of the habitat for infauna, this aspect of mangrove habitat complexity is even less studied. Little is known on how belowground mangrove roots may influence the meiofauna (Sahoo et al., 2013). For example, fine roots may either exert a negative impact by occupying space in the meiofauna habitat, but also may provide micro-habitats or support meiofauna trophically through the provision of organic exudates. Also, different mangrove roots help aerate the sediment at various levels, resulting in different sulphide concentrations among sediments colonised by co-occurring species (McKee et al., 1988; Kryger and Lee, 1996). Estuarine macrofauna such as crabs may also shape meiofaunal assemblages through physical, e.g. bioturbation, or trophic interactions (Abdullah and Lee, 2016).

The interactions between biological and physical characteristics of mangrove sediments is vital to the function of these complex habitats. The fact that different mangrove plants have different environmental niches, and affect their surroundings differently, makes it difficult to assess the extent tree diversity influences meiofaunal diversity (Nagelkerken et al., 2008). Nevertheless, data on some macrofaunal groups suggest a positive correlation between tree and faunal species richness (Lee, 2008). Most of the previous studies on mangrove-associated meiofauna generally focused on their vertical distribution (e.g. Vanhove et al., 1992; Somerfield et al., 1998; Sahoo et al., 2013) or the

broad environmental gradients influencing generic meiofaunal distributions, such as tidal height, salinity, oxygen availability, and sediment properties such as organic content and granulometry (Somerfield et al., 1998; Coull, 1999; Tolhurst et al., 2010). A few studies focused on more specific variables such as the effects of mangrove leaf litter and pneumatophores on meiofaunal assemblages (Gwyther, 2003; Gwyther and Fairweather, 2005).

In this study, we investigated meiofaunal assemblage structure of sediments colonised by three mangrove species, namely, *Avicennia marina*, *Rhizophora stylosa* and *Aegiceras corniculatum*, at three locations in subtropical eastern Australia. This study aimed to examine the variability of the sedimentary environments within each forest of the different mangrove species, and to see how meiofaunal assemblages would respond to potential environmental drivers at different scales. The environmental variables were chosen based on the hypothesis that these variables may influence the meiofauna in different ways, i.e. those acting as proxies for food availability (phaeopigments, Chl *a*, and total organic content (TOC)), habitat structure (sediment particle size, belowground root biomass, and moisture) and also deterrents (tannin content). We predict that meiofaunal assemblages would respond to differences in the environmental variables associated with different mangrove species across different locations and seasons.

2.2 Materials and methods

2.2.1 Study Area

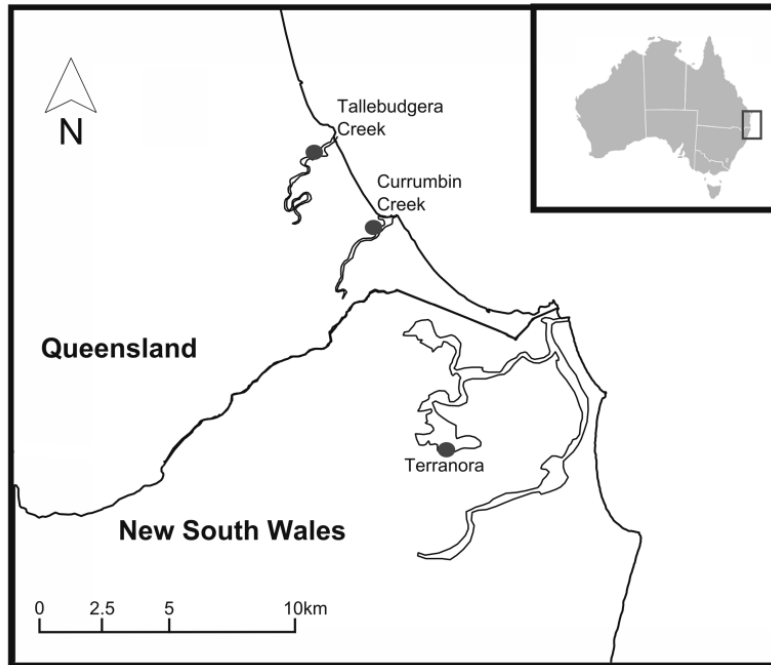


Figure 2.1: Map showing the study locations: Tallebudgera, Currumbin and Terranora mangroves located in subtropical eastern Australia.

Our study comprised three mangrove locations (Figure 2.1): Tallebudgera Creek ($28^{\circ} 6'30.77''\text{S}$ $153^{\circ}26'48.13''\text{E}$) and Currumbin Creek ($28^{\circ} 7'51.39''\text{S}$ $153^{\circ}28'44.92''\text{E}$) located in Southeast Queensland, and Terranora ($28^{\circ}13'28.38''\text{S}$ $153^{\circ}30'32.58''\text{E}$) in northern New South Wales. The former two sites have moderate dense of mangroves and much thicker in Terranora (Appendix E). This region of Australia has typical hot wet summers (December – February) and cool dry winters (June - August) with transitional conditions in autumn (March – May) and spring (September – November). The two locations in Queensland are on the southern Gold Coast and have been gazetted as Fish Habitat Area (FHA) by the Queensland government. The creeks flow directly into the Coral Sea of the South Pacific Ocean, and have low sediment trapping efficiency and low sedimentation rates. There are several distinct mangrove vegetation zones as

described by Shine et al. (1973), including *Avicennia marina* and *Rhizophora stylosa* zones located at the low intertidal areas, and the *Aegiceras corniculatum* zone located at the high intertidal area. Mangroves on Currumbin Creek are located at the low estuary and are also dominated by *A. marina*, *R. stylosa* and *A. corniculatum*.

The Terranora mangrove fringes Terranora Broadwater, located in the northeast of New South Wales, adjacent to the Queensland border. The Broadwater is a shallow estuarine lake of approximately 0.5 – 1.5 m depth and acts as the receiving waters for the freshwater catchment that discharges into Terranora Creek. It is influenced by tidal flow coming from the Tweed River estuary and freshwater inputs from the western sub-catchments from Bilambil and Duroby Creeks. *A. marina* is the dominant species, with lower occurrences of *R. stylosa* and *A. corniculatum* near to the mudflats bordering the Broadwater.

2.2.2 Field sampling

The boundaries of the mesh sizes used were 500 µm and 62 µm as upper and lower limits, respectively. Field samplings for the environmental variables and the meiofauna samples were divided into two approaches: Firstly, to measure the spatial variation of the meiofauna and the environmental variables associated with different mangrove stands (*A. marina*, *R. stylosa* and *A. corniculatum*) from the three locations (Tallebudgera, Currumbin and Terranora). These locations were used as replicates of individual mangrove species. The samplings at all three locations were performed in one season (autumn, May 2014). Secondly, temporal variations of the meiofaunal assemblages associated with the three mangrove species were measured by repetitive samplings in autumn, winter, and summer (May 2014, July 2014 and January 2015, respectively) at one location, i.e. Tallebudgera.

Nine replicate cores ($n = 9$) of mangrove soil samples were taken at each mangrove site with a cut syringe tube (internal diameter 2.67 cm). Sediment samples were collected

randomly within the aerial root zones of the *A. marina* and *R. stylosa* mangroves to reflect species-specific soil conditions down to 5 cm in depth. Meanwhile, sediment samples for the *A. corniculatum* mangrove (which does not have aerial roots) were collected within the shades of the tree. The same soil cores were used for meiofauna and root biomass analysis, to represent the biomass of the belowground roots inhabited by the meiofauna within the same sediment. At the sites with thicker fibrous roots such as within the *R. stylosa* mangrove, the syringe was pushed with the foot into deeper sediment to make sure that it did not under-sample, and the required length (5 cm) of the cored sediments were collected. Additional soil cores (n = 9) were collected for analysis of tannin, moisture and total organic contents, and for particle size analysis (PSA). For the temporal samples, all measurements of environmental variables were repeated except for the PSA, as it was intentionally used to describe the sediment texture of the sampling sites. Samples for chlorophyll a were collected by scraping the top 1 cm of mangrove surface sediment, which were then wrapped with aluminium foil and stored in an ice box. The salinity of the pore water was measured in-situ using a refractometer. At the highest intertidal area occupied by *A. corniculatum*, which received less tidal inundation and the soils were dry, sediment samples were collected and brought to the laboratory for salinity measurement. A mercury thermometer was pushed into the sediment to record the temperature.

2.2.3 Laboratory analysis

All sediment core samples were promptly stored at -20°C, except for the chlorophyll a samples, which were analysed immediately. Samples for meiofauna were processed within 48 hours to minimise the destruction of soft-bodied taxa. Soil cores were washed using the decantation technique (Giere, 2009) using 500 and 63 µm sieves as size boundaries. Samples retained on the 63 µm sieve were fixed with 70% ethanol and stained with Rose Bengal. The meiofauna was extracted using 30% LUDOX (Aldrich) solution (Eleftheriou and McIntyre, 2005) twice and counted under a dissecting

microscope. Root samples on the upper sieve (500 μm) from the meiofauna cores were washed carefully to remove any residual soil and dried at 100°C to constant weight. Chlorophyll *a* concentration was used as an indicator of microphytobenthic biomass (Ford and Honeywill, 2002). Under minimum light conditions in the laboratory, fresh soil samples were weighed (~5 g) and extracted with 10 mL of 90% aqueous acetone and active Chl *a* concentrations were measured using a spectrophotometer and calculated according to Parsons et al. (1984). Absorbance was measured at 750 and 665 nm before and after acidification of the sample to estimate the concentration of phaeopigments..

Hydrolyzable tannins were analysed following the Folin-Denis method as described in Allen (1989), using tannic acid as standard. Samples were oven-dried at 60°C and ground, and 1 g dry weight of soil was used, and tannin concentrations were calculated as percentage of tannin per sample dry weight. Soil moisture content was calculated as a percentage of wet mass, where 10 - 20 g of fresh samples were dried in an air-circulating oven at 100°C and dried to constant weight. The organic content of the sediment was estimated using the loss-on-ignition (LOI) method. Oven-dried (100 °C) sediment samples were ground and sieved through a 2 mm mesh and a known amount of sample (~ 1 g) was ignited at 550 °C in a muffle furnace for 4 hours. The samples were cooled down and then immediately weighed.

PSA was done following the wet sieving method (Buchanan, 1984; English et al., 1997). Sediment samples were oven dried at 60°C and 30 g of dried samples were treated with 30% hydrogen peroxide to remove the organic matter. Large roots or leaves were removed before analysis and 10 mL of 6.2 g L⁻¹ sodium hexametaphosphate (NaPO_3)₆ was added to aid dispersion of clay particles. Samples were washed on a 62 μm sieve by “puddling” the sieve in a basin filled with water and the water was replaced at intervals until no further fines were washed out. Samples were dried at 100°C to constant weight and transferred to the stacked series of graded sand sieves for analysis. The results were expressed as mean phi values (Krumbein and Pettijohn, 1938) and the sorting

coefficients were derived following Folk (1980). Salinity of the sediment at the *A. corniculatum* site was measured using the 1:1 (V:V) soil: water extract method and the results were expressed in electrical conductivity (EC) (Dahnke and Whitney, 1988).

2.2.4 Data analysis

Non-parametric multivariate techniques in the PRIMER (Plymouth Routines in Multivariate Ecological Research) package V7 were used (Clarke and Gorley, 2015) for analysing assemblage structure and its relationship with environmental drivers. As each of the environmental variables was measured in different scales and units, the data were normalised to provide an “equal footing”, and transformed (sediment sorting was transformed with $1/(1+V)$, and other variables were SQRT (V) transformed) to reduce skewness before multivariate analysis (Anderson et al., 2008). The similarity matrices of the environmental variables were calculated based on Euclidean distance. For the meiofaunal assemblages, Bray-Curtis similarity matrices were used on the fourth-root transformed data to reduce the influence of taxa with very high abundances, using shade plots to aid the choice of transformations (Clarke and Gorley, 2015). The spatial variations between locations and mangroves, and the temporal variations between seasons and mangroves for the environmental variables and the meiofaunal assemblages were compared using a two-way crossed PERMANOVA (9999 permutations). Principal components analysis (PCA) was used to visualize the variation of the environmental variables, with no rotation of axes used to clean up the factor loadings. The best correlation of the individual or paired environmental variables with patterns in the meiofaunal data associated with different mangrove species at different locations and seasons separately, was measured using BIOENV analysis. The individual normalized and transformed environmental variables were compared to the meiofauna similarity matrix (Bray-Curtis).

2.3 Results

2.3.1 Environmental variables

Values of the key environmental variables to be used in further analysis are summarised in Table 2.1. During autumn, the temperature of the mangrove sediment at all sites ranged from 19 to 22 °C, and the salinity ranged from 32 to 35. Mean monthly rainfall at Tallebudgera and Currumbin were 115.5 mm and in Terranora, 75.3 mm of rainfall were recorded. During winter, the sediment temperature at Tallebudgera dropped to between 13 to 15 °C, and increased during summer to between 26 to 29 °C. The salinity varied between 35 to 38 and 28 to 30 during winter and summer, respectively. Mean monthly rainfall at Tallebudgera during winter and summer were 31.9 and 283.9 mm, respectively. At the Tallebudgera *A. corniculatum* site, the mean degree of salinity as expressed by the electrical conductivity (EC) averaged at 15.8, 19.3 and 10.8 mmhos cm⁻¹ during autumn, winter and summer, respectively, which all are classified as very strongly saline (Dahnke and Whitney, 1988).

Spatial variations

PCA ordination of the environmental variables showed that the first two components accounted for 81.6% of the variability. PC1 represents an axis positively associated with all key variables while PC2 represents an axis of decreasing sorting, TOC, moisture, roots and mean phi values (Figure 2.2). In general, two major groups of conditions can be distinguished along PC1. The sites at Terranora were characterized closely into one group and were distinctively separated from those at the other two locations.

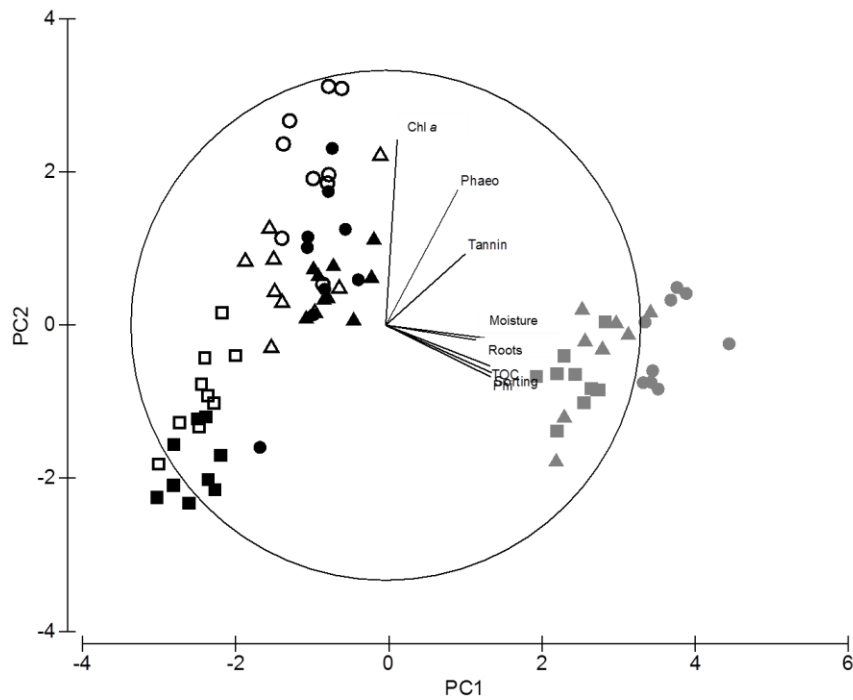


Figure 2.2: Principal Component Analysis plot describing the divergence of environmental variables, to explain the spatial variations at Tallebudgera (black-filled), Currumbin (unfilled) and Terranora (grey-filled) sites based on mangrove species. Circles = *A. marina*, triangle = *R. stylosa*, and square = *A. corniculatum*. All samples were collected in one season (autumn). Variance explained by PC1 is 61.7 % and PC2, 19.9 %.

Univariate PERMANOVA analysis indicates that the environmental variables along PC1 and PC2 were significantly different among locations and mangroves species, with a significant interaction between location and mangrove species ($P = 0.0001$, Appendix A). Pairwise tests suggest that the environmental condition along PC1 from *A. corniculatum* at Tallebudgera and Currumbin was significantly different from those associated with *A. marina* and *R. stylosa* ($P = 0.0003$ and $P = 0.0001$ respectively, Appendix A), but not significantly different between *A. marina* and *R. stylosa* ($P = 0.2237$ and $P = 0.2611$ in Tallebudgera and Currumbin respectively). In Terranora, the environmental condition of *A. marina* was significantly different from those of *R. stylosa* and *A. corniculatum* ($P = 0.0002$ and $P = 0.0001$ respectively), but not significantly different between *R. stylosa* and *A. corniculatum* ($P = 0.0732$). Along the PC2, the environmental conditions among all mangrove species from all locations were

significantly different from each other, except between *A. marina* and *R. stylosa* sites in Tallebudgera ($P = 0.0874$).

Table 2.1: Variation of the environmental variables (A) and meiofaunal density (B) at the mangrove forests in Tallebudgera, Currumbin and Terranora, and temporal variations at the Tallebudgera site. AM = *A. marina*, RS = *R. stylosa* and AC = *A. corniculatum*. Samples for the particle size descriptions were only collected during autumn, '-' symbol denotes data not available. Values are mean \pm SE (n=9).

A. Env. Variables	Tallebudgera									Currumbin			Terranora		
	Autumn			Winter			Summer			Autumn			Autumn		
	AM	RS	AC	AM	RS	AC	AM	RS	AC	AM	RS	AC	AM	RS	AC
TOC (%)	5.58 \pm 0.44	4.44 \pm 0.28	4.68 \pm 0.38	5.23 \pm 0.62	2.40 \pm 0.16	2.95 \pm 0.23	4.97 \pm 0.39	3.40 \pm 0.08	3.22 \pm 0.13	2.59 \pm 0.33	4.08 \pm 0.71	2.03 \pm 0.13	47.85 \pm 2.00	21.95 \pm 2.61	17.51 \pm 0.77
Moisture (%)	38.53 \pm 2.03	29.42 \pm 1.67	24.14 \pm 0.89	43.64 \pm 2.16	28.82 \pm 0.96	22.64 \pm 0.74	39.09 \pm 2.57	30.69 \pm 0.56	23.27 \pm 0.58	26.69 \pm 1.48	32.38 \pm 2.73	21.23 \pm 0.17	75.28 \pm 0.78	58.47 \pm 1.69	60.35 \pm 3.81
Root (g)	0.78 \pm 0.05	1.27 \pm 0.14	0.38 \pm 0.08	0.63 \pm 0.06	1.09 \pm 0.13	0.41 \pm 0.05	0.63 \pm 0.06	1.16 \pm 0.10	0.23 \pm 0.03	0.33 \pm 0.03	0.74 \pm 0.13	0.30 \pm 0.08	1.47 \pm 0.28	2.10 \pm 0.30	2.11 \pm 0.19
Chl a (mg/g)	2.59 \pm 0.55	2.11 \pm 0.15	0.77 \pm 0.08	5.47 \pm 0.43	4.92 \pm 0.43	2.42 \pm 0.33	3.29 \pm 0.18	4.44 \pm 0.27	1.52 \pm 0.09	4.15 \pm 0.38	3.23 \pm 0.42	1.84 \pm 0.16	2.33 \pm 0.20	1.86 \pm 0.17	1.80 \pm 0.14
Phaeo (mg g ⁻³)	4.94 \pm 0.31	3.59 \pm 0.32	0.94 \pm 0.07	5.94 \pm 0.51	3.61 \pm 0.41	1.34 \pm 0.12	4.63 \pm 0.25	2.89 \pm 0.17	0.93 \pm 0.06	5.46 \pm 0.46	3.67 \pm 0.39	1.56 \pm 0.25	4.56 \pm 0.28	5.47 \pm 0.60	5.18 \pm 0.24
Tannin (%)	0.38 \pm 0.06	0.90 \pm 0.07	0.09 \pm 0.03	1.84 \pm 0.01	4.90 \pm 0.03	1.16 \pm 0.01	0.15 \pm 0.04	0.31 \pm 0.05	0.06 \pm 0.01	0.85 \pm 0.01	0.15 \pm 0.04	0.03 \pm 0.01	1.76 \pm 0.11	0.77 \pm 0.04	6.76 \pm 0.03
Phi mean	1.74 \pm 0.05	2.01 \pm 0.04	1.86 \pm 0.02	-	-	-	-	-	-	2.07 \pm 0.04	2.02 \pm 0.04	2.18 \pm 0.01	4.00	4.00	4.00
Sorting	1.19 \pm 0.02	1.10 \pm 0.02	1.21 \pm 0.01	-	-	-	-	-	-	0.91 \pm 0.04	0.87 \pm 0.03	0.80 \pm 0.01	-	-	-

B. Meiofauna	Mean \pm SE (no. individual 10 cm ⁻²)														
	Tallebudgera									Currumbin			Terranora		
	Autumn			Winter			Summer			Autumn			Autumn		
	AM	RS	AC	AM	RS	AC	AM	RS	AC	AM	RS	AC	AM	RS	AC
Nematodes	280 \pm 84	176 \pm 36	732 \pm 73	1023 \pm 121	265 \pm 43	12 \pm 4	685 \pm 197	188 \pm 30	527 \pm 100	446 \pm 110	484 \pm 87	64 \pm 13	287 \pm 73	18 \pm 4	168 \pm 28
Harpacticoids	29 \pm 9	7 \pm 3	8 \pm 2	258 \pm 42	24 \pm 5	6 \pm 2	87 \pm 20	32 \pm 3	14 \pm 4	8 \pm 3	24 \pm 12	9 \pm 3	177 \pm 66	11 \pm 5	16 \pm 5
Oligochaetes	0 \pm 0	1 \pm 1	26 \pm 7	5 \pm 1	2 \pm 1	11 \pm 3	2 \pm 1	1 \pm 0	11 \pm 2	3 \pm 1	83 \pm 16	55 \pm 14	27 \pm 10	0 \pm 0	5 \pm 1
Kinorhynchs	4 \pm 1	1 \pm 1	0 \pm 0	7 \pm 2	2 \pm 1	0 \pm 0	1 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Turbellarians	2 \pm 1	0 \pm 0	6 \pm 1	7 \pm 2	2 \pm 1	13 \pm 3	13 \pm 4	6 \pm 2	20 \pm 5	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 1	0 \pm 0	7 \pm 2
Polychaetes	0 \pm 0	0 \pm 0	0 \pm 0	2 \pm 1	1 \pm 0	0 \pm 0	1 \pm 0	1 \pm 0	0 \pm 0	2 \pm 1	2 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Total	314 \pm 91	186 \pm 36	771 \pm 73	1302 \pm 125	295 \pm 49	42 \pm 8	790 \pm 201	227 \pm 33	572 \pm 103	458 \pm 112	593 \pm 89	128 \pm 23	493 \pm 140	29 \pm 9	196 \pm 30

Temporal variations of the environmental variables at Tallebudgera

PCA ordination of the environmental variables showed that the first two components accounted for 71 % of overall variability (Figure 2.3). PC1 represents an axis negatively associated with all key variables, while PC2 represents an axis of increasing root biomass, tannin, and Chl a while the other variables were decreasing. In general, there were also two major groups distinctively separated along PC1, respectively representing the environmental condition associated with *A. corniculatum* and those with the other two mangrove species.

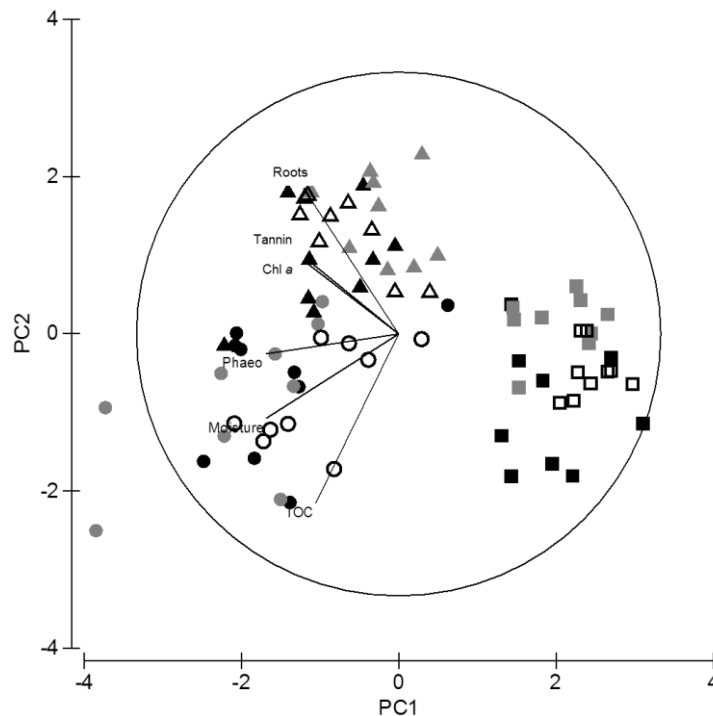


Figure 2.3: Principal Component Analysis plot describing the temporal variation of the environmental variables at the Tallebudgera site based on mangrove species. Autumn (black-filled), winter (unfilled), and summer (grey-filled). Circles = *A. marina*, triangle = *R. stylosa*, and square = *A. corniculatum*. Variance explained by PC1 is 50 % and PC2, 21 %.

Univariate PERMANOVA analysis indicates that the environmental condition along PC1 was significantly different among forests of different mangroves species ($P = 0.0001$, Appendix A) but not among seasons ($P = 0.079$), with a significant interaction between mangroves and seasons ($P = 0.0214$). Along PC2, the environmental condition was significantly different among mangrove species and seasons ($P = 0.0001$ and $P = 0.0218$ respectively), with no significant interaction between the two factors ($P = 0.1009$). Pairwise tests suggest that the environmental conditions along PC1 associated with *A. marina* and *R. stylosa* were significantly different between winter and summer ($P = 0.0345$), and between winter and autumn ($P = 0.018$), respectively (Appendix A). Meanwhile, the environmental condition at *A. corniculatum* was significantly different among all seasons except between autumn and winter ($P = 0.7103$). Along PC2, the environmental conditions were not significantly different among all seasons for *A. marina* and *R. stylosa*. The variations were only significant at the *A. corniculatum* site, between autumn and winter ($P = 0.0028$) and also between winter and summer ($P = 0.0038$) respectively.

2.3.2 Meiofaunal density and assemblage structure

Spatial variations of meiofauna

Meiofaunal assemblages were numerically dominated by nematodes (80% of total), followed by harpacticoid copepods (11%), oligochaetes (8%), with minor contributions from other meiofaunal groups, e.g. kinorhynchs, turbellarians and polychaetes (Table 2.1). On average, total meiofaunal density was highest in Tallebudgera and Currumbin and lowest in Terranora, with mean values of 424, 393 and 239 ind.10cm⁻², respectively. Meiofaunal assemblages varied significantly among locations and mangroves species, with a significant interaction between location and mangrove ($P = 0.001$, Appendix B). The highest meiofaunal density associated with *A. corniculatum*, *R. stylosa* and *A. marina* were found in Tallebudgera, Currumbin and Terranora, respectively. Meanwhile in Tallebudgera and Terranora, the lowest density was found in *R. stylosa* mangrove

while in Currumbin, the lowest density was recorded for the meiofauna associated with *A. corniculatum*. The nMDS plot (Figure 2.4) shows the groupings of the meiofaunal assemblages from different mangrove species and locations, based on the pairwise test. The pairwise test indicates that the meiofaunal density from all locations were significantly different among the different mangrove species except in Terranora, where the meiofaunal density associated with *A. marina* and *A. corniculatum* were not significantly different ($P = 0.0814$, Appendix B).

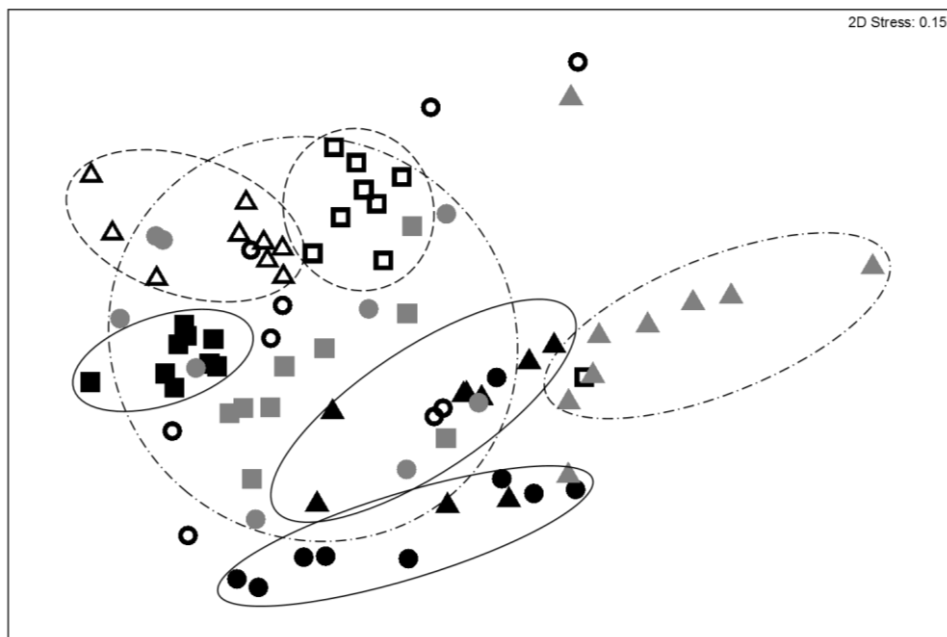


Figure 2.4: 2D Non-Metric MDS ordination showing the spatial variation of the meiofaunal assemblages collected from Tallebudgera (black-filled), Currumbin (unfilled) and Terranora (grey-filled) locations. Circles = *A. marina*, triangle = *R. stylosa*, and square = *A. corniculatum*. The solid, dashed and dash-dotted ellipses indicate the main groups that were significantly different ($P < 0.05$) according to pairwise test on Tallebudgera, Currumbin and Terranora data, respectively. No clear delineation is discernible for *A. marina* from Currumbin because of the wide variation.

Temporal variations

On average, total meiofaunal density was highest in winter and summer but lowest in autumn (mean 546, 530 and 424 ind.10cm⁻², respectively) at Tallebudgera. The meiofaunal assemblages were significantly different among all seasons and mangrove species, with a significant interaction between the two factors ($P = 0.001$, Appendix B). The nMDS plot shows the changes of the meiofaunal assemblages from the different mangrove species in response to the temporal variation (Figure 2.5). The pairwise tests indicated that temporal variation among the meiofaunal assemblages associated with different mangrove species were significant, except for the assemblage associated with *R. stylosa* (Figure 2.5B), which was not significantly different between winter and summer ($P = 0.2769$).

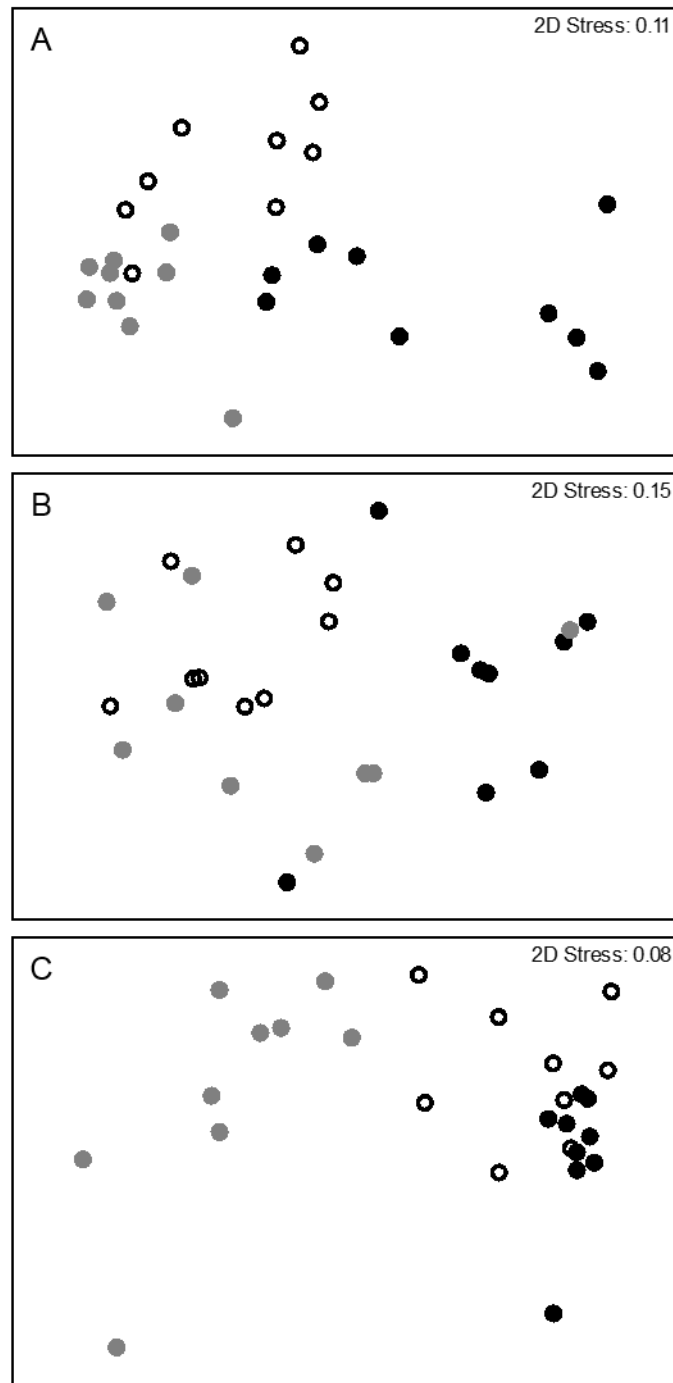


Figure 2.5: 2D Non-Metric MDS ordination for the seasonal variation of the meiofaunal assemblages in autumn (black), winter (grey) and summer (unfilled) for the samples collected from (A) *A. marina*, (B) *R. stylosa* and (C) *A. corniculatum* forests in Tallebudgera (n=9).

2.3.3 Correlations of meiofauna and the environmental variables across spatial and temporal variations

Relationships between the meiofaunal similarity matrix and the individual environmental variables (done using the BIOENV analysis) varied among locations and seasons and has been described separately according to spatial variation (Table 2.2A) and temporal variation (Table 2.2B). In Tallebudgera, the correlation was best explained by a combination of roots, phaeopigments and sediment particle size while a combination of TOC, phaeopigments and tannin best explained the correlation at Currumbin. In Terranora, the correlation was weak, with $r < 0.16$. For the comparison among different seasons in Tallebudgera, correlation between the meiofauna similarity matrix and the environmental variables was relatively weak in summer, with $r < 0.28$ as compared to the other two seasons. During autumn, the correlation was best explained by a combination of roots, Chl *a* and phaeopigments. Meanwhile during winter, a combination of moisture, roots and Chl *a* provided the best correlation.

Table 2.2: Spearman's rank correlation coefficients (r) between the single, pair and group of three environmental variables that best correlated with the meiofaunal similarity matrix across (A) spatial variation and (B) temporal variations ($n = 27$). All the environmental variables were included for spatial variation (A), but the sediment particle and sorting were excluded in temporal variation as no data is available for winter and summer (sediment particle size analysis was not repeated in the consecutive seasons). Bold type indicates the overall optimum that gives the highest rank correlations at each location and season.

	r	Single variable	r	Two variables	r	Three variables
A.						
Tallebudgera	0.62	Phaeo	0.64	Roots, Phaeo	0.64	Roots, Phaeo, Particle
Currumbin	0.41	Phaeo	0.47	Chl <i>a</i> , Tannin	0.48	TOC, Phaeo, Tannin
Terranora	0.16	Tannin	0.16	Tannin, Particle	0.13	TOC, Tannin, Particle
B.						
Autumn	0.62	Phaeo	0.60	Roots, Phaeo	0.60	Roots, Chl <i>a</i>, Phaeo
Winter	0.52	Moisture	0.57	Moisture, Chl <i>a</i>	0.62	Moisture, Roots, Chl <i>a</i>
Summer	0.26	Phaeo	0.28	Roots, Phaeo	0.26	Roots, Chl <i>a</i> , Phaeo

2.4. Discussion

The environmental variables (as indicated by the similarity matrices based on Euclidean distance) exhibit distinct variations among mangrove species across different locations but the trend was not consistent. The distinction among mangrove species varied at different locations while certain variables associated with mangrove species were not significantly different (Appendix A). Nevertheless, there are two primary groups identifiable based on the differences in environmental characteristics among locations, namely (1) Tallebudgera/Currumbin and (2) Terranora (Figure 2.2). As geophysical processes (e.g. the tidal regime, sedimentation rate) and geomorphology dictate basic mangrove forest structure (Ewel et al., 1998), it is assumed that these particular environmental characteristics at each location provide a fundamental habitat for the mangrove trees to colonize, which further modify local meiofauna habitat conditions based on their specific morphology (e.g. root biomass) and effects on sediment geochemistry (e.g. degree of aeration of sediment by aerial roots).

However, despite a strong association of meiofaunal assemblage structure with different mangrove species (as indicated by a consistent distinction in meiofaunal assemblages structure, calculated based on the Bray-Curtis similarity matrix among mangrove species across different locations), the general association of the meiofaunal assemblages with the environmental parameters selected in this study are variable. This is because the distinction of the environmental conditions among mangrove species was less consistent than do the meiofaunal assemblages. For example, even though the environmental variables at the *A. marina* stands were not significantly different from those at the *R. stylosa* stands, this lack of difference is not reflected by the meiofaunal assemblages (Appendix A and B). Variability in benthos assemblage structure is common and if this variation is related to the sediment properties, one can infer that the sediments should also vary and change at a similar scale (Tolhurst and Chapman, 2007). In addition, there was considerable variation in the

Spearman rank correlation coefficients among the meiofauna similarity matrix and the environmental variables, with weak correlations in one location, i.e. Terranora (Table 2.2). This trend indicates that the correlation was variable and site-specific, with no clear general trends that would apply to all situations, a pattern that is common among the meiofauna (Tolhurst et al., 2010). Alternatively, the divergence in meiofauna might be caused by other environmental or biotic variables that have not been included in this study e.g. dissolved oxygen, pH, position of the redox potential discontinuity (RPD) layer.

However, although the environmental variables that best correlated with the meiofaunal assemblage matrix varied among locations and seasons and do not show a general tendency, there are still discernible and interesting trends between the meiofaunal assemblages and the environmental variables at each specific location. In Tallebudgera, the correlation is best explained by a combination of the belowground roots, phaeopigments and sediment particle size/ Chl *a* ($r = 0.64$). At this location, meiofaunal abundance is highest within the *A. corniculatum* mangrove where the values of all these three variables are the lowest (Table 2.1). Meanwhile, the meiofauna associated with *R. stylosa* have the lowest density where the belowground root density is the highest and the other values are moderate. Belowground root biomass is presumably occupying space in the meiofaunal habitat and thus exerts a negative impact on meiofaunal density, at least for this particular location and/ or season. As for the phaeopigments content, the amount is highest within the *A. marina* mangrove where the harpacticoids density is apparently abundant (29 ± 9 ind. 10cm^{-2}) compared to the other two sites (< 8 ind. 10cm^{-2}). As non-photosynthetic degradation product of algal chlorophyll pigments, phaeopigments have been shown by some studies to correlate strongly with meiofaunal abundance (e.g. Danovaro et al., 2000; Skowronski and Corbisier, 2002). This positive correlation could arise from heavy consumption of the microphytobenthos by the harpacticoid copepods.

In Currumbin, a combination of total organic content, phaeopigments and tannin best explains variations in the meiofaunal assemblages ($r = 0.48$). At this location, total meiofaunal density with the highest density of harpacticoids and oligochaetes were found within the *R. stylosa* mangrove, where the TOC is the highest. Meanwhile, at the site where the phaeopigments (as a proxy for food) and the tannin (as a deterrent, Alongi, 1987b) to the meiofauna were highest within the *A. marina*, different meiofauna taxa responded in opposite ways: e.g. density of nematodes increased while oligochaetes decreased (Table 2.1).

The meiofauna also showed high responsiveness to the temporal variations, as reflected by significantly different assemblages at the three sampling times in Tallebudgera (Appendix B). This happened despite that the temporal effects are less pronounced among the environmental variables selected in this study (Appendix A). Based on the results from the Spearman's rank correlation, it is interesting to note that sediment moisture content is included in the combination of the environmental variables (along with the belowground roots and Chl *a*) that best correlated with the meiofauna during winter ($r = 0.62$) while this particular key variable did not appear in other locations or seasons (Table 2.2).

During the winter season in Tallebudgera, moisture and Chl *a* contents were peak with moderate amount of the tannin content within the *A. marina* mangrove, when the total meiofaunal density was also the highest. In fact, the nematodes, harpacticoids and total meiofaunal density recorded at this site during winter are the highest of all samples collected across different locations and seasons (Table 2.1). Meanwhile during summer, despite the relatively weak correlation compared to the other seasons ($r = 0.28$), total meiofauna was abundant within the *A. marina* mangrove where the phaeopigment content was the highest with moderate amount of the belowground root biomass.

This study has confirmed the high responsiveness of mangrove meiofauna to changing local environmental conditions, in a way that is specific to spatial and seasonal variations. Therefore, this trend supports the notion that the meiofauna are suitable bioindicators for environmental change, as has recently been demonstrated for the impact of pollution (Xu et al. 2014) as well as ecosystem recovery (Lu et al. 2011).

CHAPTER 3

MEIOFAUNA AND CRABS IN MANGROVES AND ADJOINING SANDFLATS: IS THE INTERACTION PHYSICAL OR TROPHIC?

3.1 Introduction

Due to their numerical and functional dominance (Koch and Wolff, 2002), crabs are one of the most ecologically important components of the mangrove macrofauna, and may therefore exert a large influence on the distribution and density of other animals (Lee, 2015), including the meiofauna. However, species interaction among the mangrove macrofauna and its role in shaping faunal community structure has received little attention (Lee, 1998). Despite that brachyuran crabs are dominant deposit-feeders in mangroves and the high density of meiofauna within the same habitat (Wołowicz et al., 2011), little is known about the nature of their interactions. The role of meiofauna in mangrove food chains is obscure and represents a missing link in the trophodynamics of tropical and sub-tropical soft shores. Among the crabs inhabiting mangrove and intertidal flats are members of the deposit-feeding guild, e.g. soldier crabs *Mictyris longicarpus* (Mictyridae) and fiddler crabs *Uca* spp. (Ocypodidae), which are commonly found in most tropical and sub-tropical estuaries including those in Australia and Asia (Dittmann, 1998; Rossi and Chapman, 2003).

The major activities of these crabs that may affect the meiofauna are their bioturbation (physical activities) and foraging behaviours (physical as well as trophic activities) on the surface sediment (Reinsel, 2004). *M. longicarpus* does not maintain permanent burrows (Dittmann, 1998; Rossi and Chapman, 2003) but buries and re-emerges in response to threats. This burrowing activity involves constructing an air pocket by scooping the sand in a corkscrew motion down into the sediment (Maitland and Maitland, 1992). Unlike the soldier

crab, *Uca* spp., e.g. *Uca vomeris*, build permanent burrows and normally wander no more than one meter away from it such that a quick retreat is possible when threatened (Zeil, 1998). Fiddler crab burrows are usually simple and consist of a vertical shaft extending 10 to 40 cm into the sediment. Burrows are continuously constructed, maintained and later on abandoned (Kristensen, 2008). During the burrow construction and maintenance activities by crabs, a considerable amount of sediment is excavated and mixed, altering the quality of the organic matter on the sediment surface (Gutiérrez et al., 2006; McCraith et al., 2003).

During the low tide, *M. longicarpus* emerges to feed either on or just under the surface, creating hummocks prior to their emergence (Cameron, 1966). This species uses branchial water to separate lighter organic material from the heavier inorganic material (Quinn, 1980). Fiddler crabs feed on fine particles by picking sediment from the surface using the minor chela and placing it in the mouth cavity, but its diet varies (Kristensen, 2008). Generally, as deposit-feeders, these crabs derive nutrition from a variety of foods such as fine organic detritus, the microphytobenthos, bacteria and small metazoans, e.g. the meiofauna (Dye and Lasiak, 1986; Nagelkerken et al., 2008). However, the contribution of meiofauna to the diet of these crabs is unknown. Several lines of evidence suggest a significant impact of the crab's presence on the meiofauna, especially for the fiddler crabs (Dye and Lasiak, 1986; Hoffman and Katz, 1984; Olafsson and Ndaro, 1997; Reinsel, 2004). Few studies have reported the interaction between soldier crabs and the meiofauna, but Warwick (1990) found a significant reduction in the species richness, species diversity and evenness of meiofaunal nematodes in sandflat areas within the aggregation zones of soldier crabs.

While these data clearly indicate that the presence of deposit-feeding crabs depresses meiofaunal density, the actual mechanism, i.e. whether the reduction is due to the physical disturbance effect or crab consumption of meiofauna, is not known. Assertions on the trophic interaction between crabs and the meiofauna are made solely based on the reduction in

meiofaunal density in the presence of the crabs. This top-down reduction, however, may be achieved through physical and /or trophic effects. Different crab species may bioturbate soft sediments differently, e.g. permanent versus temporary burrows, and thus may affect meiofaunal density differently. In addition, the differences of sediment characteristics may as well contribute or influence the physical interaction between the crabs and the meiofauna.

This study aimed to investigate the significance and the nature of top-down control on the density of mangrove meiofauna based on their interactions with deposit-feeding crabs; specifically, whether the interaction is mainly physical or trophic. The research questions asked in this study were 1) Does the presence of the soldier crab *M. longicarpus* on the sandflat and the fiddler crab *U. vomeris* in the mangrove, affect meiofaunal density? and 2) Is the effect of crabs due to physical or trophic interactions? To achieve this, we conducted a manipulative experiment involving exclusion/inclusion cages, with additional manipulation of the feeding appendage of the crabs to ascertain the nature of the interactions. Our hypotheses were 1) Meiofaunal density is affected by the presence of the crabs in their natural habitat; 2) Physical activities of the crabs may increase or reduce meiofaunal density, but trophic interaction will reduce meiofaunal density.

3.2 Materials and methods

3.2.1 Study area

Manipulative field experiments were conducted from December 2014 until February 2015 within the aggregation zones of soldier crabs (*M. longicarpus*) on the intertidal sandflat, and within the aggregation area of fiddler crabs (*U. vomeris*) in an open area on the mangrove forest fringe at the mouth of Tallebudgera Creek, Southeast Queensland, Australia (28° 6'18.62"S 153°26'47.80"E). Tallebudgera Creek is connected directly to the Coral Sea, and the mixed but predominantly semi-diurnal tidal regime has a range of about 2.5 m. The

mangrove fringe (*U. vomeris* site) was dominated by the mangroves *Avicennia marina* and *Rhizophora stylosa*. Significant gaps comprising clear and open areas with pneumatophores 1 – 2 cm tall occur on the sandy sediment. The aggregation area of *U. vomeris* starts at ~5 m from the lower tidal limit of the creek. Tides ranged from 0 to 1.8 m during the study period. During the experimental period, the study area received a daily average of 11.6 mm of rain (total = 1047.8 mm for the three months), with a temperature range of 16 to 37.1°C.

3.2.2 Quantification of natural crab density

The emergence and activity patterns of soldier crabs are known to vary with life stages and gender (Cameron, 1966; Unno, 2008), which may have been the main reason for the lack of a convincing method to quantify the density of this crab to date. Soldier crabs are active during the low tide when they emerge from their burrow, but the proportion of time being emergent varies between days (Cameron, 1966). Once emerged, adult soldier crabs move quickly in coordinated fast feeding movements, usually wandering around the foraging area in large groups. Soldier crabs do not maintain permanent burrows but respond to the threat by rapidly burying in the sediment. Therefore, the burrow-counting method is misleading for determining the density of soldier crabs. On the Tallebudgera sandflat, soldier crabs are abundant and live within the same microhabitat of the callianassid *Trypea australiensis*. *T. australiensis* lives in deep burrows with openings often exposed even during high tide, and might be misidentified as soldier crab burrows. Therefore, the density of the soldier crabs in this study was estimated by using the photographic counting method (Vermeiren and Sheaves, 2014) during their emergence in swarming formation. The density of fiddler crabs was quantified using the visual count method (Nobbs, 1999), where 12 of 1.5 m x 1.5 m quadrats were marked on the sediment surface, and the number of crabs counted using a pair of binoculars during the active period at low tide.

3.2.3 General experimental design

The nature of the interactions between the meiofauna and the soldier crabs and fiddler crabs and their effects on meiofaunal density was investigated using field exclusion and inclusion cages. The experimental cages were 40 cm x 20 cm internal diameter cylinders made of 5 mm plastic mesh, with the bottom 30 cm embedded in the sediment (Figure 3.1). The top and bottom of the cages were covered with mosquito netting to prevent crab movement into or out of the cages. There were five manipulative cage treatments, each with nine replicates, namely: 1) Exclusion: complete cages without crab inside to remove crab physical or trophic effects; 2) Inclusion: complete cages with one adult crab per cage, with all effects present; 3) Inclusion with 'disabled' crabs (hereafter known as Disabled): complete cages but with one 'disabled' crab to remove the trophic effect, but keeping the physical effect. Soldier crabs were disabled by removing the distal segment from both of its feeding chelipeds using small scissors. Similarly, adult male fiddler crabs *U. vomeris* were treated by removing the distal segment of the minor (feeding) chela used for picking up sediment; 4) Half-cage: half-complete cage to measure any (direct or indirect) effects due to either the material or the construction on meiofaunal density. Crabs had access to the area, i.e. there is no crab exclusion effect, but any effect due to caging is expected to be discernible by comparing the results of this treatment and the Ambient; and 5) Ambient: no manipulation was made to the activity area of the crabs, i.e. crabs exerted their effect at the natural density without interference from any procedures.

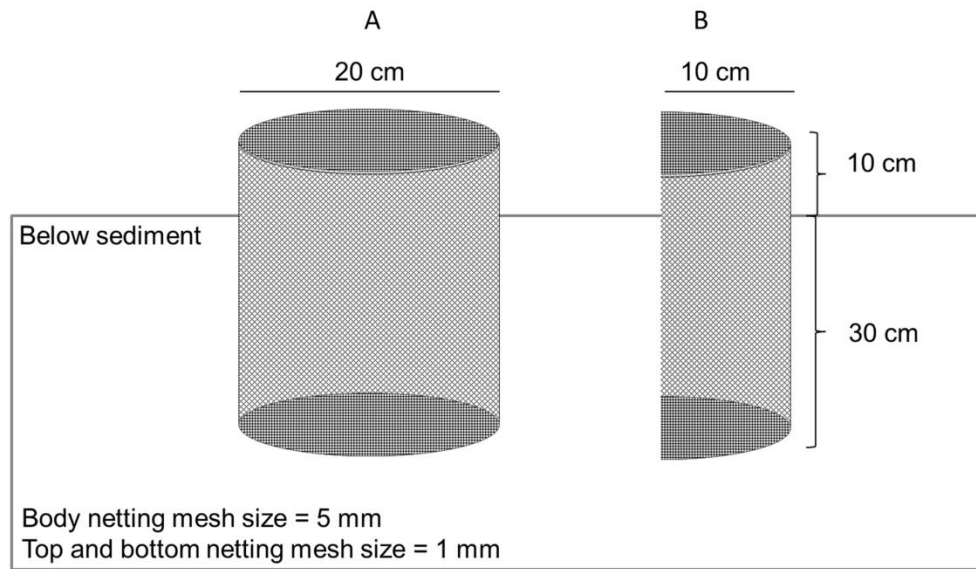


Figure 3.1: Schematic diagram of (A) Complete cage and (B) Half-cage designs of the experimental cages.

The meiofaunal density in the Exclusion and Inclusion treatments was first compared to detect any significant general impact of the crab's presence, i.e. if the crab's presence might have reduced or increased meiofaunal density (Table 3.1). The nature of the interaction between crabs and the meiofauna were further investigated by comparing the Exclusion and Inclusion treatments to look for any significant physical effect, and the Exclusion and the Disabled treatments were compared to test any significant trophic effect.

Crabs are usually able to regenerate their feeding claws in one or two ecdyses. As the impact of the crabs on the meiofauna is expected to occur over short time scales, and to avoid repeated treatment of the crabs upon claw regeneration, the experiment was conducted over a short period. After two days of disabling the crabs, both crab species were able to survive with feeding claw segments removed and left sediment working marks on the surface and continued with burrow construction and maintenance.

Key sediment variables were measured to provide basic information on sediment condition. Sediment samples (n=3) were collected from the Ambient area to describe the substrate grain size according to the Wentworth grade scale, using the dry sieving technique (Bale and Kenny, 2005). The top sediment surface (1 cm) from the Ambient and the Exclusion cages were collected (n=9) to evaluate the cage design effect and the effect of the crab's presence and absence on microphytobenthos (MPB) density (measured as Chl *a* concentration) and the total organic content (measured as loss-on-ignition, LOI). Irradiance in terms of PAR photon flux density was measured using a light meter (n=5). For chlorophyll *a* (Chl *a*) measurements, the sediment samples were collected using a corer and put on ice during transportation to the laboratory, before immediate chlorophyll extraction using 90% aqueous acetone in the dark for 24 hours. Chl *a* concentration was measured following the spectrophotometric method of Parsons *et al.* (1984). One core sample for measurement of the meiofauna was collected from each cage, frozen and washed through 500 µm and 63 µm meshes within 48 hours, and preserved in 70% alcohol and Rose Bengal for later counting.

Table 3.1: Summary of the type of effects expected to be present for each experimental treatment, namely "Physical" "Trophic" or "Cage" effect (n=9). Symbols signify the presence (+) or absence (-) of each effect.

Treatment / Effects	Exclusion	Inclusion/ Complete crab	Disabled/ Starving crab	Half-cage	Ambient
Physical	-	+	+	+	+
Trophic	-	+	-	+	+
Cage	+	+	+	+	-

3.2.4 Soldier crabs: experimental design

Sediment on the sandflat was dug out to make a depression, and the cage was deployed to get the required height inside and outside of the sediment. The sand was put back into the cage through a 5 mm mesh to remove existing macrofauna. The sediments were added to create the natural effect of humps (the raised area) and depressions (water puddles) that do not dry out during the low tide, and, therefore, providing a suitable feeding area for the crabs. Soldier crabs were observed to avoid the water puddle areas but feed only at the humps between the puddles. The cages were located within 1.5 to 2 m from each other and the experimental area located 5 m from the extreme low tide level. The top of each cage was covered with mosquito netting, and the cages left for one week to allow recovery of the disturbed sediment and the meiofauna. Adult soldier crabs of about the same size (1.5 ± 0.05 cm, carapace width and 2.2 ± 0.02 cm carapace length, mean \pm SE) were collected from the site. One individual was put into each of the inclusion or disabling treatment cages ($n=18$), and the treatments were left for two days. There were three low tide occasions within the experimental period; at 00:02 am, 13:13 pm and 12:53 pm. Sediment cores for meiofauna samples were collected on February 18, 2015.

3.2.5 Fiddler crabs: experimental design

The experimental cages were positioned randomly on the sandflat during low tide, at a minimum distance of 1.5 to 2 m from each other, a week before the experiment began. A shovel was used to dig out the sediment and were checked for crab presence and then removed. Similar to the experiment on soldier crabs, the experimental cages were put into the holes, and the cages were filled with the original sediment. The top of each cage was covered with mosquito netting, and the cages were left for one week to allow recovery of the disturbed sediment and the meiofauna. *U. vomeris* is sensitive to disturbance, and would retreat into any burrows when threatened. Therefore, it is difficult to remove fiddler crabs

without disturbing the sediments (Hoffman and Katz, 1984). Adult male fiddler crabs of about the same size (1.5 ± 0.1 cm, carapace width) were collected from the site, and one individual was put into each of the inclusion or disabling treatment cages ($n=18$). The cages were left for two days. There were three low tide occasions within the period; at 00:41 am, 13:10 pm and 01:18 am. Sediment cores for meiofauna samples were collected on March 4, 2015.

3.2.6 Data analysis

Distributions of the substrate particle sizes from the ambient of sandflat and mangrove sites were compared using the Kolmogorov-Smirnov two-sample test (K-S D), and the t-tests were used to compare the mean grain size and the sorting coefficient between the two sites. A one-sample Kolmogorov-Smirnov test was used to check normality of the data, and homogeneity of variance was evaluated using Levene's test. Data for the Chl a , LOI and Irradiance were log-transformed to satisfy the assumptions when required, and the meiofaunal density data were square-root transformed. Two-way ANOVA was used to determine the effect of crab species and cage treatment on the level of Chl a , LOI and Irradiance and the meiofaunal density. Post-hoc pairwise comparisons were applied to the significant main treatment effects to determine the pattern of difference.

3.3 Results

3.3.1 Crab density and sediment conditions

The density of soldier crabs in the swarming formation ranged from 589 to 1360 individuals per swarming group. The density of the fiddler crabs was between 8 and 13 individuals per m^2 respectively of male and female crabs in the study area. Distributions of the substrate particle size for the two study sites were not significantly different (K-S $D = 1.000$, $p = 0.270$). Medium and fine sands dominated the sediment substrate at both sites. However, the mean

grain size (ϕ) and sorting coefficients for the two sites were significantly different (Table 3.2).

Table 3.2: Description of the substrate particle sizes at the two habitats based on the Wenworth classification. Values are mean \pm SE (n=3).

Description	Particle size range (mm)	Frequency, wt % (mean \pm SE)		t-test
		Sandflat	Mangrove	
Coarse sand	0.710 - 1.0	0.33 \pm 0.03	0.00	
	0.50 - 0.710	1.95 \pm 0.07	1.37 \pm 0.28	
Medium sand	0.25 - 0.50	58.20 \pm 1.15	35.55 \pm 2.04	
Fine sand	0.125 - 0.25	38.00 \pm 1.09	46.88 \pm 0.90	
Very fine sand	0.0625 - 0.125	1.12 \pm 0.12	9.71 \pm 0.80	
Silt/Clay	<0.625	0.40 \pm 0.06	6.45 \pm 0.35	
Mean grain size (ϕ)		2.11 \pm 0.02	2.29 \pm 0.04	$t_4 = -6.76$, $p < 0.01$
Sorting		0.56 \pm 0.1	0.86 \pm 0.14	$t_4 = -19.33$, $p < 0.001$
Sorting classification		Moderately well-sorted	Moderately sorted	

3.3.2 Chl a, LOI and Irradiance

The interaction between crab species and caging was significant (Figure 3.2) in determining sediment Chl a ($F_{1,32} = 35.521$, $p < 0.001$) and LOI ($F_{1,32} = 4.528$, $p < 0.05$) but not for Irradiance ($F_{1,16} = 0.315$, $p > 0.05$). The significant interactions indicate that the effects of crab exclusion (Exclusion cage) on Chl a and LOI were different for *M. longicarpus* and *U. vomeris*. Chl a concentration (mg g⁻¹ sediment) in the Ambient within the aggregation zone of *M. longicarpus* was significantly lower ($F_{1,32} = 13.243$, $p < 0.01$) compared to those in the Exclusion cages (1.44 \pm 0.10 and 2.22 \pm 0.24, respectively, mean \pm SE). In contrast, Chl a concentration in the Exclusion cages from the *U. vomeris* experiments were significantly lower ($F_{1,32} = 22.941$, $p < 0.001$) than that in the Ambient (0.75 \pm 0.13 and 1.78 \pm 0.08, respectively).

The organic content of sediments as measured by LOI in the Ambient and Exclusion cages were not significantly different ($F_{1,32} = 0.588$, $p > 0.05$) for *M. longicarpus* but different for *U. vomeris* ($F_{1,32} = 5.029$, $p < 0.05$). LOI in the Ambient and Exclusion treatments for *M. longicarpus* was $0.663 \pm 0.07\%$ and $0.75 \pm 0.07\%$, respectively. In the experiment with *U. vomeris*, the LOI was $1.524 \pm 0.09\%$ and $1.202 \pm 0.15\%$, respectively, for the Ambient and Exclusion treatments.

There was no species effect on Irradiance ($F_{1,16} = 0.13$, $p > 0.05$), but treatment effect was significant ($F_{1,16} = 997.241$, $p < 0.001$). Irradiance inside the cages were significantly reduced ($F_{1,16} = 481.07$, $p < 0.001$), at $473.2 \pm 11.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ (soldier crab) and $466.12 \pm 14.68 \mu\text{mol m}^{-2} \text{s}^{-1}$ (fiddler crab), compared to the mean of the Ambient treatment ($F_{1,16} = 516.49$, $p < 0.001$) at $965.36 \pm 123.57 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $976.08 \pm 52.71 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the soldier and fiddler crabs, respectively.

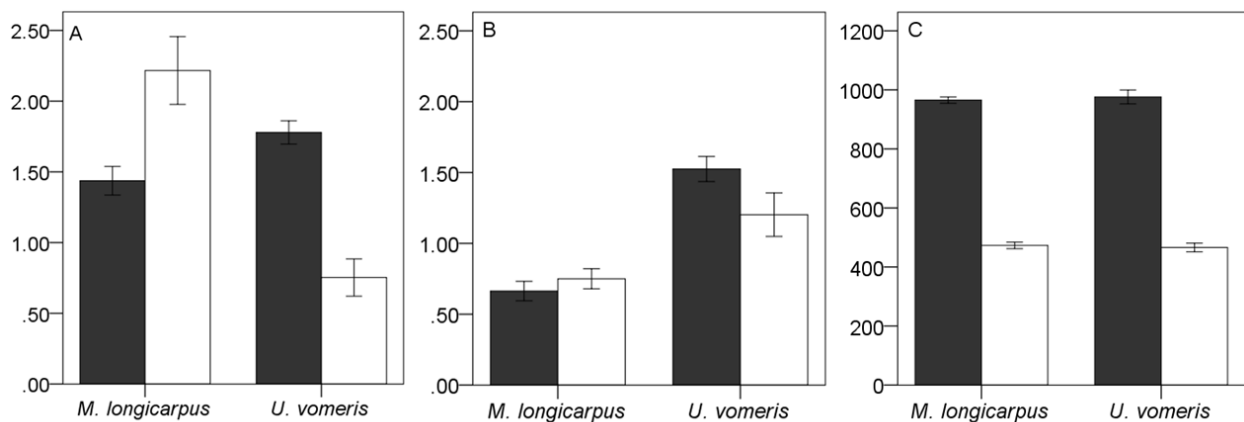


Figure 3.2: A) Chl a concentration (mg g⁻¹ sediment), B) LOI (%) and C) Irradiance (μmol m⁻² s⁻¹) in the Ambient (black bars) samples within *U. vomeris* and *M. longicarpus* activity areas compared to the Exclusion cages (white bars). All data are mean ± SE.

3.3.3 Meiofaunal density

The meiofaunal community on the Tallebudgera sandflat within the *M. longicarpus* aggregation zone was overwhelmingly numerically dominated (> 99%) by nematodes, with < 1% being harpacticoid copepods (Table 3.3). At the mangrove site within the aggregation area of *U. vomeris*, the meiofaunal community was also dominated by nematodes (> 97%), with minor contributions from harpacticoids, oligochaetes and soft-bodied meiofauna. There was a significant interaction between crab species and the experimental treatments ($F_{4,80}=14.624$, $p<0.001$).

Table 3.3: Meiofaunal density (n=9) for the *M. longicarpus* and *U. vomeris* experimental treatments. All data are mean \pm SE.

Site	Meiofaunal density (no. ind. 10cm ⁻²)				
	Exclusion	Inclusion	Disabled	Half-cage	Ambient
Sandflat (<i>M. longicarpus</i>)					
Nematode	426 \pm 46	282 \pm 22	419 \pm 46	263 \pm 26	127 \pm 14
Harpacticoid	1 \pm 0	1 \pm 0	0	1 \pm 1	2 \pm 0
Mangrove (<i>U. vomeris</i>)					
Nematode	359 \pm 57	134 \pm 25	142 \pm 15	157 \pm 22	320 \pm 52
Harpacticoid	2 \pm 1	2 \pm 1	1 \pm 1	3 \pm 1	1 \pm 1
Oligochaetes	6 \pm 1	4 \pm 1	2 \pm 1	3 \pm 1	2 \pm 1
Soft-bodied	2 \pm 1	0	0	1 \pm 0	0

There were three homogeneous sub-groups in the experimental cage treatments for *M. longicarpus* ($F_{4,80}=13.497$, $p<0.001$) (Figure 3.3). Meiofaunal density in the Exclusion (426 \pm 46 ind. 10 cm⁻²; mean \pm SE) was not significantly different from that in the Disabled treatment (420 \pm 47 ind. 10 cm⁻²). Meiofaunal density in the Inclusion (283 \pm 22 ind. 10 cm⁻²) was not significantly different from the Half-cage treatment (264 \pm 27 ind. 10 cm⁻²). The third group is represented by the Ambient treatment, where the meiofaunal density was significantly lower than those in all the other treatments (130 \pm 14 ind. 10 cm⁻²).

There was a significant treatment effect on meiofaunal density in the *U. vomeris* experiment ($F_{4,80}=11.225$, $p<0.001$). Post-hoc tests separated the treatments into two groups. The Exclusion and Ambient treatments had meiofaunal densities of 368 ± 58 and 323 ± 52 ind. 10 cm^{-2} , respectively. In the second group, meiofaunal density was lower, with Inclusion (139 ± 25 ind. 10cm^{-2}) grouped together with the Disabled and Half-cage treatments (145 ± 15 and 164 ± 23 ind. 10cm^{-2}), respectively.

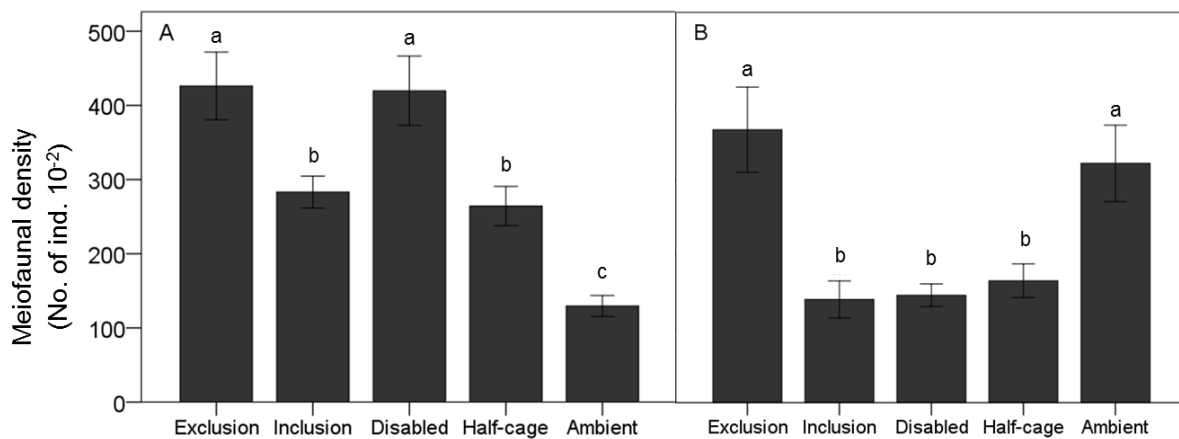


Figure 3.3: Meiofaunal density (mean \pm SE, $n=9$) in the five experimental treatments for (A) *M. longicarpus* and (B) *U. vomeris*. Treatments with different letters are significantly different from each other ($p<0.05$).

3.4 Discussion

3.4.1 Physical vs. trophic interactions

In general, the presence of either crab species at the respective habitats has a significant negative impact on meiofaunal density (Exclusion vs. Inclusion). The meiofaunal density in the Exclusion and Disabled treatments were not significantly different, suggesting that there was no significant physical effect of the soldier crabs. However, a significant trophic impact of soldier crabs occurred on the meiofauna, where the presence of the complete crab (Inclusion treatment) significantly reduced meiofaunal density compared to crabs that were

not able to feed (Disabled treatment). For the fiddler crabs, meiofaunal density in the Disabled treatment was significantly reduced compared to that in the Exclusion treatment, but not the Inclusion treatment. This pattern suggests that the effect of the fiddler crabs on meiofaunal density was mainly due to their physical but not trophic activities.

This study, which is the first to manipulate crab's feeding ability to elucidate the nature of their interaction with the meiofauna, clearly shows that soldier and fiddler crabs imposed a different type of top-down control on the meiofauna at the respective habitats. Despite being exposed to the massive physical activities of the soldier crabs, the meiofauna on the sandflat seem to be able to cope with the physical disturbance. In contrast, physical disturbance by the fiddler crabs in the mangrove habitat significantly reduced the meiofaunal density. While significant negative response by meiofauna to physical disturbances including bioturbation is common, it is not universal (Austen and Widdicombe, 2006). Several factors may account for this observation. First, the different impact of the crabs' physical activities on the meiofauna may be due to the different physical activities (e.g. temporary vs. permanent burrows, burrow maintenance) of the soldier and fiddler crabs in their natural habitats. While the difference is apparent, a fair comparison of the magnitude of the disturbances caused by the two crab species in their habitats could not be made in this study.

Second, the response of the meiofauna towards the physical disturbance suggests that the capability of the meiofauna to recover from the crab's physical disturbance is different between the two habitats. It has been shown by a previous study that the meiofauna were able to recover sooner in sandier substrates compared to muddier sediments (Dernie et al., 2003). To test this hypothesis, we compared the capability of the meiofauna to recover their density after being excluded from the Ambient (Exclusion vs. Ambient) in the two habitats. After the crab's removal, meiofauna on the sandflat were able to recover quickly by tripling

their density from that in the Ambient. However, the meiofauna from the mangrove habitat did not show such a significant recovery following the exclusion of the fiddler crabs.

However, lack of change in the overall density of the meiofauna does not necessarily show that there is no physical impact by the crab at all. This is because physical disturbance may not affect total density but the structure of the meiofauna assemblage at the lower taxonomic levels (Warwick, 1990). Future examination of the nature of the interaction between crabs and the meiofauna should preferably be conducted with higher taxonomic resolution, e.g. genus or species level, to be able to measure any crab effect on meiofaunal assemblage structure. This requirement is, however, understandably challenging to meet in ecological studies, which usually require large sample sizes.

In this study, we found a significant trophic interaction between the soldier crabs and the meiofauna (Disabled vs. Inclusion). *M. longicarpus* is reported to use predominantly the microphytobenthos as food (Cameron, 1966; Quinn, 1986; Spilmont et al., 2009), but meiofauna are occasionally found in their diet (Cameron, 1966; Lee et al., 2011a). Meiofauna offer several advantages as potential food for the soldier crabs due to their size and nutritional value, e.g. harpacticoid copepods are high in essential fatty acids (Nanton and Castell, 1998; Coull, 1999) beneficial to the higher trophic levels. There is, however, little evidence to date supporting consumption of the meiofauna by the soldier crabs. Even though the feeding mechanisms of *M. longicarpus* have been described in detail, reports on the examination of their gut content are limited (Warwick, 1990). In addition, meiofauna such as nematodes may be digested quickly with no visual remains (Coull et al., 1995).

Further, unlike the MPB which are primary producers, assessing the trophic contribution of meiofauna using the tracer approach, such as stable isotope or fatty acid analysis is more challenging. Application of the lipid biomarker and dual stable isotope approach to identifying the food sources for *M. longicarpus* could only emphasize the consumption of the

microphytobenthos and bacteria end members (Spilmont et al., 2009), but unable to confirm the contribution of the meiofauna to the diet of the crabs. However, these authors strongly suggested that meiofauna could be part of the diet of the soldier crabs, due to the distinct $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the crabs compared to the shrimps that selectively fed on the microphytobenthos. Moreover, recent reports have shown a significant trophic interaction between the meiofauna and soldier crabs using the stable isotope enrichment approach (e.g. Lee et al., 2011).

In contrast, in this experiment, we could not detect a significant trophic interaction between the fiddler crabs *U. vomeris* and the meiofauna, as the meiofaunal density in the Inclusion treatment was not significantly reduced compared to that in the Disabled treatment. Commonly referred to as a detritivore, fiddler crabs have been reported to feed on MPB, bacteria and fine organic materials either through gut contents or gut ecomorphology analysis (Robertson and Newell, 1982; Dye and Lasiak, 1986; Griffen and Mosblack, 2011), fatty acid (Meziane et al., 2006) and stable isotope analysis (Abrantes and Sheaves, 2009). The limited foraging range from their burrows (Zeil, 1998) may prevent fiddler crabs to be selective in their food. The crabs may therefore make full use of the abundant fine organic detritus or microphytobenthos close to their burrows for subsistence (di Virgilio and Ribeiro, 2012). Similar manipulative experiments covering a wider range of locations and different seasons (e.g. for variations in MPB production) may help assess the generality of these findings.

3.4.2 Experimental design

In the Exclusion experiment with *U. vomeris*, light irradiance inside the cage was reduced by 50% (Figure 3.2C), and as expected, has contributed to the reduction of Chl *a* content inside the Exclusion cage. However, the experiment with *M. longicarpus* resulted in the opposite trend, where a significant increment of 30% (over the Ambient treatment) was

found inside the Exclusion cage. This demonstrates a significant impact of soldier crab activities on the density of the microphytobenthos on the sandflat habitat. Conversely, fiddler crab activities in the mangrove habitat do not have the same significant impact on Chl *a* as that inflicted by the soldier crabs on the sandflat habitat.

The mangrove site within the aggregation of *U. vomeris* had a higher mean organic content compared to the sandflat habitat, which is attributed to the high density of organic detritus, especially from mangrove litter and root materials. Our cage design has significantly reduced the organic content in the mangrove habitat, but not on the sandflat. This indicates that within a week of the experiment, soldier crab activities did not result in a significant impact on the organic content to the same level as has been imposed on the Chl *a* content. This trend also reflects the importance of MPB to the soldier crabs as compared to the sediment organic detritus, especially within the habitat where organic detritus is limited. The reduction of the organic content inside the cages at the mangrove site was probably due to the alteration of sediment structure during the cage deployment at the beginning of the experiment. In order to remove the existing crabs inside the experimental cages, the sediments were disturbed and resulting a mixing of the organic content on the surface and the sediment below.

In both of the experiments, the presence of the Half-cages has significantly affected the meiofaunal density as compared to what have been found in their natural habitat (Ambient). On the sandflat, the meiofaunal density increased, but the density reduced in the experiment with the fiddler crabs in mangrove habitat. There are several explanations that can be related to this situation. Firstly, it may be caused by the sediment disturbance at the beginning of the experiment. However, if this is true, we should have seen significant changes in the meiofaunal density in all cage treatments including the Exclusion cage as well, in both of the experiments. However, the meiofauna in the Exclusion cage in the mangrove habitat were not significantly different with the Ambient. Secondly, there is also a probability that the

presence of the Half-cage might have changed water flow inside the cages; but this effect would not be a primary concern in our experiment as crabs are active only during low tide. Besides, the meiofaunal assemblage was overwhelmingly dominated by the nematodes. We assumed that the meiofaunal assemblage would remain stable throughout the experimental duration due to the limited movements (Austen and Widdicombe, 2006) and burrowing habit of the nematodes.

Therefore, the best explanation for the significant impact of the Half-cage treatment to the meiofaunal density was due to the crab movement and access inside the cage. The presence of the Half-cage on the sandflat habitat within the natural aggregation of the soldier crabs has probably limiting the 'marching' crab movement around the cages and, therefore, reduced the soldier crab's access to areas inside the Half-cages. On the other hand, the fiddler crabs may have been attracted to the shading and positive thigmotactic effect provided by the Half-cages, which provide cooler environment as compared to the Ambient (Nobbs, 2003; Kon et al., 2010). As a result, more fiddler crab activities occurred inside the Half-cage thus explaining the reduction of the meiofauna as compared to the Ambient. The fact that the Half-cage treatment was not significantly different with the Inclusion treatment in both experiments has supported this hypothesis. In the first experiment, the soldier crab activities inside the Half-cages became limited to about the same degree with the inclusion of one individual crab as in the Inclusion treatment, which was not enough to depress the meiofaunal density as compared to the higher impact caused by the soldier crabs at their natural density (Half-cage/ Inclusion vs. Ambient). In contrast, the magnitude of a fiddler crab activities within the Inclusion cage area is relatively higher as compared to the crabs' natural abundance in the Ambient.

CHAPTER 4

PARTITIONING ORGANIC MATTER UTILIZATION BY THE MEIOFAUNA AND MACROFAUNA IN CONNECTED SUBTROPICAL INTERTIDAL HABITATS

4.1 Introduction

Depository intertidal habitats such as mudflat, sandflat, mangrove and saltmarsh provide major ecosystem services through supporting fisheries, trapping sediment, and regulating nutrient and carbon recycling (Lovelock and Ellison, 2007; and references therein). Compared with most other habitats, sandflats are poorly studied, the physical as well as ecological processes occurring on them are not well understood, and the relative importance of various food sources that could potentially fuel food webs in this habitat has been debated (Dyer et al., 2000; Yokoyama et al., 2005; Leduc et al., 2006). Mangroves usually dominate the mid- to upper intertidal habitats on protected sub-tropical coasts, while saltmarsh communities usually occupy a position landward of mangroves in the intertidal zone, often intergrading with terrestrial vegetation on the landward edge (Adam, 1990).

The connected intertidal comprises of sandflats, mangroves and saltmarshes support high abundance and diversity of marine species including transient visitors and permanent residents. These habitats are connected through tidal inundations, animal migrations, and direct and indirect nutrient movements and trophic relationship, with tidal flow as a primary factor responsible for most of the material movement (Lee, 2008). Tidal connectivity allows access by invertebrate consumers, especially those with limited motility, to a variety of carbon and nitrogen sources including allochthonous sources such as phytoplankton and

seagrass-derived organic matter (Bouillon et al., 2004). Concomitantly, subtidal consumers such as juvenile fishes and prawns take advantage of the tidal incursion to access the local organic sedimentary pool in these intertidal habitats (Sheaves, 2005).

However, the pattern of resource utilization (e.g. relative importance of local vs imported sources for resident consumers, use of food sources by transient consumers) in these habitats is still unclear. Descriptions of the food web of soft-bottom intertidal habitats are usually focused on the macrofauna but excluded the less motile but important component of the benthic food web, e.g. meiofauna. Our knowledge of the trophodynamics of mangroves is biased by the omission of whole components of the fauna, in particular most groups of the infauna and meiofauna, leading to an underestimation of the overall role of consumers in the processing of different organic matter sources (Bouillon et al., 2008). Despite their numerical abundance (Montagna, 1984; Coull, 1999; Wolowicz et al., 2011; Nascimento et al., 2012), meiofauna are particularly under-studied, thus obscuring their potential trophic contribution and role in ecosystem functioning. Little is also known of the quantitative importance of different primary producers in the diet of meiofauna (Moens and Vincx, 1997; Demopoulos et al., 2007). In order to obtain an integrated view of the fate of primary production in soft intertidal ecosystems, it is vital to improve the understanding of routes and pathways of organic matter down to and in the sediment, and of the role benthic biota play in these processes (Moens and Vincx, 1997).

This study aimed to evaluate the meiofaunal and macrofaunal food webs associated with different but connected intertidal habitats, namely saltmarsh, mangrove and sandflat, along a tidal gradient using dual stable isotope analysis of carbon and nitrogen. Contribution of the primary food sources to the local sedimentary organic matter pool and their utilization by consumers in the three habitats were assessed using the consumer's $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to predict the potential end-members (Riekenberg et al., 2016).

4.2 Materials and methods

4.2.1 Study area

Samples were collected from the Tallebudgera Creek (28° 6' 18.62"S 153° 26' 47.80"E), Southeast Queensland, Australia, during winter 2015 (see Maizah and Lee (2016) for a detailed description of the site). The high-intertidal saltmarsh (SM), mid- to high-intertidal mangrove (MG) and low-intertidal sandflat (SF) habitats have distinct boundaries (Figure 4.1) but are connected by tidal flow for material and animal movement. Salinity ranges of the sediment porewater at the saltmarsh, mangrove and sandflat habitats were 23 to 26, 31 to 33, and 34 to 36, respectively. Different primary producers dominated the three habitats: the marine couch grass *Sporobolus virginicus* in SM, the grey mangrove *Avicennia marina* in MG, and no higher plant but only microphytobenthos (MPB) in SF (Figure 4.1).

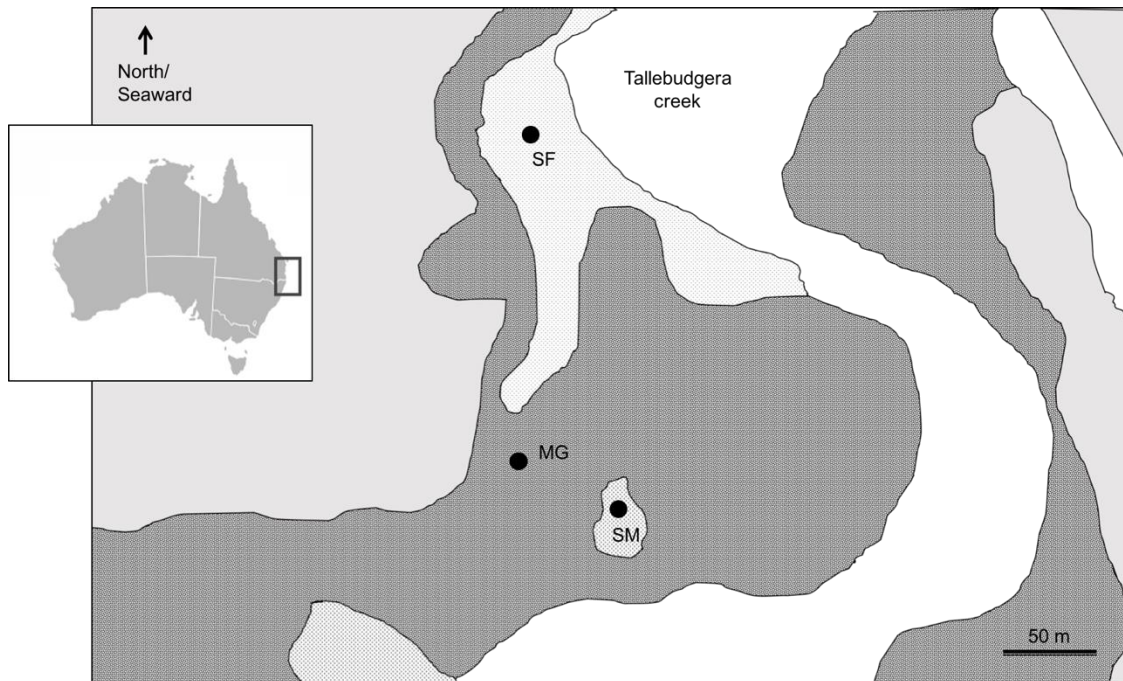


Figure 4.1: Map showing the sites where all the samples from saltmarsh (SM), mangrove (MG) and sandflat (SF) were collected at Tallebudgera creek (specific sampling site indicated as black circles).

4.2.2 Sample collection and preparation

Sediment samples for the meiofauna were collected by scraping the top 2 cm of sediments using a hand scoop. Five replicate samples (250 ml each) were collected randomly from each habitat within an area of 100 m² and kept frozen prior to extraction of the meiofauna. A small portion of these samples were set aside for stable isotope analysis of the sediment organic matter (SOM), and for total organic content by loss-on-ignition (LOI). Oven-dried sediment samples were ground and sieved through a 2 mm mesh and for LOI, 1 g samples were ignited at 550°C in a muffle furnace for 4 hours.

The infaunal macrofauna were collected by sieving the sediments in the field, and epifaunal crabs and gastropods were collected by hand. Freshly fallen leaf litter of the mangrove *A. marina* was collected from the sediment surface, and mangrove pneumatophores were gently scraped to collect the epiphytic macroalgae. *S. virginicus* samples were also collected by hand, and the microphytobenthos (MPB) were collected by scraping the top 1 cm of the sediment. Water samples for the suspended particulate organic matter (SPOM) were collected during high tide at the sandflat area, and the seagrass *Zostera mulleri* samples were collected from the seagrass patches located about 1 km away from the sampling site. All samples were frozen before analysis. The Chl *a* content of the sediment samples was also measured to assess MPB abundance. The samples were put on ice during transportation to the laboratory and Chl *a* was extracted immediately using 90% aqueous acetone in the dark for 24 hours. Chl *a* concentration was measured following the spectrophotometric method of Parsons *et al.* (1984).

4.2.3 Stable isotope analysis

The bulk sediment samples for the meiofauna were defrosted, washed with 500 and 63 µm sieves, and the meiofauna retained on the lower sieve were extracted using 30% LUDOX (colloidal silica, Sigma) solution. Meiofauna samples were washed and rinsed with distilled

water before collecting them in a petri dish filled with de-ionized water. Individuals of the meiofauna were hand-picked using a fine loop under a dissecting microscope and the samples were given a final rinse with de-ionized water. Due to their small biomass, the meiofauna samples from all replicates from each habitat were pooled to get enough samples for stable isotope analysis. About 150 to 220 and 36 to 100 individuals of nematodes and harpacticoid copepods, respectively, were collected for each replicate and three composite replicates were prepared for each habitat. Smooth wall tin capsules (flat base, size 4.5 mm x 2 mm) prewashed with methanol and acetone were used to prepare the meiofauna samples for stable isotope analysis. The tin capsules and the meiofauna samples were dried for two days at 60 °C.

For the macrofauna, generally tissue samples from one individual were prepared per replicate but on occasions when there was not enough tissue available, tissues from several individuals were pooled. The samples were dried at 60 °C, powdered and homogenized. To remove carbonates, the amphipod and sediment organic matter (SOM) samples for $\delta^{13}\text{C}$ analysis were pre-treated with dilute HCl for 24 hours then rinsed thoroughly with de-ionized water, then dried at 60 °C. However, isopod samples were excluded from the acid treatment because of insufficient material. The samples for $\delta^{15}\text{N}$ were prepared without acid treatment. Mangrove leaves were washed thoroughly, rinsed with de-ionized water and the main vein was removed before drying. Macroalgae samples were washed thoroughly with de-ionized water in a petri dish, followed by hand removal of sediment particles under a dissecting microscope. Seagrass samples were washed thoroughly, and epiphytes were removed from the seagrass blades using a cover glass. All samples were dried, powdered and homogenized for stable isotope analysis. Water samples for the SPOM were filtered onto pre-combusted GF/F filter papers and dried, then subsamples were collected by cutting the filter papers to the required amount for stable isotope analysis.

Purified MPB samples were extracted from sediment by density gradient centrifugation in colloidal silica (LUDOX). The sediment samples were washed through a 53 µm mesh to remove larger detritus and infauna. The filtrate was centrifuged at 4400 rpm for 5 min and the supernatant was removed. Pellets were resuspended in left over supernatant, divided into 5 ml aliquots in individual centrifuge tubes, mixed with 40 ml of 30% LUDOX solution (Sigma), and centrifuged again at 4400 rpm for 10 min. The distinct green layer of MPB occurred at the top of the colloidal silica was collected, and centrifuged again with deionized water to remove LUDOX before a final rinse with deionized water on a 5 µm mesh. The MPB samples were dried and collected in smooth wall tin capsules for analysis.

Dual stable isotope analyses of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ for the meiofauna were carried out on a Delta V Plus continuous flow isotope ratio mass spectrometer linked to a Flash 2000 elemental analyser (EA Thermo-Fisher Scientific, Bremen, Germany) with low volume setup (IVA Analysentechnik, Meerbusch, Germany) using a Zero Blank autosampler (Costech International, Milan, Italy) at the NIWA Ecological Stable Isotope Laboratory in Wellington, New Zealand. The other samples were analysed using a Europa EA-GSL sample preparation system interfaced to a Sercon 20-22 isotope ratio mass spectrometer (SERCON, UK). PeeDee Belemnite and atmospheric air were used as standards for C and N, respectively. Stable isotope values are reported in δ -notation (‰), i.e. $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where R is respectively $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

4.2.4 Establishment of the trophic resources from the consumer data

Trophic resources inferred from the pattern of consumer's isotope values were combined with existing knowledge of likely environmental sources consistent with the ecology of the consumers studied (Riekenberg et al., 2016) to estimate isotope mixing dynamics in the food webs. There were several steps in the mixing model analysis. Firstly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionation values of 1 and 3 ‰ respectively were used for both meiofauna and

macrofauna, based on the fractionation estimation range for small invertebrates (Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003), i.e. -1 and -3 ‰ were added to measured consumer values before mixing models were applied. In addition, the similarity between corrected isotope values of consumers and the isotopic composition of the potential food sources was considered as indicative of the importance of the respective source to the consumers' diet (Abrantes and Sheaves, 2009). Secondly, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all consumers from three habitats were plotted together in one isotope plot and a triangle was drawn tightly around the data to estimate the main food sources across the three habitats studied, at the whole landscape level. The corners of the triangle (vertices) were used to estimate aggregated mangrove habitat foods (S_1), phytoplankton foods (with high $\delta^{15}\text{N}$ values, S_2), and saltmarsh/ seagrass foods (S_3). This method estimates end-member source values of "virtual" consumers that are 100 % dependent on foods in each of the three habitats. This top-down consumer-oriented approach was used because animals did not closely correspond to sampled plant foods, so that animal data were used to estimate isotope values of foods missed in the field sampling.

This resource estimation was used mainly to estimate the phytoplankton foods that present in all three habitats. However, a further resource estimation at the ecosystem level was needed for specific end-members in each habitat. Therefore, another step was included, where a mixing triangle was plotted for each habitat to specifically estimate the local resources. At this level, the S_2 isotope values for the phytoplankton obtained from the estimation at the landscape level was used in every model. The estimated resources obtained from these models were used to calculate the proportion of contribution of resources using the IsoError mixing model.

4.2.5 IsoError mixing model

The importance of each potential source within each local habitat to the meiofauna and macrofauna was evaluated using the IsoError mixing model (version 1.04: Phillips and Gregg, 2001). While models following the Bayesian approach are recently available, e.g. MixSIR, IsoError was chosen as it does not have biased means that can occur with Bayesian approaches; it uses a more straightforward older algebra (Brett, 2014). In addition, the model incorporates the measurement error due to variability among samples, rather than measuring intra-specific variability for each taxon to provide an ecosystem-wide assessment of source utilization (Giarrizzo et al., 2011; Phillips et al., 2014). The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each potential food source was entered with the corresponding mean isotopic values of each consumer at each site, along with the standard deviation and number of samples measured. The output generated by the set of IsoError equations provides estimate contributions for each source (0-100%) with standard errors for these contribution estimates. Estimation of the 95% confidence intervals for source contributions was not generated because the potential source isotopic values were inferred as virtual end-members and were given by a single value ($n = 1$). Standard deviation for the virtual end-members were estimated by averaging the standard deviation values of all consumers, based on the assumption that the virtual end-members have about the same average variability as the consumers used to infer the virtual end-members.

4.2.6 Data analysis

Differences in the LOI and Chl *a* contents, and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) values of different samples from the three habitats were compared using one-way analysis of variance (ANOVA). Where a main treatment effect was significant, Turkey HSD post-hoc tests were applied to identify significant differences between treatments. Tests of homogeneity of variance were done using Levene's tests. Data were transformed when required to fulfil the

homogeneity and normality assumptions of the distribution. One-way PERMANOVA was used to compare the food webs of the different habitats.

4.3 Results

4.3.1 Sediment organic matter (SOM)

Sediments of the three habitats showed organic matter contents and origins. Total organic content (TOC) as indicated by LOI (Figure 4.2) was significantly different among all habitats ($F_{2,12} = 102.50$, $p < 0.001$). The LOI (%) was highest in the mangrove habitat ($10.8 \pm 1.2\%$; mean \pm SE), followed by saltmarsh and sandflat habitats (2.7 ± 0.6 and $0.72 \pm 0.05\%$ respectively). Sediments at the three habitats had significantly different $\delta^{13}\text{C}$ ($F_{2,6} = 131.52$, $p < 0.001$). Saltmarsh sediment had the highest (least negative) $\delta^{13}\text{C}$ values, followed by sandflat and mangrove. In contrast, the SOM $\delta^{15}\text{N}$ values were significantly highest on the sandflat ($F_{2,6} = 17.12$, $p < 0.01$), but $\delta^{15}\text{N}$ values were not significantly different ($p > 0.05$) between the mangrove and saltmarsh (Table 4.1 and Figure 4.5).

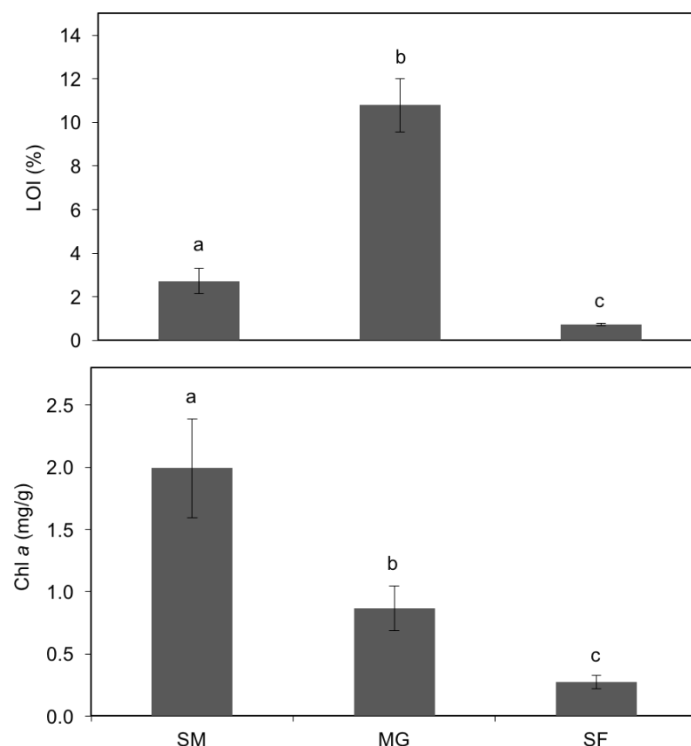


Figure 4.2: Mean values of the LOI (n = 5) and Chl a (n = 6) contents for the saltmarsh (SM), mangrove (MG) and sandflat (SF) habitats. Letters indicate samples are significantly different at $p < 0.001$. Error bars indicate standard error.

4.3.2 Primary producers

The Chl a contents, as indicator of the MPB biomass (Figure 4.2) were significantly different in the three habitats ($F_{2,15} = 25.873$, $p < 0.001$). Chl a was significantly highest at saltmarsh (1.9 ± 0.4 mg/g), followed by mangrove (0.87 ± 0.18) and lowest at the sandflat (0.27 ± 0.05). The $\delta^{13}\text{C}$ values of MPB were significantly different between habitats ($F_{2,6} = 5616.36$, $p < 0.001$). MPB from the mangrove had the most depleted $\delta^{13}\text{C}$ values, followed by those from the sandflat and then saltmarsh (Table 4.1). On the other hand, MPB $\delta^{15}\text{N}$ values were not significantly different among habitats. $\delta^{13}\text{C}$ values for the epiphytic macroalgae, mangrove leaves and *S. virginicus* were significantly different ($F_{2,6} = 19308.15$, $p < 0.001$). Epiphytic macroalgae were significantly higher in $\delta^{15}\text{N}$ values compared to the other habitats ($F_{2,6} = 99.22$, $p < 0.001$), but the $\delta^{15}\text{N}$ values from the mangrove leaves and *S. virginicus* were not significantly different ($p > 0.05$).

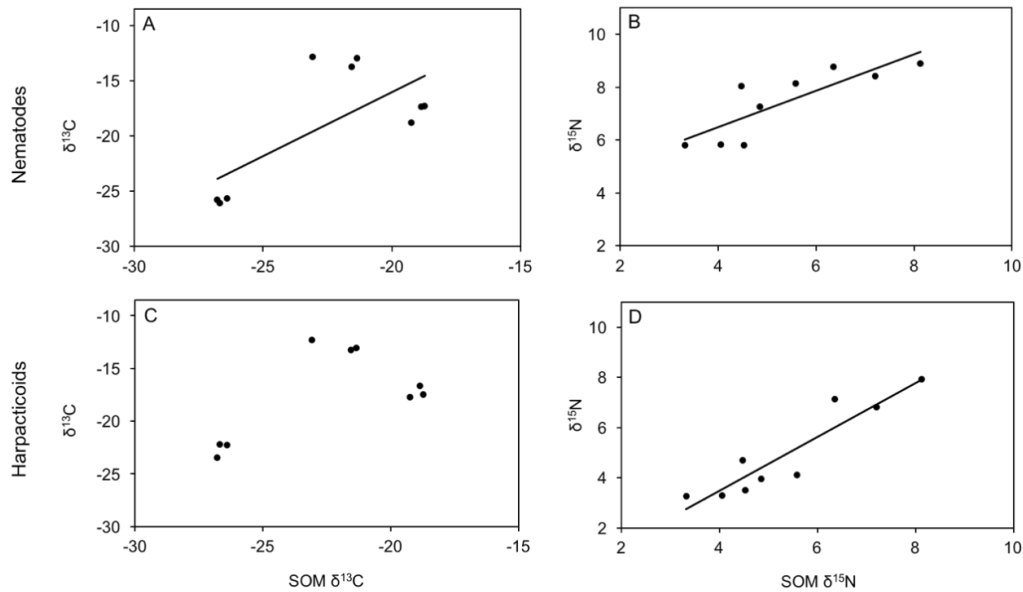


Figure 4.3: Results for the regression analysis between SOM and the meiofaunal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values ($n = 9$). (A) Relationship between the $\delta^{13}\text{C}$ values of the nematodes and SOM ($y = 1.15x + 7.02$, $R^2 = 0.49$, $p < 0.05$), (B) Relationship between the $\delta^{15}\text{N}$ values of the nematodes and SOM ($y = 0.69x + 3.71$, $R^2 = 0.69$, $p < 0.01$), (C) No significant correlation found between the $\delta^{13}\text{C}$ values of the harpacticoids and SOM ($p > 0.05$), and (D) Relationship between the $\delta^{15}\text{N}$ values of the harpacticoids and SOM ($y = 1.08x - 0.84$, $R^2 = 0.86$, $p < 0.001$).

4.3.3 Meiofauna

Both nematodes and harpacticoid copepods showed a similar trend among the habitats (Table 4.1), with lowest $\delta^{13}\text{C}$ values found at the mangrove, followed by the saltmarsh and the highest $\delta^{13}\text{C}$ values at the sandflat (ANOVA, $p < 0.01$). The $\delta^{13}\text{C}$ values for the nematodes and harpacticoids in the saltmarsh and sandflat habitats were not significantly different (Tukeys HSD test, $p > 0.05$), but the nematodes and harpacticoids from the mangrove habitat were significantly different ($p < 0.01$). The $\delta^{15}\text{N}$ values for the harpacticoids and nematodes were significantly different between habitats and taxa ($p < 0.001$), with the values of nematodes being higher than those of harpacticoids in all habitats. For both groups, the trend was similar with the highest $\delta^{15}\text{N}$ values on the sandflat, followed by the saltmarsh and lowest $\delta^{15}\text{N}$ at the mangrove site. When the meiofaunal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

values were plotted against the values of the SOM, all components showed significant positive correlations except for the $\delta^{13}\text{C}$ values of the harpacticoids and SOM (Figure 4.3).

4.3.4 Macrofauna

The mean $\delta^{13}\text{C}$ value of the macrofauna in the mangrove habitat was significantly different from those in the other two habitats (Tukeys HSD, $p < 0.001$), but mean $\delta^{13}\text{C}$ values were not significantly different between saltmarsh and sandflat ($p > 0.05$). The macrofauna at the mangrove typically had more depleted and less variable $\delta^{13}\text{C}$ values, ranging from -26.8 to -24.3 ‰, except for the juvenile shrimps and *L. scabra*, which had higher values ($-21.9 \pm 1.9\text{‰}$ and $-21.7 \pm 3.5\text{‰}$, respectively). However, the $\delta^{15}\text{N}$ values of the macrofauna at the mangrove site were more diverse, ranging from 3.1 to 7.0‰ (Table 4.1, Figure 4.5).

At the saltmarsh, the $\delta^{13}\text{C}$ values of the macrofauna (range from -15.3 to -15.1‰) were not significantly different ($p > 0.05$) except for *L. scabra* and *Ophicardelus* sp., which had lower $\delta^{13}\text{C}$ values ($-19.9 \pm 2.7\text{‰}$, and $-16.8 \pm 0.9\text{‰}$, respectively). The $\delta^{15}\text{N}$ values ranged from 4.6 to 6.8‰. The $\delta^{13}\text{C}$ values of macrofauna at the sandflat were highly variable (ranging from -17.3 to -13.8‰), and not significantly different between species. The $\delta^{15}\text{N}$ values were also highly variable, with the isopod having the lowest and the nereid polychaete, the highest values ($5.9 \pm 1.0\text{‰}$ and $12.4 \pm 0.1\text{‰}$, respectively). In general, the benthic food webs from the mangrove, sandflat and saltmarsh habitats (Figure 4.4) were significantly different (PERMANOVA, $p < 0.001$). Benthic foods (detritus + MPB) were the most dominant foods (most frequently >50% contributions in the food webs (Table 4.2).

Table 4.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SE) of the food sources and consumers collected from the Tallebudgera mangrove ecosystem. Values are not corrected for fractionation.

Species	Sample type	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	n
Saltmarsh				
Harpacticoids	Meiofauna	-17.3 \pm 0.3	4.2 \pm 0.2	3
Nematodes	Meiofauna	-17.8 \pm 0.5	7.8 \pm 0.3	3
<i>Parasesarma erythodactyla</i>	Epifauna	-15.3 \pm 0.7	6.8 \pm 0.3	3
<i>Heloecius cordiformis</i>	Epifauna	-15.1 \pm 0.1	6.1 \pm 0.1	6
<i>Littoraria scabra</i>	Epifauna	-17.2 \pm 0.3	5.9 \pm 0.2	3
<i>Phallomedusa</i> sp.	Epifauna	-15.1 \pm 0.1	5.3 \pm 0.1	3
<i>Ophicardelus</i> sp.	Epifauna	-16.8 \pm 0.9	4.6 \pm 0.5	3
<i>Sporobolus virginicus</i>	Plant	-14.4 \pm 0.1	6.1 \pm 0.3	3
MPB	Plant	-19.3 \pm 0.0	3.4 \pm 0.1	3
SOM	Soil	-18.9 \pm 0.2	5.0 \pm 0.3	3
Mangrove				
Harpacticoids	Meiofauna	-22.6 \pm 0.4	3.4 \pm 0.1	3
Nematodes	Meiofauna	-25.9 \pm 0.1	5.8 \pm 0.0	3
Juvenile shrimp (Penaeidae)	Nekton	-21.9 \pm 1.5	9.8 \pm 0.6	3
<i>Parasesarma erythodactyla</i>	Epifauna	-24.5 \pm 0.2	7.0 \pm 0.1	2
Ampharetid polychaete	Infauna	-25.8 \pm 0.1	6.6 \pm 0.1	2
Amphipod	Infauna	-26.8 \pm 0.1	4.6 \pm 0.1	3
<i>Littoraria scabra</i>	Epifauna	-25.6 \pm 0.3	5.4 \pm 0.0	2
<i>Cassidula</i> sp.	Epifauna	-24.3 \pm 0.2	3.5 \pm 0.1	3
Potamididae gastropod	Epifauna	-24.3 \pm 0.1	3.1 \pm 0.1	3
MPB	Plant	-27.5 \pm 0.0	3.2 \pm 0.2	3
SOM	Soil	-26.6 \pm 0.1	4.0 \pm 0.4	3
Mangrove leaves	Plant	-26.2 \pm 0.1	5.6 \pm 0.2	3
Epiphytic macroalgae	Plant	-32.0 \pm 0.0	2.1 \pm 0.0	3
Sandflat				
Harpacticoids	Meiofauna	-12.9 \pm 0.3	7.3 \pm 0.3	3
Nematodes	Meiofauna	-13.2 \pm 0.3	8.7 \pm 0.1	3
Nereid polychaete	Infauna	-15.9 \pm 0.3	12.3 \pm 0.1	3
Nephtyid polychaete	Infauna	-15.7 \pm 1.2	10.7 \pm 0.1	2
Ocypodidae crab	Epifauna	-14.8 \pm 0.2	9.5 \pm 0.1	3
Juvenile shrimp (Penaeidae)	Nekton	-17.3 \pm 0.9	9.3 \pm 0.0	3
<i>Mictyris longicarpus</i>	Epifauna	-13.8 \pm 0.1	8.7 \pm 0.3	3
Isopod	Infauna	-11.4 \pm 0.1	7.0 \pm 0.1	2
MPB	Plant	-23.8 \pm 0.1	5.6 \pm 1.2	3
SOM	Soil	-22.0 \pm 0.5	7.2 \pm 0.5	3
Seagrass	Plant	-12.7 \pm 0.1	6.1 \pm 0.3	3
SPOM	Particulate organic matter	-25.3 \pm 0.2	4.1 \pm 0.8	3

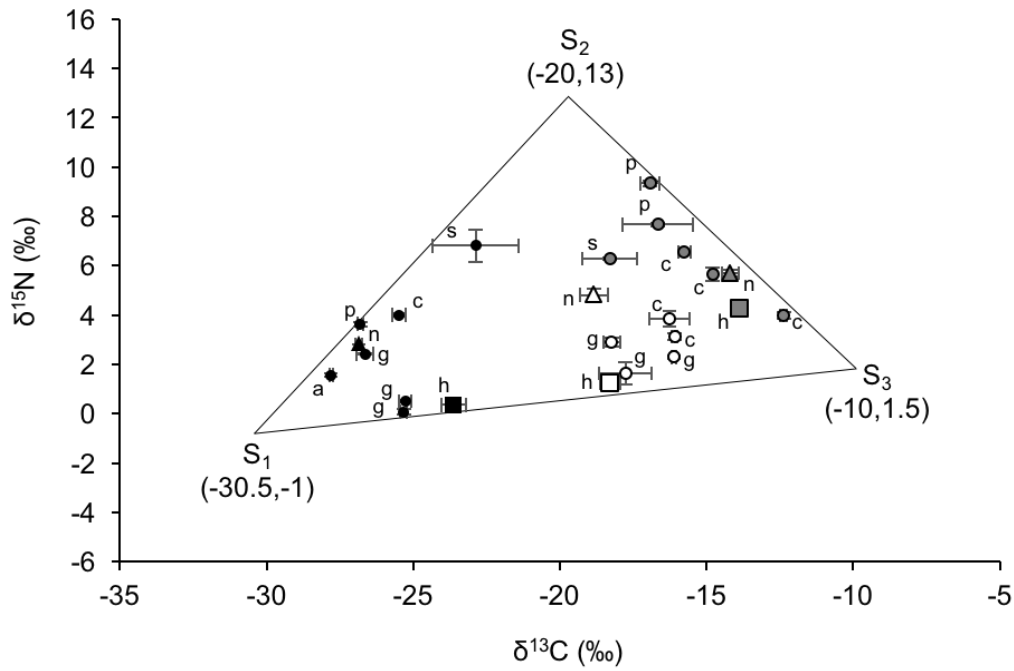


Figure 4.4: The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots of the meiofaunal nematodes (triangle) and harpacticoids (square), and also the macrofauna (circle) consumer data for the saltmarsh (unfilled), mangrove (black-filled), and sandflat (grey-filled) habitats. Data are mean \pm SE. Values are corrected for trophic fractionation. Triangle vertices (S_1 , S_2 , S_3) indicate the estimated (virtual) end-members inferred based on consumer data from the three habitats. a = amphipod, c = crabs, g = gastropod, h = harpacticoids, n = nematodes, o = ostracod, p = polychaetes, s = juvenile shrimp.

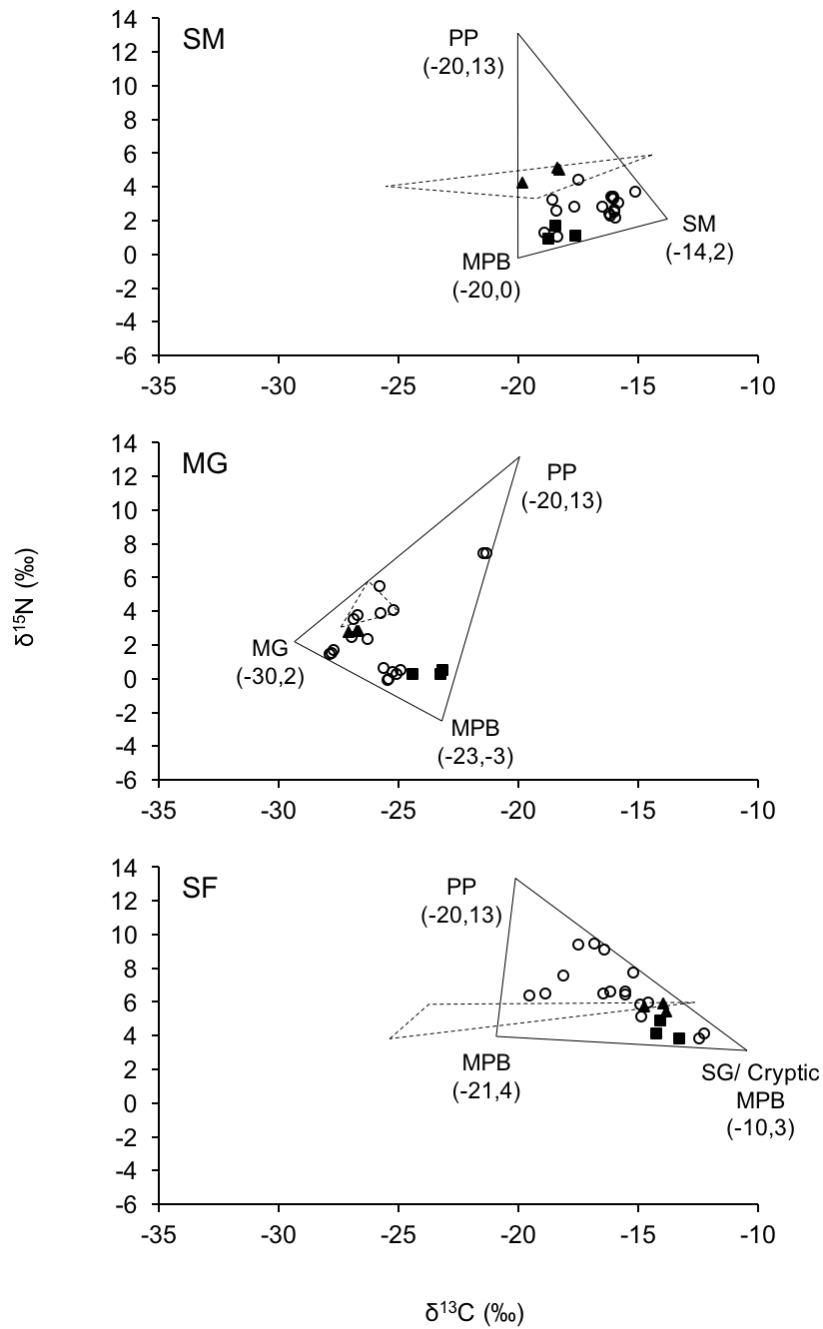


Figure 4.5: The $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots of the meiofaunal nematodes (triangle) and harpacticoids (square), and also the macrofauna (circle) consumer data for the saltmarsh (SM), mangrove (MG), and sandflat (SF) habitats. Vertices from the solid line triangle (S_1 to S_3) indicate the estimated (virtual) end-members inferred based on consumer data. Data are values of replicate samples, corrected for trophic fractionation. Dashed line triangles were plotted based on the actual end-members data collected from the local habitats for detritus (MG/ SM/ SG), SPOM and MPB resources. PP = phytoplankton, SM = saltmarsh, MG = mangrove and SG = seagrass.

4.3.5 IsoError modelling results

Table 4.2: IsoError modelling results proportional contribution of each potential food sources for consumers in saltmarsh, mangrove and sandflat habitat, as computed by the isotope mixing model IsoError (values are mean \pm SE). Bold values indicate > 50% contributions from a single resource. Isotopic values (‰) were adjusted for fractionation prior to mixing model analysis.

Habitat/ Consumer	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Proportional contribution (%), Mean \pm SE		
			S1	S2	S3
Saltmarsh			Detritus (SM)	Phytoplankton	MPB
Harpacticoids	-18.3	1.2	28 \pm 9	5 \pm 4	67 \pm 9
Nematodes	-18.8	4.8	20 \pm 7	34 \pm 3	46 \pm 7
<i>P. erythodactyla</i>	-16.3	3.8	62 \pm 8	20 \pm 3	19 \pm 8
<i>Heloeceus cordiformis</i>	-16.1	3.1	65 \pm 8	14 \pm 3	21 \pm 8
<i>Littoraria scabra</i>	-18.2	2.9	30 \pm 8	18 \pm 3	52 \pm 8
<i>Phallomedusa</i> sp.	-16.1	2.3	65 \pm 8	8 \pm 3	27 \pm 8
<i>Ophicardelus</i> sp.	-17.8	1.6	37 \pm 8	7 \pm 3	57 \pm 8
Mangrove			Detritus (MG)	Phytoplankton	MPB
Harpacticoids	-23.6	0.4	16 \pm 8	16 \pm 3	68 \pm 6
Nematodes	-26.9	2.8	63 \pm 6	17 \pm 2	21 \pm 4
Juvenile shrimp (Penaeidae)	-22.9	6.8	22 \pm 20	54 \pm 8	24 \pm 16
<i>P. erythodactyla</i>	-25.5	4.0	48 \pm 6	29 \pm 2	23 \pm 5
Ampharetid polychaete	-26.8	3.6	62 \pm 5	18 \pm 3	20 \pm 4
Amphipod	-27.8	1.6	70 \pm 4	3 \pm 2	28 \pm 3
<i>Littoraria scabra</i>	-26.6	2.4	57 \pm 7	12 \pm 3	31 \pm 5
<i>Cassidula</i> sp.	-25.3	0.5	36 \pm 6	8 \pm 3	54 \pm 4
Potamididae	-25.3	0.1	35 \pm 5	6 \pm 3	58 \pm 4
Sandflat			Detritus (SG)/ Cryptic MPB	Phytoplankton	MPB
Harpacticoids	-13.9	4.3	64 \pm 5	10 \pm 2	26 \pm 6
Nematodes	-14.2	5.7	60 \pm 5	26 \pm 2	15 \pm 7
Nereid polychaete	-16.9	9.3	32 \pm 5	62 \pm 2	6 \pm 7
Nephtyid polychaete	-16.7	7.7	35 \pm 14	45 \pm 3	20 \pm 16
Ocypodidae	-15.8	6.5	44 \pm 5	33 \pm 2	23 \pm 6
Juvenile shrimp (Penaeidae)	-18.3	6.3	22 \pm 11	28 \pm 2	50 \pm 13
<i>Mictyris longicarpus</i>	-14.8	5.7	54 \pm 4	25 \pm 4	21 \pm 6
Isopod	-12.4	4.0	77 \pm 5	9 \pm 3	14 \pm 7

4.4 Discussion

4.4.1 Differences in SOM food sources between habitats

The different habitats in this study demonstrate varying importance of nutrient sources to the local SOM pool, based on the main autochthonous primary resources and also with the influence of tidal inundation importing allochthonous materials. On the sandflat where the exposure to tidal inundation is maximum, there are no prominent autochthonous sources other than the sediment MPB, and this habitat is characterised by low Chl *a* and TOC contents (Figure 4.2). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the SOM found in this habitat (-22.0 and 7.2 ‰, respectively) suggest a significant contribution of the MPB but the values also resemble those of marine phytoplankton (Yokoyama et al., 2005; Kharlamenko et al., 2008; Bouillon et al., 2008). However, the SOM pool may as well comprise contributions from various allochthonous inputs including tidally imported organic matter from the surrounding mangroves, deposited phytoplankton or seagrass detritus, in addition to the local MPB, resulting in the isotopic values being a mixture of these resources as suggested by the depleted SPOM values found in this study. Estuaries may receive organic matter from a range of different sources (both allochthonous and autochthonous), and these different sources typically have different, if potentially overlapping, stable isotope signatures (Bouillon et al., 2011).

At the mid-tide level mangrove, the SOM $\delta^{13}\text{C}$ values are comparable to those in the literature but our data show lower $\delta^{13}\text{C}$ values for the MPB and epiphytic macroalgae. This pattern presumably reflects the lower $\delta^{13}\text{C}$ values of the local dissolved inorganic carbon (DIC) pool, where mineralization of mangrove detritus supplies the DIC pool with ^{13}C -depleted CO_2 (Bouillon et al., 2008; Maher et al., 2013). The $\delta^{13}\text{C}$ values of the SOM are similar to those of the leaves of the mangrove *A. marina*, suggesting that mangrove carbon accumulates in and dominates the SOM pool (Table 4.1). Meanwhile, the $\delta^{15}\text{N}$ values are

relatively lower than in the other two habitats, presumably due to the decomposition process of mangrove leaf litter known to reduce the $\delta^{15}\text{N}$ values (Werry and Lee, 2005). As mangrove systems mature, more mangrove detritus accumulates in the sediment, with the consequence that the stable isotope values of the sediment organic material increasingly resembling those of mangroves (Demopoulos et al., 2007).

At the highest intertidal zonation with less frequency and period of exposure of the tidal inundation, the SOM has the highest $\delta^{13}\text{C}$ values; these values are lower (by $\sim 4.5\text{‰}$) compared than those of *S. virginicus* but almost identical to those of MPB. Moreover, the SOM $\delta^{15}\text{N}$ values are between the saltmarsh and MPB values (higher than MPB but lower than saltmarsh). The SOM pool is therefore a mixture of local *S. virginicus* inputs and also the MPB. Some tidal deposition of allochthonous organic matter, e.g. phytoplankton, may be present although the high intertidal position implies relatively low contributions from this source. Even though the saltmarsh couch is the dominant species within this habitat in terms of biomass, the stable isotope values suggest a higher contribution of the local microalgal production (MPB) to the SOM pool. This inference is supported by our Chl *a* content (as indicator of the MPB biomass) data at this habitat, which is highest among all the habitats.

In this study, we found a significant correlation of the $\delta^{15}\text{N}$ values between the meiofauna and SOM (Figure 4.3), suggesting a strong dependence of the meiofauna nitrogen demand on sources that are readily available from the SOM pool in their local habitats. Even though the fact that the nematode $\delta^{15}\text{N}$ values were consistently higher than those of the harpacticoids at all habitats could be due to the nematodes having higher trophic position than the harpacticoids, this trend may as well indicate that the former group may fulfill their nitrogen requirement by assimilating food sources with high $\delta^{15}\text{N}$, e.g. heterotrophic bacteria utilizing ^{15}N -enriched ammonium pools. In contrast, the harpacticoids may partly depend on N_2 -fixing bacteria with characteristically low $\delta^{15}\text{N}$ values (Demopoulos et al., 2007;

Vafeiadou et al., 2014). However, it is possible that differences in other food sources may also be important in explaining the nematodes and harpacticoids $\delta^{15}\text{N}$ differences. IsoError calculations indicating especially higher consumption of MPB by harpacticoids (Table 4.2) could explain most of the differences. Even though a similar trend is also shown by the $\delta^{15}\text{N}$ values of the macrofauna and SOM from the sandflat, the macrofauna at the mangrove habitat exhibit a high variability in their $\delta^{15}\text{N}$ values, making it difficult to infer food sources. The sand flat is very low in organic matter, and patchy. Heterogeneous food sources may be locally important, resulting in the high $\delta^{15}\text{N}$ variability.

4.4.2 Resource utilization by the consumers

The isotopic values of food resources collected within each habitat (represented by the dotted triangles) do not match the isotopic values of the consumers (Figure 4.5), suggesting that the animals are more selective in their feeding than our sampling effort, or the trophic fractionation values were significantly different from those used in the comparison. For example, we only collected bulk samples of MPB and SPOM from each site, so that there was limited spatial and temporal replication. More comprehensive sampling may be particularly important in intertidal environments where more extreme environmental conditions may lead to higher variability in primary producer isotopic signatures (Leduc et al., 2006). In addition, isolating pure MPB and phytoplankton from bulk sediment and SPOM samples respectively is known to be technically challenging. Laboratory experimental feeding experiments suggest that some estuarine consumers may demonstrate significantly different trophic fractionation than the average values commonly used for interpretation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data (e.g. Bui and Lee 2014).

All consumers are typically have higher $\delta^{13}\text{C}$ values than the local sedimentary organic pool (SOM) within their habitats (Table 4.1). At the saltmarsh, the $\delta^{13}\text{C}$ value of the main primary producer *S. virginicus* is higher than those of the consumers, with all consumers positioned

in between the local SOM/MPB and *S. virginicus* $\delta^{13}\text{C}$. The mixing model suggests that the MPB and saltmarsh detritus are the dominant resources to the diets of the consumers, and that the phytoplankton source is less important (Table 4.2). This result is expected as the saltmarsh habitat is located at the highest intertidal area, and has limited access to the tidal inundation. Meanwhile, mangrove consumers are also utilizing mostly on the local detritus (mangrove) and MPB resources except for the juvenile shrimp, in which the phytoplankton source contributed most to its diet.

The consumers on the sandflat have highest $\delta^{13}\text{C}$ as compared to the consumers from the other habitats, and sandflat meiofauna have the highest $\delta^{13}\text{C}$ values of all consumers. IsoError mixing model suggests that the seagrass detritus or cryptic MPB sources contributed most to the consumer's diet at the sandflat (Table 4.2). While these values resemble those of seagrass (Guest et al., 2004; Bouillon et al., 2011; Vafeiadou et al., 2014), there is no large-scale seagrass occurrence in close vicinity of our sampling site except for the small patches of *Zostera mulleri* occurrence at 1 km away from the sampling location. It is possible that the seagrass detritus drifted and was carried by the tides to the sandflat, or the sources possibly contributed to the SPOM and eventually settling on the sandflat and become incorporated into the local SOM. However, it is doubtful that there is enough availability of this source to support the consumers on the sandflat.

In addition, many of the benthic invertebrates sampled in this study have limited motility and a small home-range. It is therefore likely that they derive most of their diet from locally available food sources (e.g. Guest et al., 2006), especially the meiofauna. Therefore, there is likely considerable importance of 'obscure' foods, termed here as cryptic MPB, which have not been sampled and included as a food source in the analysis. In addition to the methodological challenge to isolate pure MPB from the bulk sediment, there is also the probability that consumer food selection may leave inedible MPB left to be isolated from the

sandflat bulk sediment sample. This notion is supported by a recent related study, which reported extensive soldier crab activities on the sandflat site, and that the removal of these crabs significantly increased the Chl *a* content on the surface sediment (Abdullah and Lee, 2016). The problem of having cryptic (missing) sources is not new (Incze et al., 1982; Kitting et al., 1984). The idea behind this is that there are low-abundance, high turnover labile foods embedded in a much larger matrix of fairly indigestible organic matter (e.g. vascular plant detritus), especially sediments. The larger pool masks the isotope values of these low biomass food when the samples are analyzed as bulk materials, while the consumers are selectively eating these “chemically cryptic” foods. Therefore, the actual resources supporting most of the consumers at the sandflat, either seagrass or cryptic MPB, remain unidentified.

Overall, it has been shown that local sources (detritus, MPB) are highly utilized by the local consumers as compared to the imported source, i.e. phytoplankton at mangrove and saltmarsh habitats. At the sandflat habitat, where the isotopic values of the consumers do not reflect the utilization from the local sedimentary organic pool sources, imported resources such as seagrass detritus and phytoplankton are more prominent. However, identifying an actual resource utilization by consumers at the sandflat habitat presents a challenge, as the exposed bare sediments receive contributions from a variety of imported organic resources, or from local but unknown resources, as suggested in this study (i.e. cryptic MPB). Apart from that, certain animals showed a wider range of resource utilization while others are more selective. The sesamid crab *P. erythodactyla* and the gastropod *L. scabra* found in mangrove and saltmarsh habitats are capable of utilizing different food sources depending on their availability in the local habitat. Meanwhile for the harpacticoids, the MPB/ cryptic MPB are contributing most of its diet (Table 4.2). Certain other consumers such as the juvenile shrimps, together with certain crabs, gastropods and also polychaetes

showed high variability in their $\delta^{13}\text{C}$ values, as indicated by the high standard error values (Figure 4.4 and Table 4.1). Patchy food webs with moderate to strong local variations in food resources may be indicated for these species with limited motility.

CHAPTER 5

APPLICATION OF DUAL STABLE ISOTOPE ADDITIONS OF ^{13}C and ^{15}N TO INVESTIGATE THE IMPORTANCE OF MICROALGAE AS A FOOD SOURCE TO THE MEIOFAUNA IN A MANGROVE

5.1 Introduction

Meiofauna are ubiquitous in marine soft-bottomed habitats but their contribution to ecosystem functioning through interaction with microbes, mineralization of organic matter and also as food to the higher trophic levels, is still unclear. Investigation of resource utilization by and diet of the meiofauna is challenging due to their small sizes (62 to 500 μm). Early approaches relied on the morphology of the mouthparts for investigating resource utilization and diet of the meiofauna, resulting in classification into various feeding guilds, e.g. suspension and deposit feeders. In general, meiofauna feed on a wide variety of food sources including microalgae, protists, bacteria, fungi, yeasts, mucoid substances, detritus, dissolved organic matter (DOM), and other meiofauna (Hicks and Coull, 1983; Montagna et al., 1995; Moens and Vincx, 1997; Coull, 1999). Recent studies suggest that information on resource utilization by the meiofauna based solely on functional morphology is inadequate. This is because meiofauna are opportunistic feeders capable of changing their food sources in relation to available sources (Wolłowicz et al., 2011). In addition, significant variations exist in resource use and trophic level among nematode genera and even among congeneric nematode species from the same feeding guild, and thus interpretation of nematode feeding habits based purely on mouth morphology should be avoided (Vafeiadou et al., 2014).

Recent wide application of stable isotope analysis has shed important light on questions about meiofaunal food webs. The use of natural abundance stable isotopes has limitations in tackling detailed questions regarding exploitation of the natural resources by the meiofauna and subsequent transfer of nutrients to their predators. However, a combined natural abundance and isotope labelling approach provides a powerful tool to fill gaps of knowledge in meiofaunal food webs (Carman and Fry, 2002; Moens et al., 2002; De Troch et al., 2005; Urban-Malinga and Moens, 2006; Wyckmans et al., 2007; Franco et al., 2008; Galvan et al., 2008, 2011). Meiofauna are primarily sedentary and have short turnover time, making them good candidates for isotope labelling experiments. All the previous studies using this combined approach in estuarine habitats dealt with meiofauna from saltmarsh or seagrass habitats, with limited study on mangrove meiofauna to date (e.g Oakes et al., 2010). Further, even natural abundance isotope studies on mangrove meiofauna are uncommon, resulting in much uncertainty on their trophic ecology in mangrove-dominated habitats (Demopoulos et al., 2007).

The microphytobenthos (MPB) has generally been suggested as one of the main food sources for the meiofauna (Carman and Thistle, 1985; Caramujo et al., 2005; Wyckmans et al., 2007). Stable isotope analysis of the meiofauna from intertidal habitats, particularly saltmarsh and seagrass habitats, have shown that MPB contributed between 78 - 92 % to the diets of the harpacticoid copepods (Galvan et al., 2008), and between 34 - 82 % of the nematodes (Moens et al., 2002). This study employed a field experiment following a combined dual-element natural abundance-labelling approach to test the hypothesis that harpacticoid copepods predominantly rely on MPB while nematodes utilise a wider nutrient base. We also hypothesize that due to their higher nutritional value (compared to alternative sources such as mangrove detritus), MPB is the dominant food source to the meiofauna,

despite between-taxa variations arising from specific feeding preferences among the harpacticoid copepods and nematodes.

5.2 Materials and methods

5.2.1 Study site

The experiment was conducted in a mangrove tidal creek on Kangaroo Island (27°46'39.94"S, 153°22'48.25"E), in the Moreton Bay Marine Park (Figure 5.1). The mangrove forest is dominated by *Avicennia marina* and the tidal regime is semi-diurnal with a range of ca. 2.5m. The tidal creek is about 800 m long and has no upstream freshwater inputs (Gleeson et al., 2013).

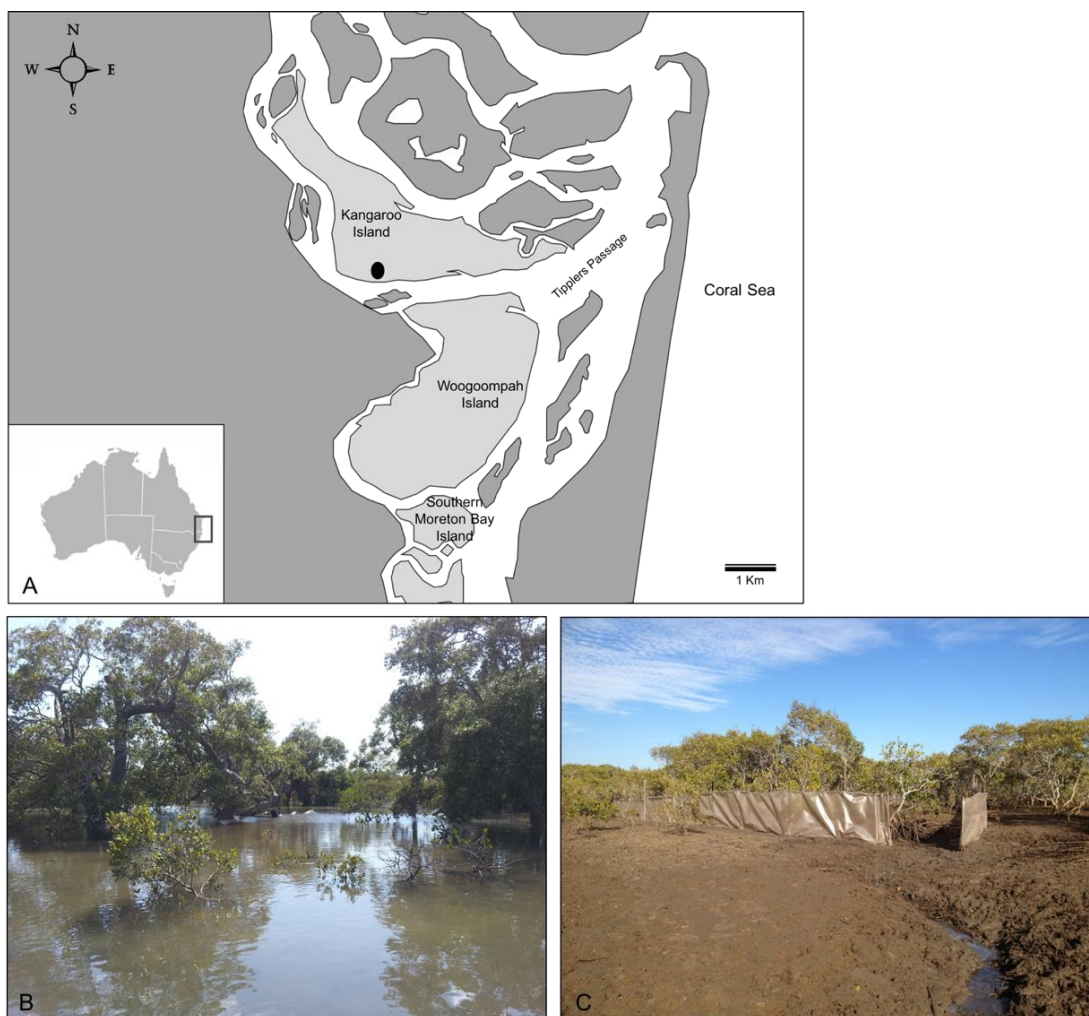


Figure 5.1: (A) Map of the study area in southern Moreton Bay, Queensland, Australia. The black circle indicates the area on Kangaroo Island. (B) and (C) are detailed photographs of site where the experiment was conducted.

5.2.2 Experimental design

About 105 g of ~99% ^{13}C -labelled sodium bicarbonate ($\text{NaH}^{13}\text{CO}_3$) and 100 g of ~99% ^{15}N -labelled potassium nitrate (K^{15}NO_3) were used as tracers. Incorporation of these labels into the MPB is expected to be rapid, a time-course enrichment in MPB, sediment organic matter (SOM) and the meiofauna would therefore indicate the strength of trophic links. The labels were dissolved in GF/F filtered site water and evenly sprayed onto the sediment surface across an enclosed experimental area of 260 m² (Figure 5.1C). The experimental area was

divided into 1 m² squares and the labels were applied to give final ¹³C and ¹⁵N label concentrations at 0.40 and 0.39 g m⁻², respectively.

Sampling time 0 (control, before enrichment) was done on the 21st November 2013 and samples were collected before the enrichment process to provide baseline data for the natural abundance of the isotope values (Day 0). The labelling was done on the 23rd November 2013 at low tide at dawn and the first sampling after the enrichment process was done 7 to 9 hours later on the same day (Day 0.5). The other sampling sessions were done at 21 (Day 1), 31 (Day 1.5), 55 (Day 2.5), 105 (Day 4.5), 142 (Day 6) and 286 hours (Day 12) later. A total of 125 ml of sediment was collected for each replicate sample of MPB, SOM (representing bulk sediment materials that includes MPB and detritus) and meiofauna, by scraping the top 1 cm layer of the sediment surface. Healthy growing mangrove leaf samples (*Avicennia marina*) were collected from multiple trees at each sample. Samples were frozen until further analysis.

5.2.3 Sample preparation for stable isotope analysis

SOM samples for $\delta^{13}\text{C}$ analysis were pre-treated with dilute HCl for 24 hours, rinsed thoroughly with de-ionized water to remove carbonates, then dried at 60°C and prepared for stable isotope analysis. The samples for $\delta^{15}\text{N}$ were prepared without acid treatment. The mangrove leaves were cut into small fractions (avoiding veins) and dried at the same temperature. For the collection of meiofauna, the sediment samples were washed with tap water over a stack of 500 μm and 63 μm sieves. Large plant debris consisting of roots, leaves and branches remaining on the 500 μm sieve was rinsed carefully and removed. Extraction of the meiofauna from fine sediments remaining on the 63 μm sieve was done using flotation extraction with Ludox (Eleftheriou and McIntyre, 2005; Nicholas et al., 1991). After initial washing, 10 times of the sample volume of Ludox were added to 50 ml tubes and centrifuged for 5 minutes. The supernatant was carefully poured through a 63 μm sieve,

rinsed with deionized water, and the meiofauna hand-picked under a dissecting microscope. The nematode and harpacticoid copepods were transferred into tin capsules and dried at 60°C for 24 hours and weighed to 0.01 mg precision for stable isotope analysis. Three replicates of each meiofauna group, each containing 70 to 80 individuals of harpacticoids and 200 to 250 of nematodes, were obtained. Complete organisms including their gut contents were used in this study.

Sediment samples for the microphytobenthos (MPB) were washed through 53 µm and 5 µm meshes to remove the infauna. 5 ml of materials retained on the lower mesh were transferred to 50 ml centrifuge tubes and 30% colloidal silica (LUDOX™ AM30, density = 1.21) was added up to 45 ml. The samples were centrifuged at 10 000 rpm for 10 min. A distinct top band containing concentrated MPB was transferred to a clean tube using a Pasteur pipette. The solution was centrifuged again with deionized water to remove residual LUDOX solution. Samples for stable isotope analysis were dried at 60°C, grounded and weighed into tin capsules.

5.2.4 Mass spectrometry

Dual stable isotope analyses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for the meiofauna were carried out on a Delta V Plus continuous flow isotope ratio mass spectrometer linked to a Flash 2000 elemental analyser (Thermo-Fisher Scientific, Bremen, Germany) with low volume setup (IVA Analysentechnik, Meerbusch, Germany) using a Zero Blank autosampler (Costech International, Milan, Italy) at the NIWA Ecological Stable Isotope Laboratory in Wellington, New Zealand. All other samples were analysed using an Automated Nitrogen Carbon Analyzer system consisting of a Sercon 20-22 mass spectrometer and an EA (SERCON, UK). PeeDee Belemnite and atmospheric air were used as standards for C and N, respectively. Stable isotope values are reported in δ -notation (‰), i.e. $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ =

$(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where R is C^{13}/C^{12} and N^{15}/N^{14} ratios for carbon and nitrogen analyses, respectively.

5.2.5 Data analysis

Enrichment above natural abundance (label uptake, δ^E) is reported in ‰, where δ^E is the enriched $\delta^{13}C$ and $\delta^{15}N$ values for that time point minus natural abundance $\delta^{13}C$ and $\delta^{15}N$ values from Day 0.

5.3 Results

5.3.1 Natural abundance stable isotope values (Day 0, before label addition)

Harpacticoid copepods have the most enriched $\delta^{13}C$ (-21.0 ‰) while the nematodes were relatively depleted at -28.3 ‰ (Table 5.1). The $\delta^{15}N$ for the harpacticoids and nematodes were 4.2 ‰ and 7.5 ‰, respectively. MPB and SOM had the most enriched $\delta^{13}C$ at -23.5 ‰ and -24.0 ‰, respectively, while mangrove leaves depleted at -30.5 ‰. The $\delta^{15}N$ for the MPB, SOM and mangrove leaves were 3.1, 3.5 and 2.2 ‰, respectively (Figure 5.2A).

5.3.2 Dual-isotope labelling ($\delta^{13}C$ and $\delta^{15}N$ average enrichment)

The ^{13}C and ^{15}N enriched labels were taken up by all samples analysed. Enrichment above background levels was observed on Day 0.5, the earliest sampling point after additions had started (Table 5.1). Harpacticoid copepods were the most enriched with average $\delta^{13}C$ at 7.0 ‰ (Figure 5.2) while the nematodes were more depleted (-13.4 ‰). The $\delta^{15}N$ for the harpacticoids and nematodes were 644.5 ‰ and 180.5 ‰ respectively. Harpacticoids were more enriched than nematodes at most times. The SOM (averaged at -2.2 ‰ and 1659.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively) became more enriched than MPB (-16.9 ‰ and 601.2 ‰). Mangrove leaves are plotted based on the natural abundance values as enrichment would not have occurred in such a short time.

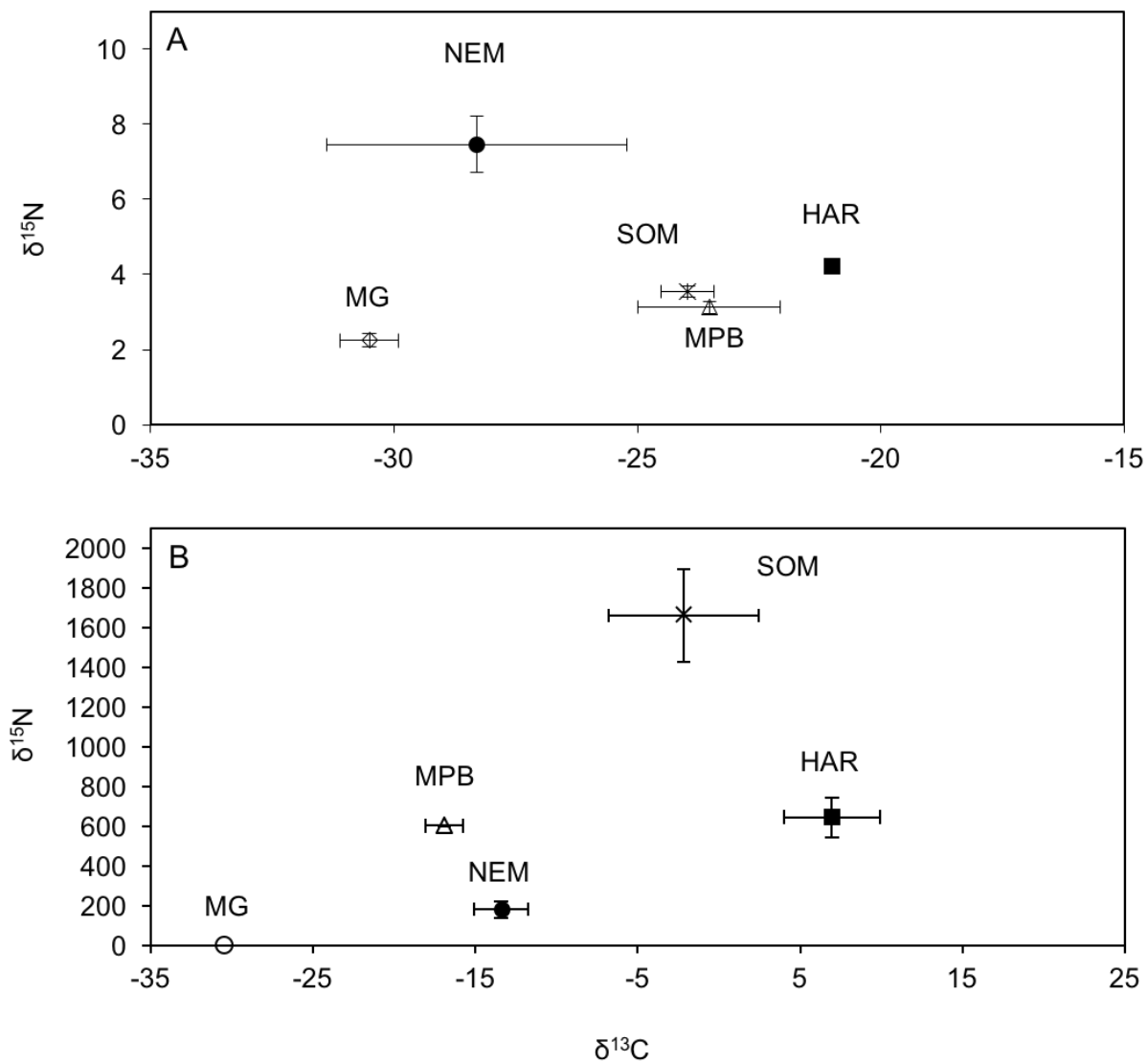


Figure 5.2: (A) Dual natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) of the nematodes (NEM), harpacticoids (HAR), MPB, SOM and mangrove leaves (MG), prior to isotope addition (Day 0). (B) The enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for each component, averaged over the sampling period after labelling. Values of the mangroves in both plots are obtained from the same site before label application ($n=3$). Values are mean \pm SE, and plotted without correction for trophic fractionation.

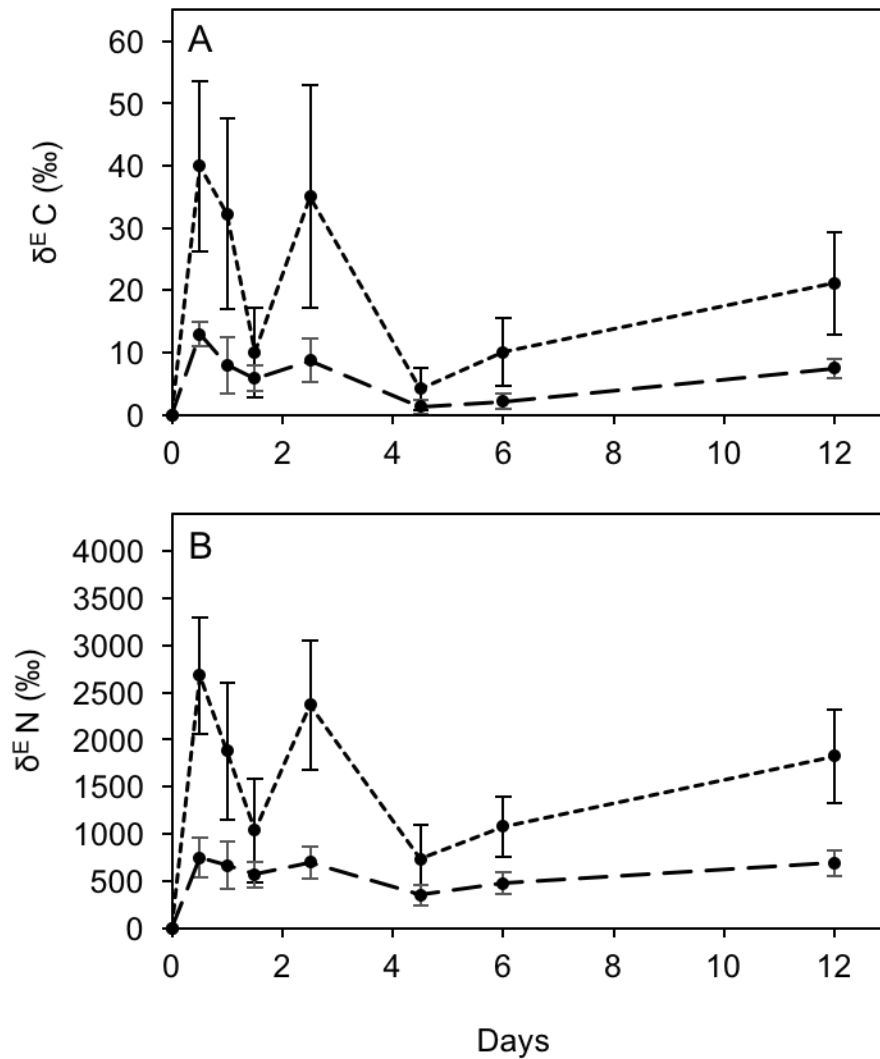


Figure 5.3: Carbon (A) and nitrogen (B) uptakes (δ^E) following dual ^{13}C and ^{15}N addition for the SOM (-----) and MPB (—) over the 12-day sampling period. Values are mean \pm SE.

5.3.3 ^{13}C and ^{15}N label uptake by SOM and MPB

Both the $\delta^{13}\text{C}$ isotope values in SOM and MPB reached a peak of enrichment on Day 0.5 with a value of 39.9‰ and 12.9‰ ($\delta^E\text{C}$) above natural abundance values, respectively (Figure 5.3, Table 5.1). Similar to the ^{13}C uptake, the $\delta^{15}\text{N}$ isotope values in both SOM and MPB reached a peak of enrichment on Day 0.5 with values of 2680.9‰ and 746.8‰ ($\delta^E\text{N}$), respectively. Even though fluctuating temporally, the uptakes showed a general slowly decreasing trend from peak enrichment until the end of the experimental period. All

components showed much higher ^{15}N than ^{13}C label uptake. On average, the ^{13}C label uptake ($\delta^{13}\text{C}$) was three-fold higher in SOM (21.8‰) than that of the MPB, which valued at 6.6‰ (Figure 5.4), while the ^{15}N label uptake was nearly three-fold higher in SOM (1655.7‰) than that of the MPB (598.1‰).

5.3.4 ^{13}C and ^{15}N label uptake by the meiofauna

In general, the ^{15}N label uptake by both of the meiofaunal groups were higher than the ^{13}C label uptake. Harpacticoids accumulated the ^{13}C label faster than did nematodes, and became highly enriched, reaching a value of 44.1‰ above the natural abundance values on Day 6. At 0.5 days after enrichment, the ^{13}C label uptake had already exceeded the averaged values of MPB, and the label uptake increased, passing the average values of SOM at Day 4.5. After reaching its peak on Day 6, the uptake started to decrease by the end day of the experiment. Nematodes accumulated the labels at a slower rate, where the ^{13}C label uptake ($\delta^{13}\text{C}$) became slightly above the averaged values of MPB at Day 1 (11.3‰). The ^{13}C label uptake increased to around but not exceeding the average values for SOM. The uptake peaked on Day 6, with a value of 24.0‰ above the natural abundance values and the uptake decreased by the end day of the experiment.

Harpacticoids also accumulated the ^{15}N labels faster than did nematodes, and became highly enriched, reaching a value of 1314.9 ‰ above the natural abundance values on Day 6 (Figure 5.4). The ^{15}N label uptake by harpacticoids exceeded the average values of MPB at Day 4.5, and the label uptake increased, peaked on Day 6, and decreased by the end day of the experiment. Different to the way harpacticoids accumulated the ^{13}C label, the ^{15}N label uptake did not exceed the average values of SOM for the whole experimental period. Nematodes accumulated the ^{15}N label at a rate much slower than that for the harpacticoids. The ^{15}N label uptake never exceeded the average values of the MPB until the last day of the experiment (Day 12), when it peaked at 457.3‰ above the natural abundance values.

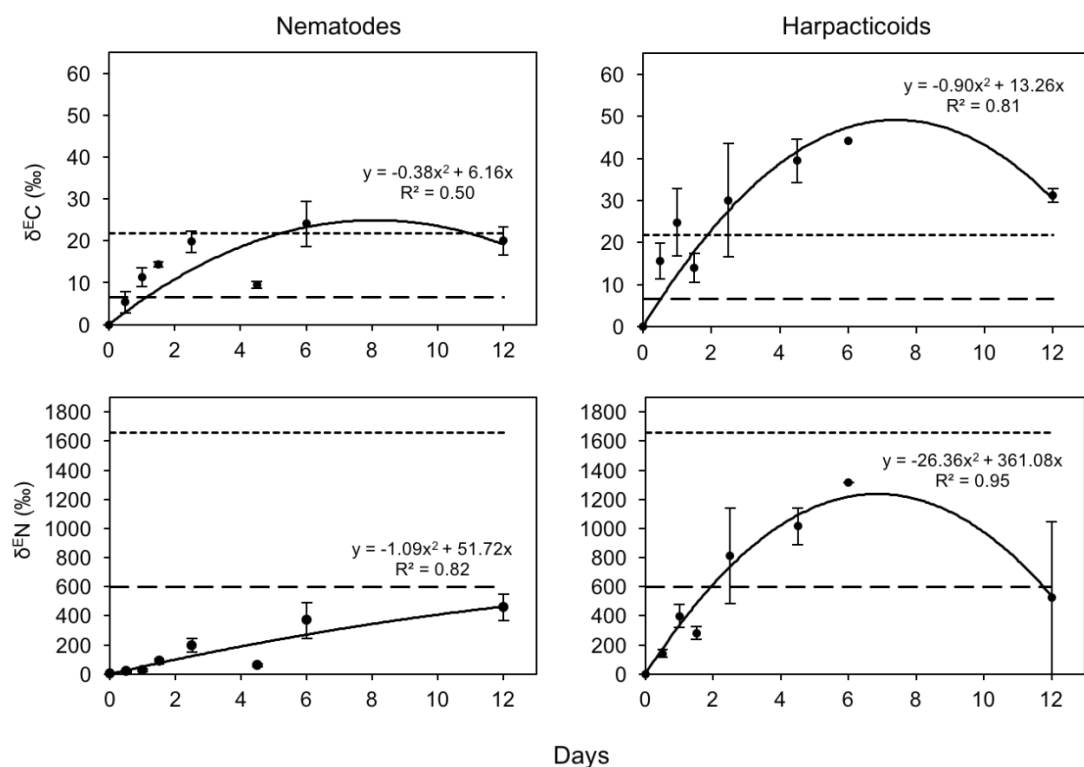


Figure 5.4: Carbon and nitrogen uptakes (δ^E , solid lines) for the nematodes and harpacticoids over the 12-day dual ^{13}C and ^{15}N addition. Average carbon and nitrogen label uptakes for the SOM (-----) and MPB (— —) are also indicated. The best fitting curves for the meiofauna are plotted with the respective quadratic functions and R^2 values are given. Values are mean \pm SE, and plotted without correction for trophic fractionation.

Table 5.1: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) of the natural abundance (Day 0), the post-labelling values and also the label uptake (δ^E) from Day 0.5 until Day 12. Values are mean \pm SE from three sample replicates ($n = 3$) unless for the harpacticoid samples on Day 6 ($n = 1$) and Day 12 ($n = 2$).

Day 0		Day 0.5		Day 1		Day 1.5		Day 2.5	
(A)	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	δ^{E}	$\delta^{13}\text{C}$	δ^{E}	$\delta^{13}\text{C}$	δ^{E}	$\delta^{13}\text{C}$	δ^{E}
Harpacticoids	-21.0 ± 0.1	-5.5 ± 4.2	15.5 ± 4.2	3.8 ± 7.9	24.8 ± 7.9	-10.5 ± 0.5	14.0 ± 3.5	9.1 ± 13.4	30.1 ± 13.4
Nematodes	-28.3 ± 3.1	-23.0 ± 2.5	5.3 ± 2.5	-17.0 ± 2.3	11.3 ± 2.3	-14.0 ± 0.6	14.3 ± 0.6	-8.5 ± 2.5	19.8 ± 2.5
MPB	-23.5 ± 1.5	-10.6 ± 2.0	12.9 ± 2.0	-15.5 ± 4.6	8.0 ± 4.6	-17.7 ± 2.0	5.8 ± 2.0	-14.8 ± 3.5	8.7 ± 3.5
SOM	-24.0 ± 0.5	15.9 ± 13.7	39.9 ± 13.7	8.3 ± 15.3	32.3 ± 15.3	-14.0 ± 7.2	10.0 ± 7.2	11.1 ± 17.9	35.0 ± 17.9
Mangrove leaves	-30.5 ± 0.6								
(B)	$\delta^{15}\text{N}$	$\delta^{15}\text{N}$	δ^{E}	$\delta^{15}\text{N}$	δ^{E}	$\delta^{15}\text{N}$	δ^{E}	$\delta^{15}\text{N}$	δ^{E}
Harpacticoids	4.2 ± 0.1	144.1 ± 26.5	139.8 ± 26.5	401.1 ± 78.5	17.7 ± 6.5	285.7 ± 45.8	281.5 ± 45.8	814.4 ± 326.8	810.2 ± 326.8
Nematodes	7.5 ± 0.7	25.1 ± 6.5	17.7 ± 6.5	33.4 ± 9.7	396.9 ± 78.5	94.5 ± 17.7	87.1 ± 17.7	204.2 ± 45.6	196.8 ± 45.6
MPB	3.1 ± 0.2	749.9 ± 213.6	746.8 ± 213.6	665.7 ± 252.6	662.5 ± 252.6	569.5 ± 134.0	566.3 ± 134.0	702.5 ± 170.0	699.4 ± 170.0
SOM	3.5 ± 0.2	2684.5 ± 616.5	2680.9 ± 616.5	1879.7 ± 726.3	1876.2 ± 726.3	1038.4 ± 545.6	1034.9 ± 545.6	2369.4 ± 689.2	2365.9 ± 689.2
Mangrove leaves	2.2 ± 0.2								

Day 4.5		Day 6		Day 12		
(A)	$\delta^{13}\text{C}$	δ^{E}	$\delta^{13}\text{C}$	δ^{E}	$\delta^{13}\text{C}$	δ^{E}
Harpacticoids	18.4 ± 5.2	39.4 ± 5.2	23.1	44.1	10.2 ± 1.6	31.2 ± 1.6
Nematodes	-18.9 ± 0.8	9.4 ± 0.8	-4.3 ± 5.3	24.0 ± 5.3	-8.3 ± 3.4	20.0 ± 3.4
MPB	-22.2 ± 1.1	1.3 ± 1.1	-21.4 ± 1.2	2.1 ± 1.2	-16.1 ± 1.6	7.5 ± 1.6
SOM	-19.8 ± 3.4	4.1 ± 3.4	-13.9 ± 5.4	10.1 ± 5.4	-5.9 ± 7.3	18.0 ± 7.3
(B)	$\delta^{15}\text{N}$	δ^{E}	$\delta^{15}\text{N}$	δ^{E}	$\delta^{15}\text{N}$	δ^{E}
Harpacticoids	1019.9 ± 126.1	1015.6 ± 126.1	1319.2	1314.9	1114.6 ± 64.5	1110.3 ± 64.5
Nematodes	67.2 ± 11.5	59.7 ± 11.5	374.5 ± 125.3	367.0 ± 125.3	464.7 ± 91.1	457.3 ± 91.1
MPB	351.3 ± 106.0	348.1 ± 106.0	477.4 ± 114.9	474.3 ± 114.9	692.0 ± 134.5	688.9 ± 134.5
SOM	734.0 ± 368.7	730.4 ± 368.7	1082.2 ± 317.7	1078.7 ± 317.7	1826.1 ± 493.0	1822.6 ± 493.0

5.4 Discussion

Results from the labelling experiment suggest that meiofauna utilize resources to fulfill their carbon and nitrogen needs in different ways. Both meiofaunal groups showed a wider range of utilized carbon sources but more limited sources for nitrogen. While this happened to both meiofaunal groups, nematodes and harpacticoids showed specific preferences of achieving this, as reflected by the different rates of label uptake for the two groups. Harpacticoids are more highly enriched (on average nearly two-fold higher) and accumulated labels at a faster rate than nematodes. The reason for this trend could not be predicted, as information on the direct comparison of the metabolic or tissue turnover rate for harpacticoids and nematodes are not available. In terms of starting biomass, individual nematodes are smaller (lighter) than the harpacticoids (Appendix C).

Utilization of the carbon resources, as indicated by the ^{13}C label uptake by nematodes is restricted to what were available within the SOM, as the maximum label uptake did not exceed the average SOM values (Figure 5.4). These data suggest that nematodes were consuming the resources available within their local sedimentary organic matter pool, probably due to their low movement capacity and their deposit-feeding habit. Consumers are expected to demonstrate $\delta^{13}\text{C}$ values approaching but not exceeding the values of their food source in pulse-chase enrichment data, if they depend solely on that particular food source. The fact that the $\delta^{13}\text{C}$ value of the nematodes was much higher than the average MPB value suggests additional reliance to the other sources available within the local SOM pool, such as mangrove detritus. However, as non-living material, mangrove detritus is assumed to be unaffected by the labels (Galvan et al., 2011).

This inference is supported by the natural abundance data (Figure 5.2), which shows the nematodes having the most depleted consumer $\delta^{13}\text{C}$ isotope values close to those of the mangrove and the SOM values. As the nematodes live within the interstitial subsurface sediment and have limited motility, the food sources available might be

restricted to what are available within the local SOM pool, which would be a mixture of labelled MPB and unlabelled mangrove detritus. The natural abundance $\delta^{13}\text{C}$ isotope values of the SOM (-24.0‰) is considered enriched as compared to the main primary source, i.e. mangrove leaves (-30.5‰). Therefore, it is assumed that the SOM pool in the study area is a mixture of mangrove organic matter, MPB and also other more enriched sources such as estuarine phytoplankton (~ -21 ‰, Bouillon et al., 2011), seagrass (~ -11.7 ‰, Guest et al., 2004) or “cryptic” MPB (as discussed in Chapter 4). In contrast, $\delta^{15}\text{N}$ of the nematodes was below the average values of the MPB, indicating a significant consumption of the MPB to fulfill their nitrogen requirements.

Meanwhile, the $\delta^{13}\text{C}$ value of the harpacticoids was much higher than the average values of both the MPB and SOM, suggesting consumption of other resources not included in the study (unknown sources), which could have efficiently incorporated the ^{13}C label introduced into the habitat. Even though not intentionally targeted, the results suggest that this unknown resources are able to incorporate the ^{13}C label even more efficiently than the targeted MPB. Harpacticoids live mostly on the topmost layer of the sediment and have higher motility than nematodes, and therefore are exposed to higher diversity of food resources other than what are available only within the subsurface sediment. Harpacticoids accumulate the ^{15}N label nearly four-fold higher than did nematodes, exceeding the average $\delta^{15}\text{N}$ value of the MPB but not that of the SOM. This pattern suggests a reliance of harpacticoids to a mixture of sources within the local sedimentary organic pool.

CHAPTER 6

GENERAL CONCLUSION

6.1 Summary of findings

Chapter 2 shows that meiofaunal assemblages exhibited significant associations with local sediment environmental variables of their habitats. High values of food proxies such as phaeopigments, Chl *a* or TOC, with moderate tannin content provide the best condition for the meiofauna to achieve the highest density. Components of habitat structure (sediment particle size, belowground root biomass and/ or moisture content) also influenced meiofaunal density. However, given the complex temporal environmental dynamics and the spatial heterogeneity of the mangrove environments, no clear generalization could be made regarding the key environmental variables that predominantly shape meiofaunal assemblage structure at the location level. Local sediment conditions at micro-scale probably play a significant role. The meiofaunal assemblage structure showed a strong distinction among the specific mangrove species they inhabit, which is an integral part of the large variations of the specific environmental characteristics driven by spatial and temporal variations.

In chapter 3, soldier crabs (*Mictyris longicarpus*) on the sandflat of Tallebudgera showed significant trophic interaction with the meiofauna, but fiddler crabs (*Uca vomeris*) in the mangrove habitat did not seem to rely on the meiofauna as food. This study suggests that while trophic interactions may be specific to the consumer's food preferences, physical interactions are not solely caused by the crab's physical bioturbation activities but is also closely related to the local sediment characteristics. Specifically, the sandflat site where the soldier crabs occurred naturally has a more fluid sediment due to the more frequent tidal inundation and thus a high water content. On the other hand, the sediment of the mangrove habitat where the fiddler crab occurred was more compact due to the

less frequent tidal inundation and potentially also the higher detritus content (e.g. humic substances that help bind sediment particles together), as well as the sediment-stabilising effects of fine mangrove roots. Different degrees of bioturbation activities may impact the ability of the meiofauna to recover from disturbances in different habitats. In the loose sediment on the sandflat, mobility of the meiofauna would be facilitated due to the larger interstitial space among the sediment particles. However, drier and compact sediments may hinder the movement of the meiofauna among the sediment particles, resulting in slower recovery of the meiofauna after the initial disturbance in the exclusion treatment. A longer recovery period after site disturbance may be appropriate for caging experiments, especially on drier and compact sediment substrates. While my inclusion and exclusion cage experiment coupled with manipulation of the crab's feeding activities (the 'Disabled' treatment) could differentiate between physical and trophic impacts, a general conclusion on the effects of these two crab species on the meiofauna could not be made due to the lacking of temporal and spatial replicates.

Meanwhile, the data in chapter 4 show strong differences in resource utilization by benthic invertebrate consumers between different habitats that are tidally connected. Overall, the results of this study support the notion that the local sources of sedimentary organic matter determine the overall trophic dependency of consumers of limited motility on different sources available at soft-sediment, depository, habitats, despite the obvious fact that different species have different feeding specializations. However, the complement of organic matter available at a location is partially influenced by tidal connectivity, which in turn drives trophic connectivity between the habitats supporting the producers and consumers. Meanwhile, the dual labeling experiment in chapter 5 has provided more insight into how meiofauna fulfill their carbon and nitrogen requirements. While the MPB are normally attributed as an important food source to the meiofauna, my results suggest that there is a strong divergence in the diet between the nematodes and harpacticoid copepods. Soft-sediment infauna have dietary needs and flexible feeding

strategies that may change over space and time and among species, making generalizations of the food web positions of infauna tenuous. The ^{13}C and ^{15}N labels used in this study were targeting the MPB but the fact that there was a higher incorporation of labels in the SOM suggests that enrichment of additional (cryptic or unknown) and microscopic organic matter sources, e.g. autotrophic bacteria, has occurred. These sources may be important in the meiofaunal food web, especially in habitats that are regularly inundated by tides and exposed to variety contribution of sources either from local or imported materials. Our data also show that meiofauna studied in this experiment have reached isotopic equilibrium within the 12-day experimental period; the trend is, however, much slower for the nematodes especially for nitrogen uptake.

6.2 Significance of this research and implication for future studies

This work has successfully answered the research questions raised at the beginning of this study. For RQ1 asking if the dynamics of the environmental conditions and the heterogeneity of the mangrove sediments affect the meiofaunal assemblages, I have shown that variability among mangrove sediments of different species is significant. Determining the relationships between the biological, chemical and physical structure of intertidal sediments is vital for improving our understanding of complex and dynamic habitats such as mangroves, which is a challenge for researchers (Tolhurst et al., 2010). It is suggested that future studies investigating the meiofauna-sediment relationship should include phaeopigments, belowground root biomass and also tannin contents in order to achieve a more comprehensive understanding of the meiofaunal assemblages in mangroves. Mangrove detritus is widely known to increase sediment tannin content but most of the studies on benthic assemblages in mangroves do not include tannin as one of the variables. Instead, the discussions on the effect of tannin was only supported by few studies, e.g. meiofauna - Alongi (1987a, b) and macrofauna - Lee (1997).

RQ2 addresses the nature of the interactions (trophic or physical) between deposit-feeding brachyuran crabs and the meiofauna, as these two abundant animal groups share the same habitat. While previous studies have suggested significant negative impacts by the crabs on meiofaunal density, the nature of interactions was poorly described. The classic approach to answering this question employed an inclusion and exclusion cage experiment, combined with a novel approach by manipulating the crab's feeding activities, suggests significant top-down effects on the meiofauna. Future studies may investigate the effect of different sediment substrates on the physical interaction between crabs and the meiofauna. Future experiments may also test if these interactions may be modified by factors such as sediment particle size and availability of alternative foods. Chemical approaches such as stable isotope analysis may help to further elucidate the nature of interactions between deposit-feeding crabs and the meiofauna on tropical soft shores. In addition, the presence of meiofauna in the foregut/proventriculus and hindgut of crabs could be investigated to see if the meiofauna, e.g. nematodes, may just pass through the intestine of crabs without being digested. A definite conclusion regarding their trophic interaction could then be made.

The third question on the trophic ecology of the meiofauna as consumers in mangroves and other connected habitats was addressed through application of stable isotope analysis in two different studies, respectively following the natural abundance and natural abundance/labelling approaches (chapters 4 and 5, respectively). In the isotopic labelling approach, the addition of highly-enriched stable isotopes introduced a large, and therefore unambiguous, isotopic signal to the targeted food source (i.e. MPB) in the mangrove food webs involving the meiofauna. At the beginning of this study, the main issue to overcome was getting enough sample weight for isotopic spectrometry analysis. Because analytical methods typically require quantities of biomass in milligrams of animal tissue for reliable detection of isotopic compositions, most studies have focused on larger animals while the smaller organisms such as meiofauna have been the subject

of relatively few stable-isotope studies (Carman and Fry, 2002). Getting sufficient biomass for the dual stable isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis is a huge challenge due to their small body size. Therefore, large numbers are required to obtain sufficient materials for analysis.

Further, even less information is available regarding the carbon and nitrogen content of individual meiofaunal groups, making the sample preparation for the analysis time consuming as well as guess-work. The number of individual meiofauna required for one sample usually depended on crude biomass estimates based on observations of length and width of selected specimens. Carbon was conservatively estimated at 10% of nematode wet weight (Sikora et al. 1977, Heip et al. 1985). For example, a range of 50 to 200 individual from the bulk nematode samples are required to provide enough biomass for 10 μg of elemental carbon per sample (Moens et al., 2002). Modifications to a conventional elemental analyser-stable isotope ratio mass spectrometer system (EA-MS system) is usually required to allow the analysis of ^{13}C and ^{15}N in small samples ($\geq 1 \mu\text{g N}$ and $2 \mu\text{g C}$) (Carman and Fry, 2002). However, the configuration of most commercial EA-IRMS normally requires at least 5 $\mu\text{g C}$ or N, which for N often corresponds to several hundred nematodes (Bouillon and Gallucci, 2005). In this study, a minimum 5 $\mu\text{g N}$ of sample was needed for the analysis, therefore, at least 43-61 of harpacticoids and 51-134 of nematodes individual must be prepared for each sample to fulfill the required N content (Appendix C). My work has demonstrated that with on-going advancement of small-sample SIA and reasonable effort devoted to sample isolation and preparation, stable isotope analysis of meiofauna can be achieved to provide a powerful tool for studying the trophodynamics of soft-sediment communities.

A thorough understanding of the energy and food web relations in benthic communities is difficult to achieve (Peterson, 1999). This is especially true about estuarine habitats, where food may originate from a large range of autochthonous (e.g. MPB, mangroves, saltmarsh plants) and allochthonous (e.g. settled phytoplankton, imported seagrass

detritus) sources. In addition, the energy supply for benthic consumers originates from a diversity of sources with the relative importance of different sources varying greatly in space and time. Through the application of different stable isotope analysis approaches to achieve these aims, two key challenges have been addressed in chapter 4 and 5, respectively.

Firstly, the isotopic values of food resources collected within each habitat did not match the isotopic values of the consumers, suggesting that the animals were either highly selective in their feeding, or the trophic fractionation values were significantly different from those used in the comparison. One important rule for quality stable isotope tracer work is to know the δ -values of the source materials that are used in mixing model equations to compute the relative contribution of the multiple end-members (Peterson, 1999). Therefore, the establishment of the trophic resources to be included in the IsoError mixing model was gained from the consumer data. This top-down consumer-oriented approach estimates the end-member source values of “virtual” consumers that are fully dependent on different foods in each habitats. Besides, identifying actual resource utilization by consumers at the sandflat presents another challenge as the consumer’s isotopic values do not reflect utilization of the local sedimentary organic pool sources. Therefore, certain cryptic or missing sources are suggested to contribute to the resource utilized by the sandflat consumers. Despite these challenges, a trend of resource partitioning by the meiofauna and macrofauna consumers in the mangroves and adjoining habitats, namely sandflat and saltmarsh, in sub-tropical eastern Australia was established.

The second challenge addressed in chapter 5 is also related to cryptic or unknown food sources, which could have efficiently incorporated the ^{13}C label introduced to the mangrove habitat. By following the incorporation of the ^{13}C and ^{15}N labels in the harpacticoid copepods and nematodes, the results suggest that nematodes utilized resources available within their local sedimentary organic pool and MPB to fulfill their

carbon and nitrogen requirements, respectively. In contrast, harpacticoids utilized a cryptic carbon source, while a mixture of sources within the local sedimentary organic pool fulfilled their nitrogen requirements. In this study, a remarkably large ^{15}N label uptake was recorded for both meiofaunal groups as compared to the ^{13}C label uptake. Nitrogen is often limiting in coastal marine ecosystems, which may explain this pattern. Therefore, the involvement of the meiofauna in nearshore N dynamics demands more attention.

Overall, this study has contributed to the understanding and knowledge of the ecological roles of the meiofauna in sub-tropical mangrove and the adjoining habitats. The role of the meiofauna, as a ubiquitous and abundant component of soft-sediment marine habitats, is complex and requires investigations to be conducted at the relevant spatial and temporal scales and whenever possible, to include finer details on the genus- or species-level identification of the meiofauna to reflect their various feeding guilds. The pulse-chase labelling experiment is an innovative attempt to unravel trophic role of the meiofauna in a mangrove. Such experiments are expensive, and following label uptake by the consumers require much sampling effort in terms of time and resources. Therefore, attention should be given to the sampling practice and extraction techniques to unravel the cryptic sources especially from the bulk sample materials such as MPB and SPOM. Despite their small body size, this study has shown that the meiofauna could be efficiently used to answer ecological questions and also to be included in trophic studies employing stable isotope analysis despite their short-life cycle, high tissue turnover rate, and benthic habit.

APPENDICES

APPENDIX A

Table A.1: Results from the univariate PERMANOVA analysis of each PC scores that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B) based on 2-way crossed design. "Perm" indicates number of unique permutations available. Bold values are significant ($\alpha = 0.05$).

A. Spatial variation

PC1							PC2					
Source	df	SS	MS	Pseudo-F	<i>P</i>	Perm	df	SS	MS	Pseudo-F	<i>P</i>	Perm
Location	2	349.0	174.5	1345.7	0.0001	9950	2	384.0	192.0	177.6	0.0001	9949
Mangrove	2	30.9	15.5	119.2	0.0001	9949	2	104.7	52.3	48.4	0.0001	9950
Location x Mangrove	4	5.8	1.5	11.2	0.0001	9954	4	54.4	13.6	12.6	0.0001	9902
Residual	72	9.3	0.1				72	77.9	1.1			
Total	80	395.0					80	621.0				

B. Temporal variation

PC1							PC2					
Source	df	SS	MS	Pseudo-F	<i>P</i>	Perm	df	SS	MS	Pseudo-F	<i>P</i>	Perm
Mangrove	2	197.7	98.8	209.2	0.0001	9945	2	62.2	31.1	70.9	0.0001	9956
Season	2	2.5	1.2	2.6	0.079	9945	2	3.7	1.8	4.2	0.0218	9954
Mangrove x Season	4	5.8	1.4	3.0	0.0214	9943	4	3.6	0.9	2.0	0.1009	9950
Residual	72	34.0	0.5				72	31.6	0.4			
Total	80	239.9					80	101.0				

Table A.2: Results from the pairwise comparison for the univariate PERMANOVA analysis of each PC scores that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B) based on 2-way crossed design. "Perm" indicates number of unique permutations available. Bold values are significant (alpha = 0.05).

A. Spatial variation

PC1									
Groups	Tallebudgera			Currumbin			Terranora		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	1.288	0.2237	8076	1.157	0.2611	7999	5.157	0.0002	8140
AM, AC	10.703	0.0003	8077	10.412	0.0003	8131	7.957	0.0001	8079
RS, AC	12.977	0.0001	8100	5.753	0.0001	8148	1.892	0.0732	8050

PC2									
Groups	Tallebudgera			Currumbin			Terranora		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	1.616	0.0874	8161	3.726	0.0003	8185	3.903	0.0001	8119
AM, AC	5.652	0.0001	8207	7.690	0.0001	8183	6.244	0.0001	8179
RS, AC	9.001	0.0001	8214	4.193	0.0002	8110	1.623	0.041	8112

B. Temporal variation

PC1									
Groups	<i>A. marina</i>			<i>R. stylosa</i>			<i>A. corniculatum</i>		
	t	P	Perm	t	P	Perm	t	P	Perm
Aut, Win	1.095	0.2867	8120	2.603	0.018	8055	0.369	0.7103	8208
Aut, Sum	1.257	0.2352	8043	0.827	0.4191	8048	2.207	0.0437	8083
Win, Sum	2.299	0.0345	8107	1.892	0.0764	8127	2.216	0.0441	8118

PC2									
Groups	<i>A. marina</i>			<i>R. stylosa</i>			<i>A. corniculatum</i>		
	t	P	Perm	t	P	Perm	t	P	Perm
Aut, Win	0.320	0.7477	8051	2.120	0.0517	8093	3.798	0.0028	8127
Aut, Sum	0.207	0.8439	8054	1.563	0.1336	8104	1.671	0.1158	8129
Win, Sum	0.165	0.8723	8127	0.773	0.4417	8018	3.702	0.0038	8093

PC1									
Groups	Autumn			Winter			Summer		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	1.633	0.1235	8188	4.663	0.0002	8128	1.157	0.2656	8063
AM, AC	9.486	0.0002	8062	10.389	0.0003	7997	13.107	0.0004	8076
RS, AC	9.417	0.0004	8081	9.860	0.0001	8092	14.661	0.0002	8132

PC2									
Groups	Autumn			Winter			Summer		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	4.368	0.0003	8005	6.277	0.0002	8010	7.813	0.0002	8089
AM, AC	0.603	0.5432	8106	2.860	0.0075	8021	1.278	0.2176	8114
RS, AC	5.343	0.0002	8048	6.033	0.0001	8048	9.201	0.0002	8069

APPENDIX B

Table B.1: Results from the univariate PERMANOVA analysis (Table C) and pairwise comparison of the meiofaunal density that tests the factors of location and mangrove species for spatial comparison (A) and mangrove species and season for temporal comparison (B) based on 2-way crossed design. "Perm" indicates number of unique permutations available. Bold values are significant (alpha = 0.05).

Table C

A. Spatial variation						
Source	df	SS	MS	Pseudo-F	P	Perm
Location	2	7957.0	3978.5	14.0	0.0001	9956
Mangrove	2	6513.1	3256.5	11.4	0.0001	9947
Location x Mangrove	4	14991.0	3747.7	13.1	0.0001	9926
Residual	72	20532.0	285.2			
Total	80	49992.0				

B. Temporal variation						
Source	df	SS	MS	Pseudo-F	P	Perm
Mangrove	2	15463.0	7731.7	43.1	0.0001	9951
Season	2	5841.2	2920.6	16.3	0.0001	9957
Mangrove x Season	4	7235.6	1808.9	10.1	0.0001	9931
Residual	72	12930.0	179.6			
Total	80	41470.0				

Table D

A. Spatial variation									
Groups	Tallebudgera			Currumbin			Terranora		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	2.017	0.0342	8149	2.274	0.0008	8120	4.011	0.0001	8187
AM, AC	5.603	0.0001	8228	2.626	0.0016	8211	1.548	0.0814	8193
RS, AC	6.235	0.0002	8137	3.502	0.0002	8161	4.319	0.0001	8170

B. Temporal variation									
Groups	<i>A. marina</i>			<i>R. stylosa</i>			<i>A. corniculatum</i>		
	t	P	Perm	t	P	Perm	t	P	Perm
Aut, Win	4.794	0.0002	8232	2.341	0.0144	8176	5.823	0.0001	8087
Aut, Sum	3.445	0.0001	8164	3.848	0.0002	8163	2.012	0.0034	8151
Win, Sum	2.848	0.0011	8175	1.179	0.2769	8178	4.645	0.0001	8249

Groups	Autumn			Winter			Summer		
	t	P	Perm	t	P	Perm	t	P	Perm
AM, RS	2.017	0.0335	8252	3.633	0.0002	8140	2.155	0.0051	8150
AM, AC	5.603	0.0001	8073	8.906	0.0001	8155	2.336	0.0003	8134
RS, AC	6.235	0.0002	8126	4.936	0.0002	8223	3.734	0.0001	8168

APPENDIX C

Table C.1: Carbon (C) and nitrogen (N) content (mean \pm SE) of harpacticoids and nematodes and the resultant number of individual animals required for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. 5 μg is the minimum amount of nitrogen required for the stable isotope analysis in this study.

Meiofauna	Element content (%)			Individual biomass (µg ind. ⁻¹)	Element content (µg ind. ⁻¹)		Min N, ind. required in this study
	C	N	C:N		C	N	
Kangaroo Island mangrove site (n = 20 and 24 for harpacticoids and nematodes respectively)							
Harpac	32.31 ± 1.35	7.39 ± 0.32	4.38 ± 0.03	1.46 ± 0.07	0.47 ± 0.02	0.11 ± 0.01	47
Nem	45.25 ± 0.88	10.66 ± 0.24	4.26 ± 0.05	0.92 ± 0.04	0.41 ± 0.02	0.10 ± 0.00	51
Tallebudgera mangrove site (n = 3)							
Mangrove							
Harpac	54.35 ± 1.59	11.28 ± 0.29	4.82 ± 0.05	0.77 ± 0.03	0.42 ± 0.01	0.09 ± 0.00	58
Nem	51.47 ± 8.43	9.92 ± 1.51	5.17 ± 0.06	0.39 ± 0.06	0.19 ± 0.01	0.04 ± 0.00	134
Sandflat							
Harpac	34.85 ± 1.56	7.42 ± 0.32	4.70 ± 0.02	1.08 ± 0.16	0.38 ± 0.08	0.08 ± 0.02	61
Nem	55.79 ± 2.27	11.64 ± 0.16	4.79 ± 0.19	0.54 ± 0.09	0.30 ± 0.05	0.06 ± 0.01	79
Saltmarsh							
Harpac	31.71 ± 3.64	7.30 ± 0.90	4.35 ± 0.04	1.51 ± 0.34	0.50 ± 0.11	0.12 ± 0.04	43
Nem	55.59 ± 6.93	10.70 ± 1.34	5.19 ± 0.01	0.38 ± 0.04	0.20 ± 0.01	0.04 ± 0.00	127

APPENDIX D

Journal of Experimental Marine Biology and Ecology 479 (2016) 69–75



Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe



Meiofauna and crabs in mangroves and adjoining sandflats: Is the interaction physical or trophic?



Maizah M. Abdullah^{a,b,*}, S.Y. Lee^a

^a Australian Rivers Institute & School of Environment, Griffith University Gold Coast Campus, Southport, Queensland 4222, Australia

^b School of Marine and Environmental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

ARTICLE INFO

Article history:

Received 2 December 2015

Received in revised form 6 February 2016

Accepted 11 March 2016

Available online xxxx

Keywords:

Meiofauna

Crabs

Top-down control

Bioturbation

Trophic impact

Caging experiments

ABSTRACT

Meiofauna distribute widely in most soft substrates in the marine and freshwater realms. Given their small body size (63 to 500 μm) and high density, meiofauna are potential food items for predators such as deposit-feeding brachyuran crabs. Crab bioturbation may also affect meiofaunal assemblages through effects such as translocation to unsuitable microhabitats. This study aimed to investigate the significance and nature of top-down control on the density of meiofauna based on their interactions with deposit-feeding crabs in a mangrove and adjoining sandflat; specifically, whether the interaction is primarily physical or trophic. Field manipulative experiments were conducted within the aggregation zones of soldier crabs (*Mictyris longicarpus*) and fiddler crabs (*Uca vomeris*) in a mangrove-lined creek in Southeast Queensland, Australia. Meiofaunal density in five experimental cage treatments (Exclusion, Inclusion with complete crab ('Inclusion'), Inclusion with 'disabled' crab (feeding claw removed, 'Disabled'), Half-cage, and Ambient) was compared. Removal of soldier crabs from the cages (Exclusion) increased meiofaunal density ($426 \pm 46 \text{ ind./10 cm}^2$; mean \pm SE) by 50% over that in the Inclusion treatment (283 ± 22). The nature of the interactions was further investigated by comparing meiofaunal density in the Inclusion treatment (with both physical and trophic effects present) with that in the Disabled treatment (with physical but no trophic effect present). Removal of trophic effect by 'disabling' the crab increased meiofaunal density by 30% compared to that in the Inclusion treatment, but at a similar density to the Exclusion treatment. This pattern suggests that the top-down control by soldier crabs on the meiofauna is fundamentally trophic, i.e. predation. In the experiment with fiddler crabs, meiofaunal densities in the inclusion treatments (Inclusion and Disabled) were not significantly different from each other, but density was reduced by more than 50% in the Exclusion treatment. Fiddler crabs significantly impact the meiofauna through their bioturbation activities such as sediment turnover and burrowing, but their trophic activities did not significantly reduce meiofaunal density. Different crab species at different habitats, therefore, may influence meiofaunal density through different processes on sub-tropical soft shores.

© 2016 Elsevier B.V. All rights reserved.

Figure D.1: Screenshot of a published manuscript based on the content from chapter three.

APPENDIX E



Figure E.1: Screenshots of the aerial views of the sampling sites in (A) Tallebudgera, (B) Currumbin and (C) Terranora, showing different degree of canopy cover and mangrove tree abundance. Images retrieved from Google Earth on 10 October 2016.

REFERENCES

- Abdullah, M.M., Lee, S.Y., 2016. Meiofauna and crabs in mangroves and adjoining sandflats: Is the interaction physical or trophic? *Journal of Experimental Marine Biology and Ecology* 479, 69–75.
- Abrantes, Sheaves, 2009. Food web structure in a near-pristine mangrove area of the Australian Wet Tropics. *Estuarine, Coastal and Marine Science* 82, 597–607.
- Ackman, R.G., Tocher, C., McLachlan, J., 1968. Marine phytoplankter fatty acids. *Journal of the Fisheries Board of Canada* 25, 1603-1620.
- Adam, P., 1990. Saltmarsh ecology. Cambridge University Press, Melbourne.
- Akoto, L., Stellaard, F., Irth, H., Vreuls, R.J.J., Pel, R., 2008. Improved fatty acid detection in micro-algae and aquatic meiofauna species using a direct thermal desorption interface combined with comprehensive gas chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A* 1186, 254-261.
- Allen, S.E., 1989. Chemical analysis of ecological materials, 2nd. ed. Blackwell Scientific Publications. Oxford.
- Alongi, D.M., 1987a. Intertidal zonation and seasonality of meiobenthos in tropical mangrove estuaries. *Marine Biology* 95, 447–458.
- Alongi, D.M., 1987b. The influence of mangrove-derived tannins on intertidal meiobenthos in tropical estuaries. *Oecologia* 71, 537–540.
- Alongi, D.M., 2009. The energetics of mangrove forests, Springer Science & Business Media. Springer, Netherlands.
- Anderson, J.M., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: Guide to software and statistical methods. PRIMER- E Ltd. Plymouth.
- Andersen, F.O., Kristensen, E., 1992. The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. *Limnology of Oceanography* 37, 1392-1403.
- Austen, M.C., Widdicombe, S., 2006. Comparison of the response of meio- and macrobenthos to disturbance and organic enrichment. *Journal of Experimental Marine Biology and Ecology* 330, 96–104.
- Bale, A.J., Kenny, A.J., 2005. Sediment Analysis and Seabed Characterisation, in: Eleftheriou, A., McIntyre, A. (Eds.), *Methods for the Study of Marine Benthos*.

Blackwell Science Ltd, Oxford, UK, pp. 43-86.

- Banta, G.T., Holmer, M., Jensen, M.H., Kristensen, E., 1999. Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment. *Aquatic Microbial Ecology* 19, 189-204.
- Bec, A., Perga, M.E., Koussoroplis, A., Bardoux, G., Desvillettes, C., Bourdier, G., Mariotti, A., 2011. Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies. *Methods in Ecology and Evolution* 2, 651-659.
- Bell, S., Coull, B., 1978. Field evidence that shrimp predation regulates meiofauna. *Oecologia* 35, 141-148.
- Bell, S.S., 1980. Meiofauna-Macrofauna Interactions in a High Salt Marsh Habitat. *Ecological Monographs* 50, 487-505.
- Bligh, E., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37, 911-917.
- Bouillon, S., Raman, A.V., Dauby, P., Dehairs, F., 2002. Carbon and Nitrogen Stable Isotope Ratios of Subtidal Benthic Invertebrates in an Estuarine Mangrove Ecosystem (Andhra Pradesh, India). *Estuarine, Coastal and Shelf Science* 54, 901-913.
- Bouillon, S., Moens, T., Dehairs, F., 2004. Carbon sources supporting benthic mineralization in mangrove and adjacent seagrass sediments (Gazi Bay, Kenya). *Biogeosciences Discussions* 1, 311-333.
- Bouillon, S., Connolly, R.M., Lee, S.Y., 2008. Organic matter exchange and cycling in mangrove ecosystems: Recent insights from stable isotope studies. *Journal of Sea Research* 59, 44-58.
- Bouillon, S., Connolly, R.M., Gillikin, D.P., 2011. Use of Stable Isotopes to Understand Food Webs and Ecosystem Functioning in Estuaries. *Treatise on Estuarine and Coastal Science*. Elsevier Inc.
- Brett, M.T., 2014. Resource polygon geometry predicts Bayesian stable isotope mixing model bias. *Marine Ecology Progress Series* 514, 1-12.
- Buchanan, J.B., 1984. Sediment analysis, in: Eleftheriou, A., McIntyre, A. (Eds). *Methods for the study of marine benthos*. Blackwell Science Ltd, Oxford, pp. 41-65.
- Bui, T.H.H., Lee, S.Y., 2014. Does “you are what you eat” apply to mangrove grapsid

crabs? *PLoS One* 9,2: e89074.

- Cameron, A.M., 1966. Some aspects of the behaviour of the soldier crab, *Mictyris longicarpus*. *Pacific Science* 20, 224–234.
- Caramujo, M.J., Van der Grinten, E., Admiraal, W., 2005. Trophic interactions between benthic copepods and algal assemblages: a laboratory study. *Journal of North American Benthological Society* 24, 890–903.
- Carman, K.R., Thistle, D., 1985. Microbial food partitioning by three species of benthic copepods. *Marine Biology* 88, 143-148.
- Carman, K.R., Fry, B., 2002. Small-sample methods for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. *Marine Ecology Progress Series* 240, 85-92.
- Chandler, G.T., Fleeger, J.W., 1984. Tube-building by a marine meiobenthic harpacticoid copepod. *Marine Biology* 82, 15-19.
- Chapman, M.G., Tolhurst, T.J., 2007. Relationships between benthic macrofauna and biogeochemical properties of sediments at different spatial scales and among different habitats in mangrove forests. *Journal of Experimental Marine Biology and Ecology* 343, 96–109.
- Chinnadurai, G., Fernando, O. 2006. Meiobenthos of Cochin mangroves (southwest coast of India) with emphasis on free living marine nematode assemblages. *Russian Journal of Nematology* 14, 127-137.
- Chinnadurai, G., Fernando, O.J., 2007. Meiofauna of mangroves of the southeast coast of India with special reference to the free-living marine nematode assemblage. *Estuarine, Coastal and Shelf Science* 72, 329–336.
- Chong, V.C. and Sasekumar, A., 1981. Food and feeding habits of the white prawn *Penaeus merguensis*. *Marine ecology progress series* 5, 185-191.
- Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User manual/ tutorial, 1st ed. PRIMER-E Ltd. Plymouth.
- Connolly, R.M., Guest, M.A., Melville, A.J. and Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, 138, 161-167.
- Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., 2004. Assessing the influence and distribution of shrimp pond effluent in a tidal mangrove creek in north-east Australia. *Marine Pollution Bulletin* 48, 514-525.

- Couch, C.A., 1989. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Estuarine, Coastal and Shelf Science* 28, 433-441.
- Coull, B.C., 1999. Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology* 24, 327–343.
- Coull, B.C., Greenwood, J.G., Fielder, D.R., Coull, B.A., 1995. Subtropical Australian juvenile fish eat meiofauna: experiments with winter whiting *Sillago maculata* and observations on other species. *Marine Ecology Progress Series* 125, 13–19.
- Coull, B., Palmer, M., 1984. Field experimentation in meiofaunal ecology. *Hydrobiologia* 118, 1-19.
- Coull, B.C., 1990. Are members of the meiofauna food for higher trophic levels? *Transactions of the American Microscopical Society* 109, 233-246.
- Coull, B.C., 1999. Role of meiofauna in estuarine soft-bottom habitats. *Australian Journal of Ecology* 24, 327-343.
- Cullen, D.J., 1973. Bioturbation of superficial marine sediments by interstitial meiobenthos. *Nature* 242, 323-324.
- Dahnke, W.C., Whitney, D.A., 1988. Measurement of soil salinity. *Recommended chemical soil test procedures for the North Central Region* 221, 32-34.
- Danovaro, R., 1996. Detritus-bacteria-meiofauna interactions in a seagrass bed (*Posidonia oceanica*) of the NW Mediterranean. *Marine Biology* 127, 1-13.
- Danovaro, R., Gambi, C., Manini, E., Fabiano, M., 2000. Meiofauna response to a dynamic river plume front. *Marine Biology* 137, 359–370.
- Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., Gooday, A.J., 2008. Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. *Current Biology* 18, 1-8.
- De Troch, M., Steinarsdóttir, M.B., Chepurnov, V., Ólafsson, E., 2005. Grazing on diatoms by harpacticoid copepods: Species-specific density-dependent uptake and microbial gardening. *Aquatic Microbial Ecology* 39, 135–144.
- Demopoulos, A.W., Fry, B., Smith, C.R., 2007. Food web structure in exotic and native mangroves: a Hawaii–Puerto Rico comparison. *Oecologia* 153, 675-686.
- Dernie, K.M., Kaiser, M.J., Richardson, E.A., Warwick, R.M., 2003. Recovery of soft sediment communities and habitats following physical disturbance. *Journal of Experimental Marine Biology and Ecology* 286, 415–434.

- di Virgilio, A., Ribeiro, P.D., 2012. Spatial and temporal patterns in the feeding behaviour of a fiddler crab. *Marine Biology* 160, 1001–1013.
- Dittmann, S., 1996. Effects of macrobenthic burrows on infaunal communities in tropical tidal flats. *Marine Ecology Progress Series* 134, 119-130.
- Dittmann, S., 1998. Behaviour and population structure of soldier crabs *Mictyris longicarpus* (LATREILLE): observation from a tidal flat in tropical North Queensland, Australia. *Senckenbergiana maritime* 28, 177–184.
- Duke, N.C., Ball, M.C., Ellison, J.C., Ballt, M.C., 1998. Factors influencing gradients and distributional biodiversity in mangroves. *Global Ecology and Biogeography Letters* 7, 27–47.
- Dye, A.H., 1983. Vertical and horizontal distribution of meiofauna in mangrove sediments in Transkei, southern Africa. *Estuarine, Coastal and Shelf Science* 16, 591-598.
- Dye, A.H., Lasiak, T.A., 1986. Microbenthos, meiobenthos and fiddler crabs: trophic interactions in a tropical mangrove sediment. *Marine Ecology Progress Series* 32, 259–264.
- Dye, A. 2005. Meiobenthos in intermittently open/closed coastal lakes in New South Wales: spatial and temporal patterns in densities of major taxa. *Marine and Freshwater Research* 56, 1055-1067.
- Dyer, K.R., Christie, M.C., Wright, E.W., 2000. The classification of intertidal mudflats. *Continental Shelf Research* 20, 1039–1060.
- Eleftheriou, A., McIntyre, A., 2005. Methods for the study of marine benthos, 3rd ed. Blackwell Science Ltd. Oxford.
- El-Sabaawi, R., Dower, J. F., Kainz, M., Mazumder, A., 2008. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Marine Biology* 156, 225-237.
- English, S., Wilkinson, C., Baker, V., 1997. Survey manual for tropical marine resources, 2nd ed. Australian Institute of Marine Science, Townsville.
- Ewel, K.C., Twilley, R.R., Ong, J.E., 1998. Different kinds of mangrove forests provide different goods and services. *Global Ecology and Biogeography Letters* 7, 83–94.
- Feller, R.J. 2006. Weak meiofaunal trophic linkages in *Crangon crangon* and *Carcinus maenus*. *Journal of Experimental Marine Biology and Ecology* 330, 274-283.

- Findlay, R.H., King, G.M., Watling, L., 1989. Efficacy of phospholipid analysis in determining microbial biomass in sediments. *Applied and Environmental Microbiology* 55, 2888-2893.
- Findlay, S., Tenore, K.R., 1982. Effect of a Free-Living Marine Nematode (*Diplolaimella chitwoodi*) on detrital carbon mineralization. *Marine Ecology Progress Series* 8, 161-166.
- Folk, R.L., 1980. Petrology of sedimentary rocks, Hemphill Publishing Company. Texas.
- Ford, R.B., Honeywill, C., 2002. Grazing on intertidal microphytobenthos by macrofauna: is pheophorbide a useful marker? *Marine Ecology Progress Series* 229, 33–42.
- Franco, M. A., Soetaert, K., Costa, M.J., Vincx, M., Vanaverbeke, J., 2008. Uptake of phytodetritus by meiobenthos using ¹³C labelled diatoms and Phaeocystis in two contrasting sediments from the North Sea. *Journal of Experimental Marine Biology and Ecology* 362, 1–8.
- Gaever, S., Moodley, L., Pasotti, F., Houtekamer, M., Middelburg, J.J., Danovaro, R., Vanreusel, A., 2009. Trophic specialisation of metazoan meiofauna at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence. *Marine Biology* 156, 1289-1296.
- Galván, K., Fleeger, J., Fry, B., 2008. Stable isotope addition reveals dietary importance of phytoplankton and microphytobenthos to saltmarsh infauna. *Marine Ecology Progress Series* 359, 37–49.
- Galvan, K., Fleeger, J.W., Peterson, B., Drake, D., Deegan, L. a., Johnson, D.S., 2011. Natural abundance stable isotopes and dual isotope tracer additions help to resolve resources supporting a saltmarsh food web. *Journal of Experimental Marine Biology and Ecology* 410, 1–11.
- Gaston, G.R., 1992. Green-winged teal ingest epibenthic meiofauna. *Estuaries* 15, 227-229.
- Gerlach, S.A., 1971. On the importance of meiofauna for benthic communities. *Oecologia* 6, 176–190.
- Giarrizzo, T., Schwamborn, R. and Saint-Paul, U., 2011. Utilization of carbon sources in a northern Brazilian mangrove ecosystem. *Estuarine, Coastal and Shelf Science* 95, 447-457.
- Giere, O., 2009. Meiobenthology, Second. ed. Springer-Verlag Berlin Heidelberg, Berlin.
- Griffen, B.D., Mosblack, H., 2011. Predicting diet and consumption rate differences

- between and within species using gut ecomorphology. *Journal of Animal Ecology* 80:854–863.
- Guest, M.A., Connolly, R.M., Loneragan, N.R., 2004. Within and among-site variability in ^{13}C and ^{15}N for three estuarine producers, *Sporobolus virginicus*, *Zostera capricorni*, and epiphytes of *Z. capricorni*. *Aquatic Botany* 79, 87–94.
- Guest, M.A., Connolly, R.M., Lee, S.Y., Loneragan, N.R., Breitfuss, M.J., 2006. Mechanism for the small-scale movement of carbon among estuarine habitats: Organic matter transfer not crab movement. *Oecologia* 148, 88–96.
- Gutiérrez, J.L., Jones, C.G., Groffman, P.M., Findlay, S.E.G., Iribarne, O.O., Ribeiro, P.D., Bruschetti, C.M., 2006. The contribution of crab burrow excavation to carbon availability in surficial salt-marsh sediments. *Ecosystems* 9, 647–658.
- Gwyther, J., 2003. Nematode assemblages from *Avicennia marina* leaf litter in a temperate mangrove forest in south-eastern Australia, *Marine Biology* 142, 289–297.
- Gwyther, J., Fairweather, P.G., 2005. Meiofaunal recruitment to mimic pneumatophores in a cool-temperate mangrove forest: Spatial context and biofilm effects. *Journal of Experimental Marine Biology and Ecology* 317, 69–85.
- Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. *Oceanography and Marine Biology* 23, 399–489.
- Hicks, G.R., Coull, B.C., 1983. The ecology of marine meiobenthic harpacticoid copepods. *Oceanography and Marine Biology* 21, 67–175.
- Hodda, M., Nicholas, W.L., 1985. Meiofauna Associated with Mangroves in the Hunter River Estuary and Fullerton Cove, South-eastern Australia. *Marine and Freshwater Research* 36, 41–50.
- Hoffman, J.A., Katz, J., 1984. Fiddler crab deposit-feeding and meiofaunal density in salt marsh habitats. *Journal of Experimental Marine Biology and Ecology* 82, 161–174.
- Hughes, J.E., Deegan, L.A., Peterson, B.J., Holmes, R.M., Fry, B., 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. *Ecology* 81, 433–452.
- Incze, L.S., Mayer, L.M., Sherr, E.B., Macko, S.A., 1982. Carbon inputs to bivalve mollusks: A comparison of two estuaries. *Canadian Journal of Fisheries and Aquatic Sciences* 39, 1348–1352.

- Jensen, P., 1987. Feeding ecology of free-living aquatic nematodes. *Marine Ecology Progress Series* 35, 187-196.
- Kamal, S., Lee, S.Y., Warnken, J., 2014. Investigating three-dimensional mesoscale habitat complexity and its ecological implications using low-cost RGB-D sensor technology. *Methods in Ecology and Evolution* 5, 845-853.
- Kennedy, A.D., 1993. Minimal predation upon meiofauna by endobenthic macrofauna in the Exe Estuary, south west England. *Marine Biology* 117, 311-319.
- Kennedy, A.D., 1994. Predation within meiofaunal communities: description and results of a rapid-freezing method of investigation. *Marine Ecology Progress Series* 114, 71-71.
- Kennedy, A.D., Jacoby, C.A., 1999. Biological indicators of marine environmental health: meiofauna - a neglected benthic component? *Environmental Monitoring and Assessment* 54, 47-68.
- Kharlamenko, V., Kiyashko, S., Imbs, A., Vyshkvartzev, D., 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series* 220, 103-117.
- Koch, V., Wolff, M., 2002. Energy budget and ecological role of mangrove epibenthos in the Caeté estuary, North Brazil. *Marine Ecology Progress Series* 228, 119-130.
- Kon, K., Kurokura, H., Tongnunui, P., 2010. Effects of the physical structure of mangrove vegetation on a benthic faunal community. *Journal of Experimental Marine Biology and Ecology* 2, 171-180.
- Krauss, K.W., Lovelock, C.E., McKee, K.L., López-Hoffman, L., Ewe, S.M.L., Sousa, W.P., 2008. Environmental drivers in mangrove establishment and early development: a review. *Aquatic Botany* 89, 105-127.
- Kristensen, E., 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. *Journal of Sea Research* 59, 30-43.
- Krumbein, W.C., Pettijohn, F.J., 1938. Manual of sedimentary petrography. Appleton-Century-Crofts, New York.
- Kryger, L., Lee, S.K., 1996. Effects of mangrove soil ageing on the accumulation of hydrogen sulphide in roots of *Avicennia* spp. *Biogeochemistry* 35, 367-375.
- Kurashov, E., 2002. The role of meiobenthos in lake ecosystems. *Aquatic Ecology* 36, 447-463.

- Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., . . . Yeager, L.A., 2011. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews* 87, 545-562.
- Leduc, D., Probert, P.K., Frew, R.D., Hurd, C.L., 2006. Macroinvertebrate diet in intertidal seagrass and sandflat communities: A study using C, N, and S stable isotopes. *New Zealand Journal of Marine and Freshwater Research* 40, 615–629.
- Leduc, D., & Probert, P. K. (2009). The effect of bacterivorous nematodes on detritus incorporation by macrofaunal detritivores: A study using stable isotope and fatty acid analyses. *Journal of Experimental Marine Biology and Ecology* 371, 130-139.
- Leduc, D., Probert, P.K., Duncan, A., 2009. A multi method approach for identifying meiofaunal trophic connections. *Journal of Experimental Marine Biology and Ecology* 371,130-139.
- Lee, K.M., Lee, S.Y., Connolly, R.M., 2011. Combining stable isotope enrichment, compartmental modelling and ecological network analysis for quantitative measurement of food web dynamics. *Methods in Ecology and Evolution* 2, 56–65.
- Lee, K.M., Lee, S.Y., Connolly, R.M., 2012. Combining process indices from network analysis with structural population measures to indicate response of estuarine trophodynamics to pulse organic enrichment. *Ecological Indicators*. 18, 652-658.
- Lee, S.Y. 1997. The effect of mangrove leaf litter enrichment on macrobenthic colonization of defaunated sandy substrates. *Estuarine, Coastal and Shelf Science* 49, 703-712.
- Lee, S.Y., 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. *Marine and Freshwater Research* 49, 335–343.
- Lee, S.Y., Kwok, P.W., 2002. The importance of mangrove species association to the population biology of the sesarmine crabs *Parasesarma affinis* and *Perisesarma bidens*. *Wetland Ecology and Management* 10, 215–226.
- Lee, S.Y., Dunn, R.J.K., Young, R.A., Connolly, R.M., Dale, P.E.R., Dehayr, R., Lemckert, C.J., McKinnon, S., Powell, B., Teasdale, P.R. and Welsh, D.T., 2006. Impact of urbanization on coastal wetland structure and function. *Austral Ecology* 31, 149-163.
- Lee, S.Y., 2008. Mangrove macrobenthos: assemblages, services, and linkages. *Journal of Sea Research* 59, 16–29.
- Lee, S.Y., 2015. Ecology of Brachyura, in: Castro, P., Davie, P., Guinot, D., Schram, F.,

- Von Vaupel Klein, J. (Eds.), *Treatise in Zoology. The Crustacea*. Brill, Leiden, The Netherlands, pp. 469–543.
- Leh, C.M. and Sasekumar, A., 1980. Feeding ecology of prawns in shallow waters adjoining mangrove shores. In *Asian Symposium on Mangrove Environment: Research and Management, Kuala Lumpur (Malaysia), 25-29 Aug 1980*.
- Levin, L.A., Neira, C., Grosholz, E.D., 2006. Invasive cordgrass modifies wetland trophic function. *Ecology* 87, 419–432
- Litulo, C., 2005. Population biology of the fiddler crab *Uca annulipes* (Brachyura: Ocypodidae) in a tropical East African mangrove (Mozambique). *Estuarine, Coastal and Shelf Science* 62, 283-290.
- Lovelock, C., Ellison, J., 2007. Vulnerability of mangroves and tidal wetlands of the Great Barrier Reef to climate change. *Climate Change and the Great Barrier Reef: A Vulnerability Assessment* 237–269.
- Lu, X.-S., Xu, W.-Z., Cheung, S.G., Shin, P.K.S. 2011. Marine meiobenthic and nematode community structure in Victoria Harbour, Hong Kong upon recovery from sewage pollution. *Marine Pollution Bulletin* 63, 318-325.
- Maddi, P., Carman, K.R., Wissel, B., 2006. Use of primary production by harpacticoid copepods in a Louisiana salt-marsh food web. In: Kromkamp, J.C., de Brouwer, J.F.C., Blanchard, G.F., Forster, R.M., Cr  ch, V. (Eds.), *Functioning of Benthic Microalgae in Estuarine Environments*. Royal Dutch Academy of Arts and Sciences, Amsterdam, pp. 65–81.
- Maitland, D.P., Maitland, A., 1992. Penetration of water into blind-ended capillary tubes and its bearing on the functional design of the lungs of soldier crabs *Mictyris longicarpus*. *Journal of Experimental Biology* 344, 333–344.
- Maher, D.T., Santos, I.R., Golsby-Smith, L., Gleeson, J., Eyre, B.D., 2013. Groundwater-derived dissolved inorganic and organic carbon exports from a mangrove tidal creek: The missing mangrove carbon sink? *Limnology and Oceanography* 58, 475–488.
- McCraith, B.J., Gardner, L.R., Wetthey, D.S., Moore, W.S., 2003. The effect of fiddler crab burrowing on sediment mixing and radionuclide profiles along a topographic gradient in a southeastern salt marsh. *Journal of Marine Research* 61, 359–390.
- McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378-390.

- McIntyre, A., 1969. Ecology of marine meiobenthos. *Biological Reviews* 44, 245-288.
- McKee, K.L., Mendelssohn, I. A., Hester, M.W., 1988. Reexamination of pore water sulfide concentrations and redox potentials near the aerial roots of *Rhizophora mangle* and *Avicennia germinans*. *American Journal of Botany* 75, 1352.
- McPhee, D., 2001. A Comparison of discharge quality and quantity into Queensland east coast catchments (pp. 49). Australian Prawn Farmers Association.
- Mermillod-Blondin, F., 2011. The functional significance of bioturbation and biodeposition on biogeochemical processes at the water–sediment interface in freshwater and marine ecosystems. *Journal of the North American Benthological Society* 30, 770-778.
- Meziane, T., Tsuchiya, M., 2000. Fatty acids as tracers of organic matter in the sediment and food web of a mangrove intertidal flat ecosystem, Okinawa, Japan. *Marine Ecology Progress Series* 200, 49-57.
- Meziane, T., D'Agata, F., Lee, S.Y., 2006. Fate of mangrove organic matter along a subtropical estuary: small-scale exportation and contribution to the food of crab communities. *Marine Ecology Progress Series* 312, 15–27.
- Mfilinge, P.L., Meziane, T., Bachok, Z., Tsuchiya, M., 2005. Total lipid and fatty acid classes in decomposing mangrove leaves of *Bruguiera gymnorhiza* and *Kandelia candel*: Significance with respect to lipid input. *Journal of Oceanography* 61, 613-622.
- Middelburg, J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T., Heip, C.H.R., 2000. The fate of intertidal benthic microalgae carbon: An in situ ¹³C-labeling study. *Limnology and Oceanography* 45, 1224–1234
- Moens, T., Vincx, M., 1996. Do meiofauna consume primary production?: about many questions and how to answer them. In: Baeyens, J., et al. (Eds.), Integrated Marine System Analysis. European Network for Integrated Marine System Analysis. FWO Vlaanderen: Minutes of the First Network Meeting, pp. 188-202.
- Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom* 77, 211-228.
- Moens, T., Luyten, C., Middelburg, J.J., Herman, P.M.J., Vincx, M., 2002. Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. *Marine Ecology Progress Series* 234, 127–137.

- Montagna, P.A., 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. *Marine Ecology Progress Series* 18, 119–130.
- Montagna, P.A., Blanchard, G.F., Dinert, A., 1995. Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. *Journal of Experimental Marine Biology and Ecology* 185, 149–165.
- Nagelkerken, I., Blaber, S.J.M., Bouillon, S., Green, P., Haywood, M., Kirton, L.G., Meynecke, J.-O., Pawlik, J., Penrose, H.M., Sasekumar, A., Somerfield, P.J., 2008. The habitat function of mangroves for terrestrial and marine fauna: a review. *Aquatic Botany* 89, 155–185.
- Nanton, D.A., Castell, J.D., 1998. The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae. *Aquaculture* 163, 251–261.
- Napolitano, G.E., Pollero, R.J., Gayoso, A.M., Macdonald, B.A., Thompson, R.J., 1997. Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology* 25, 739–755.
- Nascimento, F.J.A., Naslund, J., Elmgren, R., 2012. Meiofauna enhances organic matter mineralization in soft sediment ecosystems. *Limnology and Oceanography* 57, 338–346.
- Nicholas, W.L., Elek, J.A., Stewart, A.C., Marples, T.G., 1991. The nematode fauna of a temperate Australian mangrove mudflat; its population density, diversity and distribution. *Hydrobiologia* 209, 13–27.
- Nielsen, O.I., Kristensen, E., Macintosh, D.J., 2003. Impact of fiddler crabs (*Uca* spp.) on rates and pathways of benthic mineralization in deposited mangrove shrimp pond waste. *Journal of Experimental Marine Biology and Ecology* 289, 59–81.
- Nielsen, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. *European Journal of Soil Science* 62, 105–116.
- Netto, S.A., Gallucci, F., 2003. Meiofauna and macrofauna communities in a mangrove from the Island of Santa Catarina, South Brazil. *Hydrobiologia* 505, 159–170.
- Nobbs, M., 1999. Developing methods for quantifying the apparent density of fiddler crabs (Ocypodidae : *Uca*) in mangrove habitats. *Australian Journal of Ecology* 24, 43–49.

- Nobbs, M., 2003. Effects of vegetation differ among three species of fiddler crabs (*Uca* spp.). *Journal of Experimental Marine Biology and Ecology* 284, 41-50.
- Ólafsson, E., 1995. Meiobenthos in mangrove areas in eastern Africa with emphasis on assemblage structure of free-living marine nematodes. *Hydrobiologia* 312, 47–57.
- Ólafsson, E., Ndaro, S.G.M., 1997. Impact of the mangrove crabs *Uca annulipes* and *Dotilla fenestrata* on meiobenthos. *Marine Ecology Progress Series* 158, 225–231.
- Ólafsson, E., Carlström, S., Ndaro, S.G.M., 2000. Meiobenthos of hypersaline tropical mangrove sediment in relation to spring tide inundation. *Hydrobiologia* 426, 57-64.
- Parrish, C., Abrajano, T., Budge, S., Helleur, R., Hudson, E., Pulchan, K., Ramos, C., 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In *Marine Chemistry*, pp. 193-223: Springer.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984: A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, 173 pp.
- Peterson, B.J., 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. *Acta Oecologica* 20, 479–487.
- Phillips D.L. and Gregg, J.W., 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127, 171–179.
- Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X. and Ward, E.J., 2014. Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92, 823-835.
- Pinckney, J.L., Carman, K.R., Lumsden, S.E. and Hymel, S.N., 2003. Microalgal-meiofaunal trophic relationships in muddy intertidal estuarine sediments. *Aquatic Microbial Ecology* 31, 99-108.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703-718.
- Quinn, R.H., 1980. Mechanisms for obtaining water for floatation feeding in the soldier crab, *Mictyris longicarpus* Latreille, 1806 (Decapoda, Mictyridae). *Journal of Experimental Marine Biology and Ecology* 43, 49–60.
- Quinn, R.H., 1986. Experimental studies of food ingestion and assimilation of the soldier crab, *Mictyris longicarpus* Latreille (Decapoda, Mictyridae). *Journal of Experimental Marine Biology and Ecology* 102, 167–181.

- Raffaelli, D., 1987. The behaviour of the nematode/copepod ratio in organic pollution studies. *Marine Environmental Research* 23, 135-152.
- Raffaelli, D., Mason, C., 1981. Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Marine Pollution Bulletin* 12, 158-163.
- Reinsel, K.A., 2004. Impact of fiddler crab foraging and tidal inundation on an intertidal sandflat: season-dependent effects in one tidal cycle. *Journal of Experimental Marine Biology and Ecology* 313, 1–17.
- Reise, K., 1979. Moderate predation on meiofauna by the macrobenthos of the Wadden Sea. *Helgolaender Wissenschaftliche Meeresuntersuchungen*, 32, 453-465.
- Riekenberg, P., Carney, R., Fry, B., 2016. Trophic plasticity of the methanotrophic mussel *Bathymodiolus childressi* in the Gulf of Mexico. *Marine Ecology Progress Series* 547, 91–106.
- Rieper, M., 1978. Bacteria as food for marine harpacticoid copepods. *Marine Biology* 45, 337-345.
- Rieper, M., 1982. Feeding preferences of marine harpacticoid. *Marine Ecology Progress Series* 7, 303-307.
- Robertson and Newell, 1982. A study of particle ingestion by three fiddler crab species foraging on sandy sediments. *Journal of Experimental Marine Biology and Ecology* 65, 11–17.
- Robertson, A.I., Phillips, M.J., 1995. Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. *Hydrobiologia* 295, 311-321.
- Rosado-Solórzano, R., Guzmán del Prío, S.A., 1998. Preliminary trophic structure model for Tampamachoco lagoon, Veracruz, Mexico. *Ecological Modelling* 109, 141-154.
- Rossi, F., Chapman, M.G., 2003. Influence of sediment on burrowing by the soldier crab *Mictyris longicarpus* Latreille. *Journal of Experimental Marine Biology and Ecology* 289, 181–195.
- Sahoo, G., Suchiang, S.R., Ansari, Z.A., 2013. Meiofauna-mangrove interaction: A pilot study from a tropical mangrove habitat. *Cahiers de Biologie Marine* 54, 349–358.
- Sasekumar, A., 1994. Meiofauna of a mangrove shore on the west coast of peninsular Malaysia. *Raffles Bulletin of Zoology* 42, 901–915.

- Scharler, U.M., 2011. Whole food-web studies: mangroves. In W. Editors-in-Chief: Eric & M. Donald (Eds.), *Treatise on Estuarine and Coastal Science* pp. 271-286. Waltham: Academic Press.
- Schratzberger, M., Warwick, R.M., 1998. Effects of the intensity and frequency of organic enrichment on two estuarine nematode communities. *Marine Ecology Progress Series* 164, 83-94.
- Sheaves, M., 2005. Nature and consequences of biological connectivity in mangrove systems. *Marine Ecology Progress Series* 302, 293–305.
- Shine, R., Ellway, C.P., Hegerl, E.J., 1973. A Biological Survey of the Tallebudgera Creek Estuary. *Operculum* 3, 59–83.
- Sikora J. P., Sikora W. B., Erkenbrecher C. W. & Coull B. C., 1977. The significance of ATP, carbon and caloric content of meiobenthic nematodes in partitioning benthic biomass. *Marine Biology* 44, 7–14.
- Skowronski, R.S.P., Corbisier, T.N., 2002. Meiofauna distribution in Martel Inlet, King George Island (Antarctica): sediment features versus food availability. *Polar Biology* 25, 126–134.
- Somerfield, P.J., Gee, J.M., Aryuthaka, C., 1998. Meiofaunal communities in a Malaysian mangrove. *Journal of the Marine Biological Association of the United Kingdom* 78, 717–732.
- Soto, D.X., Wassenaar, L.I. and Hobson, K.A., 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Functional Ecology* 27, 535-543.
- Spilmont, N., Meziane, T., Seuront, L., Welsh, D.T., 2009. Identification of the food sources of sympatric ghost shrimp (*Trypaea australiensis*) and soldier crab (*Mictyris longicarpus*) populations using a lipid biomarker, dual stable isotope approach. *Austral Ecology* 34, 878–888.
- Sutherland, T., Shepherd, P., Elner, R., 2000. Predation on meiofaunal and macrofaunal invertebrates by western sandpipers (*Calidris mauri*): evidence for dual foraging modes. *Marine Biology* 137, 983-993.
- Tolhurst, T.J., Defew, E.C., Dye, A., 2010. Lack of correlation between surface macrofauna, meiofauna, erosion threshold and biogeochemical properties of sediments within an intertidal mudflat and mangrove forest. *Hydrobiologia* 652, 1–13.

- Torres-Pratts, H., Schizas, N.V., 2007. Meiofaunal colonization of decaying leaves of the red mangrove *Rhizophora mangle*, in southwestern Puerto Rico. *Caribbean Journal of Science* 43, 127.
- Unno, J., 2008. The Western Australian soldier crab, *Mictyris occidentalis* Unno 2008 (Brachyura : Decapoda : Mictyridae): the importance of behaviour in design of sampling methods. *Journal of the Royal Society of Western Australia* 91, 243–263.
- Urban-Malinga, B., Moens, T., 2006. Fate of organic matter in Arctic intertidal sediments: Is utilisation by meiofauna important? *Journal of Sea Research* 56, 239–248.
- Vafeiadou, A.-M., Materatski, P., Adão, H., De Troch, M., Moens, T., 2014. Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii* beds. *Biogeosciences* 11, 4001–4014.
- Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* 46, 2061–2066.
- Vander Zanden, H.B., Soto, D.X., Bowen, G.J. and Hobson, K.A., 2016. Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. *Frontiers in Ecology and Evolution* 4, 20.
- Vanhove, S., Vincx, M., Gansbeke, D. Van, Gijssels, W., Schram, D., 1992. The meiobenthos of five mangrove vegetation types in Gazi Bay, Kenya. *Hydrobiologia* 247, 99–108.
- Vermeiren, P., Sheaves, M., 2014. A remote photographic technique for high replication, large scale understanding of spatial distribution patterns of intertidal crabs. *Hydrobiologia* 724, 79–89.
- Volkman, J., Jeffrey, S., Nichols, P., Rogers, G., Garland, C., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology* 128, 219–240.
- Wang, J., Zhou, H., Zhang, Z., Cong, B., Xu, S., 2011. Effects of organic enrichment on sandy beach meiofauna: A laboratory microcosm experiment. *Journal of Ocean University of China* 10, 246–254.
- Warwick, R.M., 1990. The effect of disturbance by soldier crabs *Mictyris platycheles* H. Milne Edwards on meiobenthic community structure. *Journal of Experimental Marine Biology and Ecology* 135, 19–33.
- Wieser, W. and Kanwisher, J., 1961. Ecological and physiological studies on marine

- nematodes from a small salt marsh near Woods Hole, Massachusetts. *Limnology and Oceanography* 6, 262-270.
- Werry, J., Lee, S.Y., 2005. Grapsid crabs mediate link between mangrove litter production and estuarine planktonic food chains. *Marine Ecology Progress Series* 293, 165–176.
- Wilson, J.G., Luczkovich, J.J., 2011. Introduction to food webs in coastal and estuarine ecosystems. In W. Editors-in-Chief: Eric & M. Donald (Eds.), *Treatise on Estuarine and Coastal Science* pp. 1-4. Waltham: Academic Press.
- Wolanski, E., Spagnol, S., Thomas, S., Moore, K., Alongi, D.M., Trott, L., Davidson, A., 2000. Modelling and visualizing the fate of shrimp pond effluent in a mangrove-fringed tidal creek. *Estuarine, Coastal and Shelf Science* 50, 85-97.
- Wołowicz, M., Sokołowski, A., Urban-Malinga, B., Szymelfenig, M., 2011. Meiofauna as Consumers in Coastal Food Webs, in: Wolansky, E. and McLuscy, D.S. (Eds.), *Treatise on Estuarine and Coastal Science*. Waltham: Academic Press, Elsevier, pp. 173–202.
- Wyckmans, M., Chepurnov, V.A., Vanreusel, A., De Troch, M., 2007. Effects of food diversity on diatom selection by harpacticoid copepods. *Journal of Experimental Marine Biology and Ecology* 345, 119-128.
- Xu, W.Z., Cheung, S.G., Shin, P.K.S. 2014. Structure and taxonomic composition of free-living nematode and macrofaunal assemblages in a eutrophic subtropical harbour, Hong Kong. *Marine Pollution Bulletin* 85, 764-773.
- Yokoyama, H., Tamaki, A., Koyama, K., Ishihi, Y., Shimoda, K., Harada, K., 2005. Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. *Marine Ecology Progress Series* 304, 101–116.
- Zeil, J., 1998. Homing in fiddler crabs (*Uca lactea annulipes* and *Uca vomeris*: Ocypodidae). *Journal of Comparative Physiology A - Sensory, Neural, and Behavioural Physiology* 183, 367–377.
- Zhou, H., 2001. Effects of leaf litter addition on meiofaunal colonization of azoic sediments in a subtropical mangrove in Hong Kong. *Journal of Experimental Marine Biology and Ecology* 256, 99–121.
- Zhou, X., Cai, L., Fu, S., 2015. Comparison of meiofaunal abundance in two mangrove wetlands in Tong'an Bay, Xiamen, China. *Journal of Ocean University, China* 14,

816–822.