Contribution of seagrass (*Zostera muelleri*) to estuarine food webs revealed by carbon and nitrogen stable isotope analysis

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Abstract

Seagrass is a conspicuous marine angiosperm that forms extensive beds in coastal and intertidal estuarine areas. In many regions worldwide seagrass is in decline and there is a pressing need to understand the function and importance of seagrasses to coastal ecosystems. To assess the importance of seagrass (*Zostera muelleri*) to secondary production, a dual carbon and nitrogen stable isotope study was conducted using potential food sources (seagrass live blades and detritus, phytoplankton and microphytobenthos) and selected macro-invertebrate consumers at four study sites in three estuaries (Raglan, Tauranga and Whangapoua Harbours). Sampling focused on bivalves, gastropods, crustaceans and annelid polychaetes, representing a range of functional feedings groups (suspension- and deposit-feeders) that were found in seagrass beds and adjacent non-vegetated sediment. In addition, macro-invertebrate community analysis determined the abundances of species selected for isotope analysis and their relative importance to the macro-invertebrate communities.

Macro-invertebrate species analysed for stable isotope analysis accounted for between 26 - 63% and 37 - 95% of the macro-invertebrate abundance within seagrass and non-vegetated sediment, respectively. The distinctive $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of *Z. muelleri* ($\delta^{13}C$ = -10 and $\delta^{15}N$ = 6‰), phytoplankton (-23 and 7‰) and microphytobenthos (-20 and 5‰) allowed these potential food sources to be traced through the food webs studied. The herbivorous gastropods *Diloma subrostrata* and *Zeacumantus lutulentus* had similar $\delta^{13}C$ values to seagrass (mean

$\delta^{13}C$ = -10‰) and the $\delta^{13}C$ values of the deposit feeding bivalve *Macomona liliana*, crustacean *Helice crassa* and annelid polychaete species *Orbinia papillosa* and *Aquilaspio aucklandica* were generally intermediate between microphytobenthos and *Z. muelleri*. *Austrovenus stutchburyi* (suspension feeding bivalve) was depleted in ^{13}C and had a mean $\delta^{13}C$ value similar to microphytobenthos. Polychaetes *Aglaophamus macroura*, *Aonides oxycephala* and *Glycera americana* consistently recorded $\delta^{13}C$ values similar to the other consumers (excluding *A. stutchburyi*) but had $\delta^{15}N$ values characteristic of a higher trophic level and a predatory existence. Differences in the isotopic ratios of macro-invertebrate species between sampling locations within seagrass beds and adjacent non-vegetated sediment were not significant, indicating that seagrass contributes to the food chain in nearby (< 20 m) non-vegetated sediment. Significant differences in isotopic signatures existed between east and west coast sites, with food sources and benthic consumers consistently displaying enriched $\delta^{15}N$ and depleted $\delta^{13}C$ values at Raglan on the west coast suggesting differences in the inorganic nutrient inputs fuelling primary production.

Results suggest that secondary production was fuelled by benthic (seagrass and microphytobenthos) rather than pelagic organic matter (phytoplankton). *Z. muelleri* was an exclusive food source for gastropods *D. subrostrata* and *Z. lutulentus* and also contributed to the diets of *M. liliana*, *H. crassa*, *O. papillosa* and *A. aucklandica*. Spatial differences occurred between study sites but not between sampling locations. This study confirms that *Z. muelleri* plays a role in the production of benthic macro-invertebrate communities and its loss from estuarine ecosystems will impact negatively on secondary production.

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1 Introduction

1.1 Seagrass: Ecological Role and Population Dynamics in New Zealand

Seagrass is a conspicuous and ecologically important marine angiosperm that forms extensive beds intertidally in estuaries and coastal areas. However, many seagrass species are in decline worldwide (Inglis 2003). Seagrasses are an important pool of organic matter, contributing to the food web either through direct consumption by consumers of fresh blades (Vizzini et al. 2002) or detritus (Hyndes and Lavery 2005, Kharlamenko et al. 2001, Kurata et al. 2001). For example, Jones et al. (2003) reported that seagrasses are important primary producers, contributing about 30% of total ecosystem net primary production estuarine systems. The productivity of seagrass is enhanced by the presence of epiphytes that are known to be readily consumed by micro- and macro-invertebrates (Kitting et al 1984, Loneragan et al. 1997, Nichols et al. 1985, Smit et al. 2006). In addition, low current velocities associated with seagrass beds cause the sedimentation of fine-grained particles from the water column. Productivity of seagrass is further enhanced by the settled particles, which are utilised by benthic consumers. Furthermore, the dense above-ground biomass inhibits resuspension, retaining the organic matter within the system (Inglis 2003, Howard 1982, Heiss et al. 2000).

As well as being a potential food source for the local food web, seagrass beds increase habitat complexity and alter benthic community structure and function (Smit et al. 2006). Positive correlations exist between the abundance and diversity of macro-invertebrates within seagrass beds compared to adjacent non-vegetated sediment (e.g. Hackney and Durako 2004, van Houte-Howes et al. 2004, Vizzini et al. 2002). Seagrass provides a three-dimensional habitat, whereby micro- and macro-fauna are able live within the sediment and root-rhizome system, on the blades, or between the sediment surface and blades (Heck and Valentine 2006, Inglis 2003). This three-dimensional habitat increases macro-invertebrate abundance and diversity and provides a nursery for commercially important fish and shellfish species (Heck and Valentine 2006, Klumpp and Nichols 1983). The decline of seagrasses worldwide has stimulated global interest in understanding seagrass ecosystems and in particular their role in supporting marine food webs (Heck and Valentine 2006, Nichols et al. 1985).

As of 1996, approximately 90,000 ha of seagrass loss had been documented (Short and Wyllie-Echeverria 1996). The decline of seagrass has been directly related to increasing population densities in coastal areas, intensified coastal development, agricultural practices and commercial sediment dredging. Increased loads of suspended particulate matter in the water column can smother the seagrass and excess nutrients can stimulate algal growth (Hadwen and Bunn 2005, Inglis 2003, Short and Wyllie-Echeverria 1996). Consequently, both lead to reduced light attenuation, inhibiting the photosynthetic capacity of the seagrass. Organisms living within and exploiting seagrass as a food source are directly affected by catchment practices and loss of seagrass. Therefore, it is important to

understand the role of seagrass in estuarine ecosystems and specifically to the associated organisms, in order to better conserve this resource.

In New Zealand, seagrass is abundant from Parengarenga Harbour (34°31′S, 172°58′E) in the north to Stewart Island (47°5′S, 167°55′E) in the south. Until recently, two species were recognised, *Zostera capricorni* Aschers (1876) and *Zostera novazealandica* Setchell (1933) (Inglis 2003). However, Jones (2004) recently found that *Zostera muelleri* Irmisch ex Aschers is the only species of seagrass in New Zealand. Information of the demography and ecology of *Zostera* within New Zealand is limited, but a large decline in New Zealand has also been recorded (Inglis 2003). For example, in Tauranga Harbour, North Island, 69% of the seagrass biomass was lost between 1959 and 1996 (Park 1999a, b). Macro-invertebrate abundance and diversity within seagrass beds is greater than in non-vegetated sediments, consistent with international findings (Inglis 2003). However, van Houte-Howles et al. (2004) found that the presence of seagrass had an influence on macro-invertebrate community composition, which varied spatially within and between estuarine study sites (Alfaro 2006). Characterisation of the abundance and diversity of benthic species within seagrass beds and knowledge of the contribution seagrass makes to the associated food web will facilitate management strategies within New Zealand and internationally (Brito et al. 2005).

1.2 Stable Isotopes and Applications in Ecological Studies

Natural abundance of stable isotopes of carbon (C) and nitrogen (N), serve as potentially useful markers of ecosystem processes (Peterson and Fry 1987, McClelland et al., 1997). Isotopic fractionation results in preferential respiration of the lighter isotope (^{12}C and ^{14}N) over the heavier isotope (^{13}C and ^{15}N), leaving a higher concentration of the heavier isotope within the cells of consumer tissues compared to the food source (Fry and Sherr 1984, Lajtha and Michener 1994). Diet controls the overall isotopic compositions of animals but considerable isotopic variation still exists due to fractionation (Vander Zanden and Rasmussen 2001). However, by making assumptions about the extent of trophic fractionation, food sources can be inferred (Peterson and Fry, 1987). $\delta^{13}C$ and $\delta^{15}N$ isotope values of potential food sources (i.e. primary producers) are often distinct, making N and C sources identifiable and traceable within an ecosystem (DeNiro and Epstein 1981).

Stable isotopes are widely used in ecological research to elucidate both aquatic and terrestrial food webs. In the past, food web studies have relied on gut analysis, but this method provides only a 'snap-shot' of consumer diet (Hyslop 1980, Kharlamenko et al. 2001). Stable isotopes integrate assimilated diet over a long time period (Logan et al. 2006), and microscopic food sources, unidentifiable by eye, may be identified by their $\delta^{13}C$ signature (Fry et al. 1983).

Stable isotopes are commonly used to delineate C and N flow through the assimilated diets of animals (Adin and Riera 2003, Hobson *et al.* 1995, Kurata et

al. 2001, Sauriau and Kang 2000, Vizzini et al. 2002). Aquatic plants often possess carbon isotope ratios that are distinct from each other because of differences in C uptake mechanisms. Because the trophic fractionation between animals and their food source is relatively consistent, it is possible to assess the relative dependence of animals on these isotopically distinct food sources (Tieszen et al. 1983).

Estuarine food web systems are extremely complex, mainly because the number of potential food sources available to macro-invertebrate consumers is numerous and vary both spatially and temporally (Peterson 1999). Therefore, it is beneficial to measure at least two stable isotopes (i.e. carbon and nitrogen), which provide additional information to help resolve the complexities of food webs (Fry and Sherr 1984, Macko et al. 1982, Peterson 1999). Animal tissues generally have more ^{15}N and ^{13}C than their food source and this enrichment increases by 3-5‰ for nitrogen and approximately 1-2‰ for carbon for each trophic transfer (Cabana and Rasmussen 1996, Carmichael et al. 2004, Fry and Sherr 1984, Kurata et al. 2001, McCutchan et al. 2003, Page and Lastra 2003, Peterson and Fry 1987, Vanderklift and Ponsard 2003, Vizzini et al. 2002).

The decline of seagrass has increased the desire for knowledge regarding their role and importance in coastal ecosystems. $\delta^{13}C$ values of seagrass range between -3‰ and -19‰ and are generally enriched in ^{13}C compared to other food sources, for example phytoplankton and microphytobenthos (Nichols et al. 1985) (Appendices: Figures 5 and 6). This distinction between seagrass and other potential food sources allows the flow of elements to be traced. Past studies have

supported the role of seagrass as a source of organic matter for consumers, while others also dismiss its importance as a food source. However, it is commonly agreed that fresh seagrass makes a minor contribution to estuarine consumers due to its refractory nature and high ligninocellulose content (Dauby 1995, Fry 1983, McConnaughey and McRoy 1979, Peterson et al. 1985). On the other hand, seagrass carbon and nitrogen may penetrate the food web via a detrital pathway through bacterial and fungal intermediates (Smit et al. 2006, Vizzini et al. 2002).

1.3 Study Species

Species representing a wide variety of functional feeding groups were selected from within seagrass and adjacent non-vegetated sediments at four study sites. The analysis of $\delta^{13}C$ ratios of the organisms sampled provided an indication of the food sources contributing to the diet and $\delta^{15}N$ values were used to investigate trophic levels within the food web.

1.3.1 Suspension feeders

The endemic suspension-feeding Venerid bivalve, *Austrovenus stutchburyi*, is common intertidally throughout New Zealand (Morton and Miller 1973, Sherwood and Nelson, 1979) and lives just beneath the surface of the sediment. Short siphons are extended into the overlying water column and organic particles within the water column are utilised (Crowe 1999, Gill 1998, Lundquist et al. 2004, Morton and Miller 1973), including phytoplankton (Hackney and Haines 1980) and resuspended microphytobenthos (de Jonge and van Beusekom 1995,

Delgado et al 1991, Lucas et al. 2000, MacIntyre et al. 1996). Marsden (2004) reported that the food sources utilised by *A. stutchburyi* is uncertain and may vary temporally because estuarine particulate matter has different origins and therefore the diet is variable with natural and anthropogenic nutrient inputs (Kasai et al. 2004).

1.3.2 Surface deposit feeders

The Tellinid deposit feeding bivalve, *Macomona liliana* lives 2 to 25 cm below the sediment surface and feeds on sediment organic matter via two long siphons (Crowe 1999, Gill 1998, Lundquist et al. 2004, Roper et al 1992). *M. liliana* are commonly found with *A. stutchburyi*, occupying a niche deeper within the sediment however, *M. liliana* is absent when densities of *A. stutchburyi* are high (Morton and Miller 1973).

The herbivorous grazer and deposit feeding Trochid gastropod *Diloma subrostrata* is also common on New Zealand's intertidal regions. *D. subrostrata* is known to consume seaweeds, sponges and micro- and macro-algae (Morton and Miller 1973). Close examination of the constituents of its gut showed sand grains were present and that subsequently microphytobenthos may be an important source of food (Crowe 1999, Logan 1976, Miller and Poulin 2001). The herbivorous deposit feeding Batillariid gastropod *Zeacumantus lutulentus* are also extremely abundant on mud flats (Gill 1998) and consume large amounts of surface sediment, surface algae and organic matter (Crowe 1999, Morton and Miller 1973). Herbivorous grazers such as *D. subrostrata* and *Z. lutulentus* are unselective feeders and provide an important link between primary producers and

higher trophic levels (Jernakoff and Nielsen 1997, Kurata et al. 2001, Thom et al, 1995).

The Grapsid mud crab *Helice crassa* is exceedingly common in harbours around New Zealand (Gibbs et al. 2001, McLay 1988). Living in burrows, it is believed that it is an opportunistic omnivore, emerging to feed on any live or dead particulate organic matter associated with the sediment (Gill 1998, McLay 1988). Food sources known to be utilised by *H. crassa* include *Ulva lactuca.* and the bodies of annelids, ascidians, and when reared in the laboratory, *A. stutchburyi*. Juvenile crabs are often found within seagrass due to the protection it provides and the abundance of crabs is also positively correlated to high densities of bivalves (Seitz et al. 2005).

Macro-invertebrate communities are typically dominated by polychaetes and in particular Spionids, which play a major role in the functioning of benthic communities by reworking and recycling the sediment (Hutchings 1998, Probert 2001). *Aonides oxycephala* and *Aquilaspio aucklandica* belong to the Family Spionidae, and are opportunistic surface deposit feeders but may switch to suspension feeding allowing them to exploit a variety of food sources (Dauer et al. 1981, NIWA unpublished, Probert 2001). *Orbinia papillosa* belongs to the family Orbiniidae and is a deposit feeder living throughout sandy sediment (Bleidorn 2005, NIWA, unpublished). *Aglaophamus macroura* and *Glycera americana* are large predatory polychaetes belonging to the families Nephtyidae and Glyceridae, respectively (NIWA unpublished). While burrowing though the sand, *G. americana* captures food using four strong teeth at the base of an extendable

proboscis (Morton and Miller 1973). Polychaetes are represented at all levels of the food chain and are known assimilate a wide variety of food sources including algae and organic matter in the sediments and like *A. macroura* and *G. americana*, other polychaetes and macro-invertebrates (Hutchings 1998).

1.4 Study Objectives

In the present study, four potential food sources (*Z. muelleri* live blades and detritus, phytoplankton and microphytobenthos) of benthic macro-invertebrates were identified and sampled for carbon and nitrogen isotope analysis. Four study sites were selected from Tauranga, Whangapoua and Raglan estuaries at sampling locations within seagrass and on adjacent non-vegetated sediment. The main objective of the study was to determine to trophic significance of *Z. muelleri* live blades and detritus to macro-invertebrate consumers. Specifically to, (1) determine spatial differences in the stable isotope signatures of potential food sources and macro-invertebrates within (two sites in Tauranga Harbour) and among estuaries and (2) identify any trophic relationships, (3) determine the macro-invertebrate community composition at each study site and location and (4) establish the abundances of species selected for isotope analysis and their relative importance to the macro-invertebrate communities. Determining the importance of seagrass as a food source for benthic production is crucial because the continuing effects of coastal development impacting on seagrasses and accelerating their decline have a direct impact on estuarine organisms.

2 Methods

2.1 Study Sites

Four sites located in three North Island estuaries (Tauranga, Whangapoua, Raglan; Figure 2.1) were selected to assess differences in the isotopic signatures of benthic macro-invertebrates inside and outside *Zostera muelleri* beds. Tauranga Harbour, situated on the east of the North Island is impounded by a large sandy low-lying barrier island (Matakana Island). The harbour has two tidal inlets, and the water flow at high tide converges in the middle of the harbour essentially forming two distinct basins (Davis-Colley 1976, Roper 1990). Two sampling sites were selected within this harbour to quantify intra-estuary variations in isotopic signatures of potential food sources and macro-invertebrate consumers; Tauranga Upper (TU) and Tauranga Lower (TL) (Table 2.1 and Figure 2.1). TU was located at Tuapiro Point in the northern basin of Tauranga Harbour where the surrounding catchment is primarily rural. In contrast TL in the southern basin of the Harbour has approximately 90,000 people residing within the catchment. Whangapoua (WP) and Raglan (RG) estuaries, situated on the east and west of the North Island, respectively, have primarily rural catchments with some forestry and minimal urbanised areas.

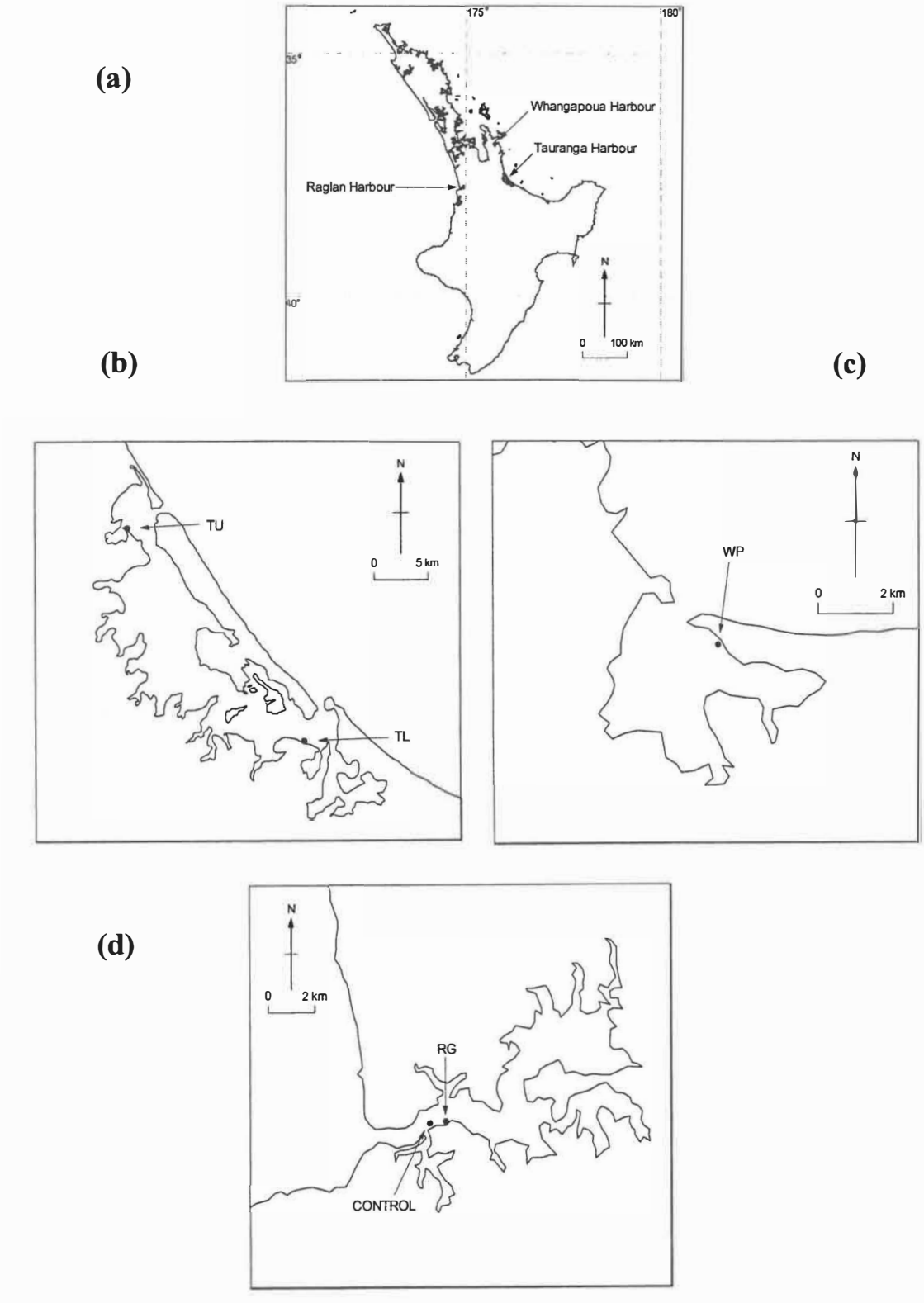

Figure 2.1: (a) Location of study sites and sampling locations (●) in (b), Tauranga (c), Whangapoua (d) and Raglan Estuaries

Table 2.1: Total area (km^2), % intertidal and seagrass cover of each estuary; the area of the seagrass beds (m^2) at each site and sampling location coordinates (Heath 1976, Park 1999a, b, Sherwood and Nelson 1979, van Houte-Howes et al. 2004). – indicates data was unavailable.

Site	Abbrev.	Total area (km^2)	Intertidal (%)	Seagrass cover (%)	Seagrass sampled (m^2)	Sampling location	Latitude (S)	Longitude (E)
Whangapoua	WP	13.1	83	23	36,000	IN	36°44.099′	175°38.429′
						OUT	36°44.090′	175°38.512′
Tauranga Upper	TU	85	39	52	12,782	IN	37°28.960′	175°57.214′
						OUT	37°29.045′	175°57.214′
Tauranga Lower	TL	116	54	48	25,530	IN	37°40.030′	176°09.642′
						OUT	37°40.021′	176°09.552′
Raglan	RG	33	24	-	10,738	IN	39°47.688′	174°52.684′
						OUT	37°47.688′	174°52.601′
	Control	33	24	N/A	N/A	Site A (0 m)	37°47.733′	174°52.370′
						Site B (50 m)	37°47.737′	174°52.320′

2.2 Sampling Design

At each study site a large continuous seagrass (*Zostera muelleri*) bed (>10,000 m^2) with a similar sized area of adjacent non-vegetated sediment was selected. Sampling locations inside the seagrass and adjacent non-vegetated sediment were selected at equal distances relative to the edge of the seagrass bed (Figure 2.2). Distances of sampling locations from the edge of the seagrass at each site were 43 m at TU, 45 m at TL, 42 m at WP and 42 m at RG.

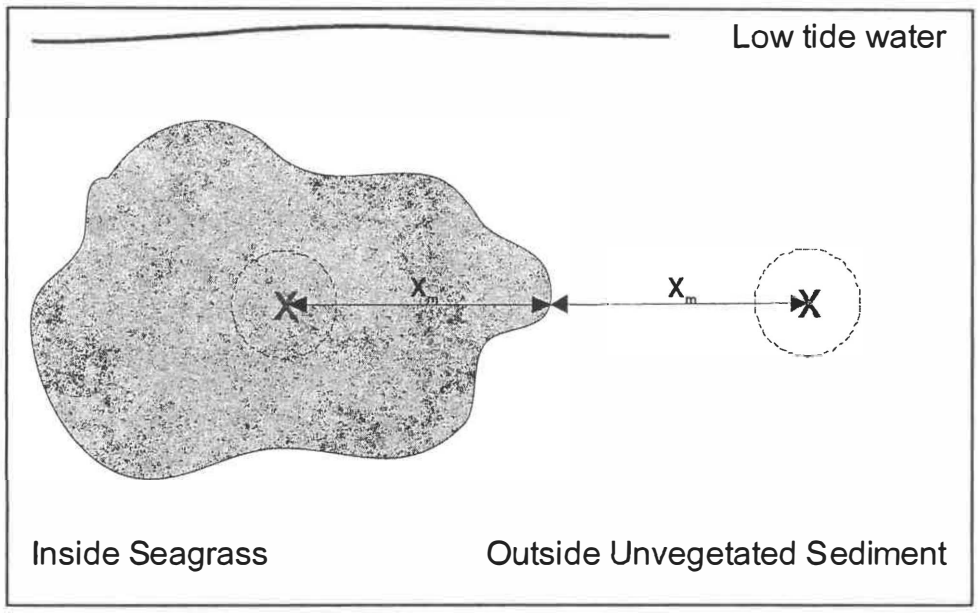

Figure 2.2: Schematic of the sampling locations at each site. The outside and inside sampling locations are the same distance from the edge of the seagrass bed (X m).

It was essential that the potential food sources chosen for stable isotope analysis had distinct stable isotope signatures so that the contribution of each food source to consumers may be easily traceable (Behringer and Butler 2006). Based on unpublished preliminary data collected from TU in 1999, *Z. muelleri* live blades and detritus, phytoplankton and microphytobenthos were selected as potential

food sources. Benthic macro-invertebrate consumer species, including suspension- and deposit- feeding bivalves, deposit-feeding gastropods, annelids and an omnivorous crustacean were selected for isotopic analysis based on their presence inside and outside the seagrass beds at all study sites, and to cover a broad range of functional feeding groups (Table 2.2). At all sites bivalves, gastropods and crustacean species were easily obtained. However, five species of annelids were collected to account for their inconsistent abundances at each site and location.

Table 2.2: Food sources and macro-invertebrate consumers sampled for stable isotope analysis, the type of tissue used for analysis and the amount thereof per replicate.

Species	Taxa	Tissue	Amount of tissue /replicate
Potential Food Sources			
Zostera muelleri		Live blades	10 blades
Zostera muelleri (detritus)		Decaying blades	10-15 blades
Phytoplankton		Whole cells	1 precombusted GF/F filter
Microphytobenthos (MPB)		Whole cells	1 precombusted GF/F filter paper
Suspension feeders			
Austrovenus stutchburyi	Bivalve	Foot muscle	20 'feet'
Surface Deposit Feeders			
Macomona liliana	Bivalve	Foot muscle	20 'feet'
Diloma subrostrata	Gastropod	Operculum	20 operculum
Zeacumantus lutulentus	Gastropod	Operculum	25 operculum
Orbinia papillosa	Annelid	Whole body	3 worms
Aquliaspio aucklandica	Annelid	Whole body	6 worms
Aonides oxycephala	Annelid	Whole body	5 worms
Aglaphamus macroura	Annelid	Whole body	3 worms
Glycera Americana	Annelid	Whole body	5 worms
Omnivores			
Helice crassa	Crustacean	Whole body	4 crabs

The research aimed to determine spatial differences in the isotope ratios of macro-invertebrate consumers between seagrass and non-vegetated sediment at four sites within three estuaries. Furthermore, sampling was conducted during the winter months of June to August to avoid temporal variation.

To test whether any observed differences in the isotope signatures of macro-invertebrate consumers were a function of spatial differences, an additional site (control) of non-vegetated sediment in the Raglan Estuary was sampled (Figure 2.1 and Table 2.1). The control consisted of two sampling locations (A and B) separated by 50 m and macro-invertebrates were collected within a 12 m^2 area at each location.

2.3 Field and Laboratory Methods

2.3.1 Sediment characteristics and seagrass biomass

Sediment samples were collected from each location (IN and OUT) at each site (TU, TL, WP and RG) using syringe cores (2.5 cm diameter) to measure sediment grain size, sediment chlorophyll *a* (Chl *a*) and pheophytin (phaeo.) and sediment organic matter content (OC). In the laboratory, sediment grain size samples were treated with 10 ml of 10% hydrogen peroxide (H_2O_2) for 24 h to digest organic matter (Singer et al. 1988). After 24 h, if the samples had become dried out or were still bubbling, the addition of H_2O_2 was repeated until bubbling ceased. Grain sizes were measured using Malvern Mastersizer-S (laser diffraction particle size analyser). Sediment cores were sectioned at 0-5 mm using a sharp razor blade for both the determination OC and of pigment (Chl *a* and phaeo.) concentrations. For

determination of OC, the water content of the sediment was determined from the weight loss after drying at 60°C for 24 h and then OC was determined by weight loss after the samples were combusted at 450°C for 7 h. Pigment samples were freeze dried for 45 h then homogenised using a ceramic mortar and pestle and then approximately 30 mg was placed into centrifuge tubes with a 10 ml solution of 90% acetone and 10% magnesium carbonate. Samples were placed in the dark at 4°C for 20 h. The tubes were then centrifuged at 3300 rpm for 10 minutes before Chl *a* and phaeo. was measured (μg g dw^{-1}) using a 10-AU fluorometer (Arar and Collins 1997).

Five benthic cores (13 cm diameter, 7 cm depth) were randomly collected from each seagrass bed for estimates of seagrass biomass and blade measurements (Mattila et al. 1999). Seagrass from each benthic core was washed free of sediment and the length and width of five of the longest blades were measured using electronic callipers. In the laboratory, the roots and blades were separated, washed again before drying at 80˚C for 24 h and then weighed. The above step was repeated to ensure the seagrass material was completely dry (Hackney and Durako 2004).

2.3.2 Macro-invertebrate community identification

Three benthic cores were taken at each location from each site to obtain an estimate of macrofaunal community structure. The core material was transported back to the laboratory, sieved on a 500μm mesh, preserved using 80% isopropyl alcohol and stained using rose Bengal (Mason and Yevich 1967). The material was sorted in the laboratory using a dissecting microscope and identified using a variety of taxonomic guides. Some species were grouped together due to the difficulty in

identifying to species level. For example, many of the amphipods were grouped as the sub-families Gammeridae and Paracalliope.

2.3.3 Sample preparation for isotope analysis

Approximately 30 fresh seagrass blades and decaying blades (detritus) found on the sediment surface were collected from inside the seagrass beds at each site. Water samples (6 L) for phytoplankton were collected above the seagrass beds during a flood tide because according to Yin and Harrison (2000) Chl *a* is higher during this part of the tidal cycle. Microphytobenthos was collected from just outside the seagrass bed by scraping surficial sediment (to 1 cm depth) using a hand trowel and then placing it into labelled zip-lock bags. Adult macro-invertebrate consumers were collected by hand from the sediment surface and within the sediment within a 12 m^2 area at each location (Figure 2.2 and Table 2.2). Adults of all species were collected to avoid any isotopic variation due to animal age (e.g. Vizzini and Mazzola 2003). All samples were put immediately on ice and then transported back to the laboratory within 4 h of collection.

Immediately on arrival to the laboratory microphytobenthos samples were violently agitated with filtered seawater to resuspend the algae from the sediment and then placed into a 5 L glass beaker for between 3 to 4 hours to allow the sediment to settle (Hyndes and Lavery 2005, Smit et al. 2006). Approximately 500 ml of the supernatant water was decanted and as with phytoplankton water samples was passed through a 63 μm mesh sieve to remove large detritus and sediment particles. Phytoplankton and microphytobenthos samples were then filtered through pre-washed, pre-combusted (450°C for 4 h) Whatman GF/F glass fibre filter paper (25 mm diameter, 0.7μm

retention size) until the filters were blocked and colouration was visible (Lepoint et al. 2000, McConnaughey and McRoy 1979, Page and Lastra 2003, Vizzini et al. 2002, Vizzini and Mazola 2003, Vizzini and Mazzola 2006). The filters were then placed in a glass dessicator for 24 h over 1.2 N HCl to remove carbonates (Vizzini and Mazzola 2006). Lastly, after decarbonation, the filters were dried at 60°C for 4 h and then placed into screw-top glass vials and left in the freezer until isotope analysis. To act as a control, filtered seawater was put through the same process.

Live seagrass and detritus were washed with RO water and epiphytes were removed manually using a blunt razor blade and forceps (Dauby, 1995; Dauby and Poulicek, 1995, Hyndes and Lavery 2005, Vizzini and Mazzola 2003). The seagrass blades were then rinsed again in RO and Milli-Q water before drying at 60 °C for 48 h. Samples were then ground and homogenised into a fine powder using a ceramic mortar and pestle. The powder was then carefully placed into a labelled glass vial and left in the freezer until isotope analysis.

Individuals of each macro-invertebrate species collected (Table 2.2) from each site and location were pooled together thereby reducing variability in the isotope signatures of each species (Dauby, 1995; Lee, 2000; Vizzini and Mazzola, 2006). The foot muscle and operculum of bivalves (*M. liliana* and *A. stutchburyi*) and gastropods (*D. subrostrata* and *Z. lutulentus*), respectively, were extracted immediate after freezing (Fry et al. 1983a). The whole bodies of the crab *H. crassa* and annelid polychaetes *A. aucklandica*, *A. oxycephala*, *A. macroura*, *O. papillosa* and *G. americana* were used for isotopic analysis because they were too small to dissect enough muscle tissue for a sample. Crabs and polychaetes were placed into separate aerated aquaria for

approximately 24 h to allow the contents of their guts to be expelled (Hyndes and Lavery 2005). Prior to drying at 60°C for 48 h, bivalve, gastropod and polychaete tissue were washed twice in RO water and then once in Milli-Q water. Crabs were treated the same way, except they were first submerged in 1.2N HCl until the samples stopped bubbling to ensure all the carbonates were removed (Dauby 1995, Vizzini et al. 2002). According to Bunn *et al.* (1995), acid washing is thought to alter the chemical composition of natural materials and in particular nitrogen. To minimise this effect, samples were acidified prior to drying and grinding (Vizzini and Mazzola, 2006). Following washing and drying, all samples were ground and homogenised into a fine powder using a ceramic mortar and pestle, then stored in labelled glass vials and placed in the freezer pending isotope analysis.

2.3.4 Stable isotope analysis

Ground samples were weighed (seagrass and detritus N, 8.5 mg, C, 3.0 mg and animal tissue N, 2.0 mg, C, 3.0 mg) into small aluminium capsules, while whole filters containing phytoplankton and microphytobenthos were folded into the capsules which were then closed and folded into small spheres. Great care was taken to ensure that encapsulated material did not escape. The $\delta^{13}C$ and $\delta^{15}N$ of samples was determined at the University of Waikato Stable Isotope Unit using a Dumas Elemental Analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser) (Nevins et al., 1985). $\delta^{13}C$ and $\delta^{15}N$ measured has a precision of ±0.5‰ and ±1.0‰, respectively. Carbon and nitrogen were analysed against standardised pre-calibrated C_4 sucrose which was cross-referenced to Pee Dee belemnite (-10.8‰) and to a urea standard that is traceable to atmospheric nitrogen (-0.45‰), respectively.

2.4 Data Analysis

Sediment characteristics, seagrass biomass and macrofaunal composition data were initially analysed using univariate statistics and then multivariate techniques were employed. Using STATISTICA 6.0, data was checked for normality and homogeneity of variances using Levene's test and visual observation of residuals, respectively; and the data was transformed using ln or square-root where necessary (Ashcroft and Pereira 2003). One-way analysis of variance (ANOVA) was used to identify the effect of site on seagrass biomass (total seagrass biomass and above- and below-ground seagrass biomass). Furthermore, two-way ANOVA was used to identify the effects of site and location on all sediment characteristics (median grain size, %silt/clay, OC, Chl *a* and phaeo.) and indices of macro-invertebrate community structure (abundance, number of taxa and Simpson's Index of Diversity (D-1)). Post-hoc LSD tests were performed if differences were significant (α= 0.05). PRIMER V5.2.2 was used for multivariate data analysis (Plymouth Routines in Multivariate Ecological Research; Clarke and Warwick, 1994). A similarity matrix was constructed based on Bray-Curtis similarity measures of square-root transformed data and then an ordination plot was produced by non-metric multidimensional scaling (MDS). In addition a two-way nested analysis of similarities (ANOSIM) was conducted to determine any differences between macro-invertebrate communities at each site and location. Furthermore, a SIMPLER (similarity percentages) analysis was conducted to determine the species responsible for the significant differences detected in the ANOSIM.

Dual $\delta^{13}C$ and $\delta^{15}N$ isotope plots were constructed to graphically observe the isotope data for food sources and macro-invertebrate consumers from each study site and sampling location. Furthermore, the flow of ^{13}C and ^{15}N through the food webs

was identified. Stable $\delta^{13}C$ and $\delta^{15}N$ isotope data of potential food sources and macro-invertebrate consumers was analysed using univariate statistical analyses. Using STATISTCA 6.0, one-way ANOVA tested significant differences of the effect of site on the $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources and two-way ANOVA was used to test the effect of site and location on the $\delta^{13}C$ and $\delta^{15}N$ values of macro-invertebrate consumers (Ashcroft and Pereira 2003). The isotope data was also checked for normality and homogeneity of variances and post-hoc LSD tests were performed if differences were significant ($\alpha = 0.05$).

3 Results

3.1 Sediment Characteristics and Seagrass Biomass

At all study sites and locations sediments were classified as muddy sands and median grain size ranged from 105 to 203 μm with silt/clay content from 3.1 to 30.3 % (Table 3.1). WP and TL had coarser sediments compared to the finer sediments at TU and RG. Similarly, sediment organic content (OC) was consistent with the differences in grain size, where sites with finer sediments were approximately 1.6 times greater in percentage OC compared to sites with coarser sediments. The highest and lowest concentrations of sediment Chl *a* were at TU and WP and phaeophytin at RG and WP, respectively (Table 3.1). There were significant differences between sites for Chl *a* and phaeo. but not for OC (ANOVA; $P<0.05$; Table 3.2). The percentage silt/clay at RG was 3.7 times greater compared to sites on the east coast and the percent silt/clay of non-vegetated sediment was higher compared to sediment inside the seagrass bed at RG. However, the opposite trend was observed at sites TU, TL and WP. Sampling location was not a significant factor affecting sediment characteristics. However, there were significant site × location interactions for Chl *a* and phaeo. ($P<0.05$), indicating that there was no consistent variation in these characteristics between inside and outside the seagrass beds among study sites (Table 3.2).

Total seagrass biomass (above- and below-ground) was 74% greater at TL (11.94 g dw 0.01 m^{-2}) compared to other study sites (Table 3.3). At all sites below-ground seagrass biomass represented between 73 and 83% of the total biomass and above-ground biomass was greatest at TL (25.5%) and lowest at WP (17.3%) (Figure 3.1 and Table 3.3). Consistent with total seagrass biomass, TL also recorded the largest mean blade length (163 mm) but did not have the largest mean blade width (Table 3.3). Mean seagrass blade widths from RG (3.15 mm) and TU (1.74 mm) were the highest and lowest recorded, respectively. Site was a significant factor for above-ground biomass and blade length ($P<0.05$) but not for total seagrass biomass, indicating that patterns in above-ground seagrass biomass and blade length varied among sites (Table 3.4). Subsequently, post-hoc LSD results indicated that the significant differences were driven by the large difference between TL and the other study sites.

Table 3.1: Mean sediment characteristics for all study sites and locations. OC is the sediment organic content and Chl. *a* and Phaeo. are the chlorophyll *a* and phaeophytin concentrations, respectively. Standard deviations are in brackets and n=2 for all sediment characteristics except for median grain size, % silt/clay and OC at WP where n=1.

Site/ Location	Median grain size (µm)	Silt/Clay (% < 63 µm)	OC (%)	Chl. *a* (µg g dw^{-1})	Phaeo. (µg g dw^{-1})
WP IN	203	4.6	4.68	17.2 (0.70)	10 (0.34)
WP OUT	195	3.1	5.22	16.0(0.44)	7 (0.28)
TU IN	163	12.8	11.40 (0.26)	28.5 (1.36)	15 (3.22)
TU OUT	177	9.5	6.99 (0.92)	18.7 (2.31)	10 (1.86)
TL IN	193	8.7	6.74 (0.55)	16.7 (5.02)	12 (2.32)
TL OUT	203	7.3	6.26 (0.47)	21.6 (2.68)	13 (0.64)
RG IN	105	27.4	8.14 (3.50)	20.8 (0.55)	15 (0.88)
RG OUT	107	30.3	9.34 (0.85)	23.6 (1.07)	17 (1.21)

Table 3.2: Two-way ANOVA and post-hoc LSD results of the effects of site and location on sediment characteristics. Significant results are marked: * (P <0.05); [a] indicates that data was ln transformed prior to analysis. Refer to Table 3.1 for an explanation of abbreviations used.

Variable	Source of variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD post-hoc test
OC (%)	Site	3	0.002	3.57	0.067	
	Location	1	0.000	0.478	0.509	
	Site × Location	3	0.001	1.17	0.381	
	Error	8	0.000			
Chl *a* (μm g dw $^{-1}$)	Site	3	38.8	7.43	0.011*	
	Location	1	2.77	0.531	0.487	
	Site × Location	3	41.8	8.01	0.009*	TL(IN)>TL(OUT)
	Error	8	5.22			
Phaeo (μm g dw $^{-1}$) [a]	Site	3	0.313	18.7	0.001*	
	Location	1	0.053	3.17	0.113	
	Site × Location	3	0.088	5.26	0.027*	TL(IN)>TL(OUT)
	Error	8	0.017			WP(IN)>WP(OUT)

Table 3.3: Mean total seagrass biomass, above-ground biomass (n=5) and mean blade length and width (n=25) for each study site. Standard deviations are in brackets.

Site	Seagrass Biomass (g dw 0.01 m^{-2})	Above ground (%)	Mean blade length (mm)	Mean blade width (mm)
TU	7.15 (2.83)	27.3	155 (20.98)	1.74 (0.28)
TL	11.9 (2.97)	25.5	163 (23.01)	2.77 (0.61)
WP	10.3 (4.67)	17.3	122 (14.00)	2.55 (0.32)
RG	8.95 (2.61)	20.6	146 (16.84)	3.15 (0.22)

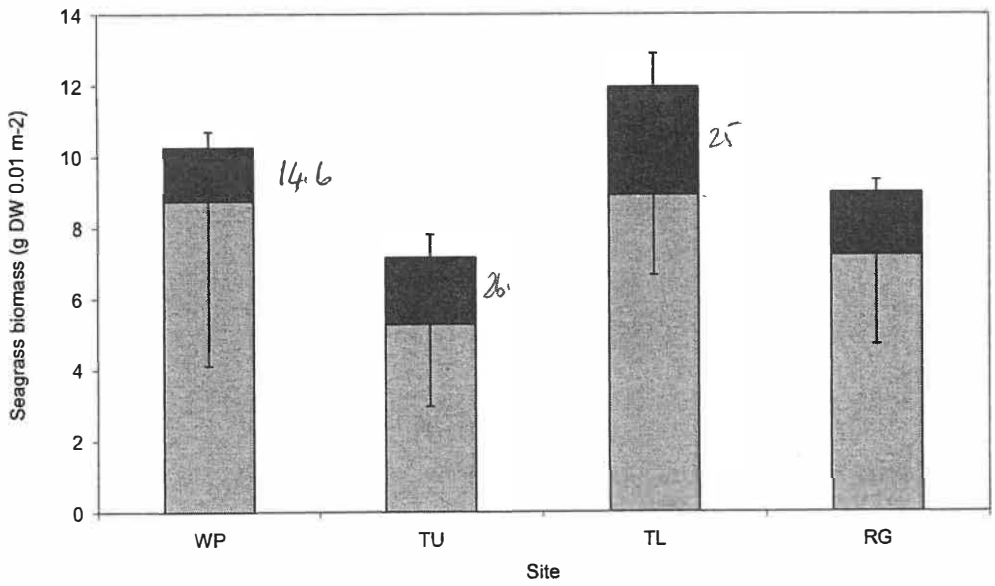

Figure 3.1: Mean above- (■) (+1SD) and below-ground (□) (-1SD) seagrass biomass (g DW 0.01 m^{-2}) at each study site (n=5).

Table 3.4: ANOVA and post-hoc LSD results of the effects of site on total seagrass biomass (SG) and above- (AG) and below- (BG) ground seagrass biomass. Significant results are marked: * (P <0.05); [a] indicates that data were ln transformed prior to analysis.

Variable	Source of variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD post-hoc test
SG	Site	3	20.5	1.80	0.187	
	Error	16	11.4			
AG [a]	Site	3	2.26	5.67	0.008*	TL>WP=TU=RG
	Error	16	0.40			
BG [a]	Site	3	14.2	1.50	0.253	
	Error	16	9.49			

3.2 Macro-Invertebrate Analysis

3.2.1 Indices of community structure

A total of 2,270 individuals, representing 43 taxa, were identified from the three replicate benthic core samples collected from each sampling location (Appendices: Tables 1 and 2). Species were grouped into the following major taxonomic groups; bivalve, gastropod, crustacean, annelid and others (Figure 3.2). Bivalves were the dominant taxa at most locations, especially at TL OUT where bivalves constituted 69% of all taxa. Crustaceans and annelids were also well represented and at WP IN crustaceans represented 50 % and RG IN annelids were 35% of all taxa, for example. Collectively, proportions of each taxa across all sites and locations was 41% bivalves, 26% annelids, 20% crustaceans, 9% gastropods and 3% other.

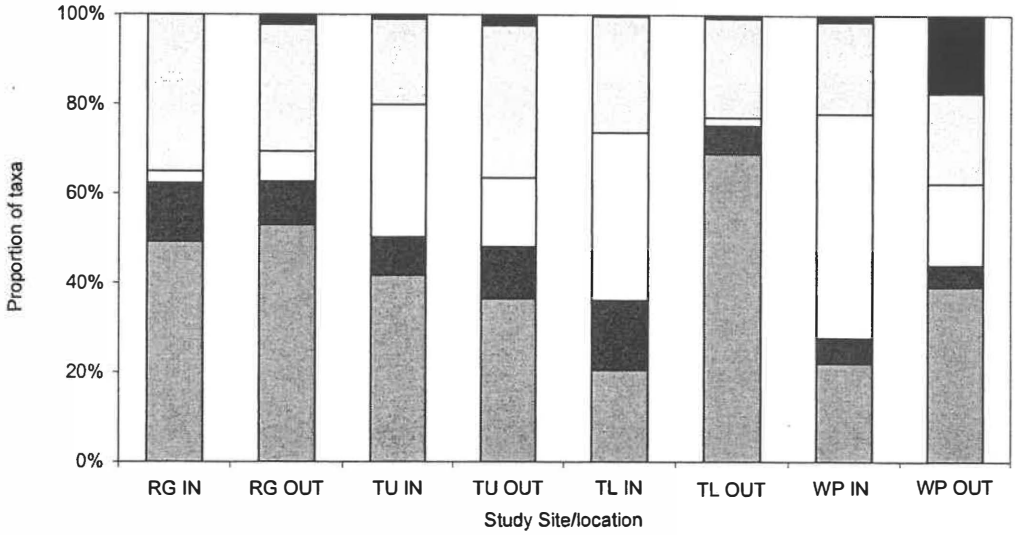

Figure 3.2: Proportions of bivalves (▩), gastropods (■), crustaceans (□), annelids (□) and other macro-invertebrates (■) from all sites and locations.

The mean number of individuals among study sites (across sampling locations) was highest at WP, closely followed by TU and TL and lowest at RG (Table 3.5). However, the mean number of taxa collected at each site was greatest at TU (35 0.01 m^{-2}) followed by WP (34.6 0.01 m^{-2}), TL (28.6 0.01 m^{-2}) and RG (24.3 0.01 m^{-2}). The mean number of taxa and mean number of individuals was higher inside compared to outside the seagrass beds at RG, TU, TL and lower inside the seagrass bed compared to outside at WP. Species diversity, calculated using Simpson's Index of Diversity (1-D), generates values ranging between 0 and 1, whereby the greater the number (i.e. closer to 1) the higher the species diversity. Mean species diversity (across sampling locations) was greatest at WP (0.84) and lowest at RG (0.78) (Table 3.5). Diversity was higher inside the seagrass bed than outside at TU and WP but the inverse was observed at RG and TL. Two-way ANOVA results revealed that location and site had no effect on

species diversity but there was a significant site × location interaction (Table 3.6) indicating that there was no consistent variation in species diversity between locations among study sites. Furthermore, LSD post-hoc test results indicate that the significant interaction was a result of higher species diversity at TL IN and RG IN compared to OUT locations.

Table 3.5: Univariate indices of macro-invertebrate community structure, including mean number of taxa, number of individuals and Simpson's Index of Diversity (1-D) for each study site and sampling location (n=3). Standard deviations are in brackets.

Site	Sampling Location	Mean Number of Taxa (# 0.01 m^{-2})	Mean Number of Individuals (# 0.01 m^{-2})	Diversity (1-D)
WP IN	IN	16.3 (1.2)	117 (18)	0.83 (0.02)
WP OUT	OUT	18.3 (6.8)	124 (28)	0.86 (0.06)
TU IN	IN	18.0 (1.0)	133 (54)	0.81 (0.03)
TU OUT	OUT	17.0 (2.6)	95 (37)	0.86 (0.03)
TL IN	IN	16.3 (4.7)	90 (31)	0.86 (0.05)
TL OUT	OUT	12.3 (3.2)	90 (3)	0.75 (0.09)
RG IN	IN	13.3 (3.8)	64 (16)	0.84 (0.03)
RG OUT	OUT	11.0 (1.0)	45 (10)	0.73 (0.09)

Table 3.6: Two-way ANOVA and post-hoc LSD results assessing differences in univariate indices of macro-invertebrate community structure among study sites and sampling locations. Significant results are marked: * ($P<0.05$)

Community Index	Source of Variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD Post-hoc test
Number of taxa (# 0.01 m^{-2})	Site	3	39.4	3.04	0.059	
	Location	1	10.7	0.823	0.378	
	Site × Location	3	9.67	0.746	0.540	
	Error	16	13.0			
Number of individuals (# 0.01 m^{-2})	Site	3	5376	6.49	0.004*	(TU=WP>RG)=TL
	Location	1	888	1.07	0.316	
	Site × Location	3	621	0.750	0.538	
	Error	16	828			
Diversity (1-D)	Site	3	0.004	1.35	0.293	
	Location	1	0.009	2.90	0.108	
	Site × Location	3	0.012	4.06	0.025*	TL IN>TL OUT
	Error	16	0.003			RG IN>RG OUT

3.2.2 Macro-invertebrate community analyses

Macro-invertebrate community data at each study site and location was square-root transformed and used to construct a 2-dimentional MDS ordination plot (Figure 3.3). The stress level of 0.11 indicated that a reasonable representation of the species present was similar between each site and location. Three replicate benthic cores were collected and average abundances of macro-invertebrates collected from each location and site are plotted on the MDS. East coast study sites (TU, TL and WP) from both locations (except for TL OUT) were generally clustered together (to the left of the plot) and separated from RG. Close clustering implies similarity whilst macro-invertebrate communities that are not clustered are relatively dissimilar. The clustering between all IN locations (situated at the bottom left) at each site is much tighter than communities in all OUT locations. At all study sites on the east coast TU, TL and WP) macro-invertebrate communities inside the seagrass were dissimilar than those from adjacent non-vegetated sediment. However, there was no significant difference in the communities between locations at RG. Based on results from TU, TL and WP macro-invertebrate community composition inside seagrass beds independent of site are relatively similar and maybe more predictable than communities on non-vegetated sediment where species composition is site dependent and more variable.

A two-way nested analysis of similarities (ANOSIM) on the macro-invertebrate community data for each site and location was conducted to detect any significant differences. Differences observed between sites (averaged across all locations) were significant ($R=0.69$, $P<0.05$), meanwhile differences observed

between locations (using site as samples) was not significant (R=0.1, P>0.05). This result implies that there are differences in the macro-invertebrate communities between estuaries but no significant differences in communities from in seagrass compared to non-vegetated sediment. A SIMPLER analysis was conducted to determine the species responsible for the significant differences (Table 3.7). The polychaete *Aquilaspio aucklandica* was abundant at all study sites and locations (except RG OUT), in particular TU OUT (21.7 / 0.01 m^{-2}), (14.3/0.01 m^{-2}) and WP IN (15.0/0.01 m^{-2}). Bivalves *Austrovenus stutchburyi* and *Nucula hartvigiana* were also abundant at all sites and locations, except for TL IN and RG OUT, respectively. *A. aucklandica*, *A. stutchburyi* and *N. hartvigiana* consistently had the greatest abundances and contribution to macro-invertebrate communities at all sites and locations. The average dissimilarity between locations among sites was variable with the greatest being at TL (65%), then in decreasing order, WP (56%), TU (54%) and the lowest at RG (52%). The percent contribution of five species at each site and location ranged between 76 and 91%, accounting for differences in the average dissimilarity among sites. Changes in the abundance of bivalves (greater OUT compared to IN), annelid polychaetes (generally similar or greater OUT compared to IN) and crustaceans (generally greater IN compared to OUT) appear to be driving the significant difference in community composition between seagrass and adjacent unvegetated sediment.

The abundances of species sampled for stable isotope analysis within each site and locations were determined. Bivalves, *A. stutchburyi* and *Macomona liliana*, the gastropod *Diloma subrostrata* and the polychaete *A. aucklandica* had

relatively high abundances and contributed to the average dissimilarity between sites and locations (Table 3.7). This suggests that these species contribute to differences in the community structure between locations and may also play significant roles within the communities. The mean percentage abundance of all species selected for isotope analysis is displayed in Table 3.8. Abundances of *A. stutchburyi* and *A. aucklandica* support the results of the SIMPLER analysis having the highest mean percentage abundance at most sites and locations, indicating their importance within communities. *A. stutchburyi*, *M. liliana*, *D. subrostrata* and *Zeacumantus lutulentus* are abundant at all locations among sites. *Helice crassa* is also abundant except at TU IN and OUT and TL OUT. Furthermore, *A. aucklandica* is abundant at all sites and locations. However, percent abundances of the other polychaete species were variable.

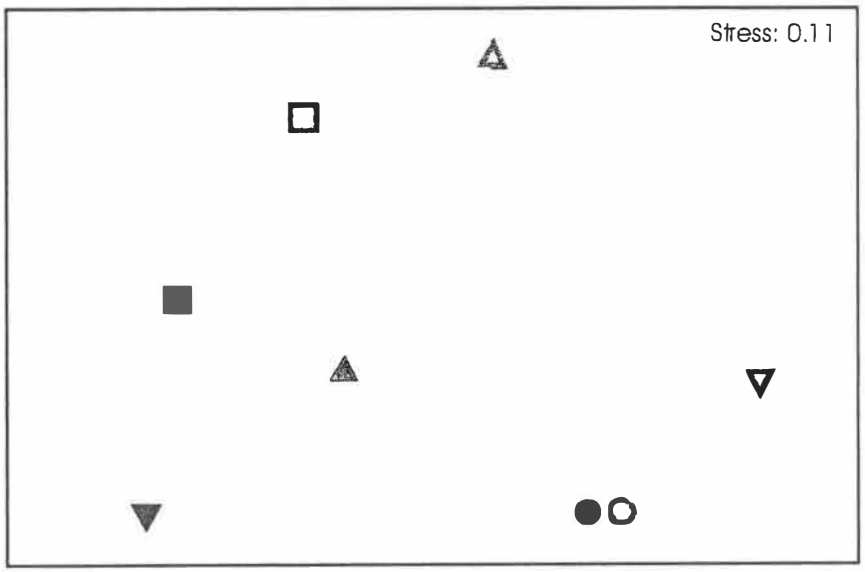

Figure 3.3: Multidimensional scaling (MDS) ordination of macro-invertebrate data from each sampling site and location: TU IN (■), TU OUT (◘), TL IN (▼), TL OUT (▽), WP IN (▲), WP OUT (△), RG IN (●) and RG OUT (○) (n=3). Symbol positions are based on Bray-Curtis similarities which are transformed using square-root. The distance between samples is proportional to their relative similarity and the axes have no units.

Table 3.7: The five dominant species contributing to the dissimilarity between the macro-invertebrate assemblages from each site and location as revealed using a SIMPLER analysis. Abund. refers to the average abundance /0.01 m^{-2} and Contr. % is percent contribution to the community. See Appendicies: Table 1 and 2 for full names of species; – indicates that the species was not dominant.

Location	Species	IN		OUT	
		Abund.	Contr. %	Abund.	Contr. %
Tauranga Upper	*N. hartvigiana*	40.3	36.0	17.3	22.2
	Ostrocod	32.3	28.3	-	-
	A. aucklandica	13.7	10.3	21.7	20.7
	A. stutchburyi	12.3	6.26	11.7	18.9
	Oligochaetes	4.33	5.40	-	-
	M. liliana	-	-	5.33	9.07
	Gammeridia sp.	-	-	8.33	8.22
	Cumulative %		**86.3**		**79.09**
Tauranga Lower	*A. aucklandica*	14.3	31.4	7.00	8.65
	N. hartvigiana	12.0	21.2	25.7	35.5
	Gammeridia sp.	12.0	8.39	-	-
	Paracalliope sp.	10.67	8.39	-	-
	D. subrostrata	4.67	6.47	-	-
	A. stutchburyi	-	-	34.3	39.5
	Neridae sp.	-	-	4.00	5.09
	M. liliana	-	-	2.33	3.05
	Cumulative %		**75.8**		**91.8**
Whangapoua	Gammeridia sp.	33.7	44.7	-	-
	A. aucklandica	15.0	16.6	10.7	8.18
	N. hartvigiana	14.3	12.3	24.0	25.1
	A. stutchburyi	9.00	8.74	11.3	15.7
	Ostrocod	9.33	7.26	-	-
	A. aureoradiata	-	-	21.0	20.9
	M. liliana	-	-	13.3	7.89
	Cumulative %		**89.6**		**77.77**
Raglan	*A. stutchburyi*	16.0	28.2	20.0	69.3
	N. hartvigiana	12.3	27.7	-	-
	A. aucklandica	8.00	19.5	-	-
	M. liliana	3.00	8.21	-	-
	Neridae sp.	4.33	5.17	-	-
	Nemertean	-	-	3.33	11.6
	A. aureoradiata	-	-	1.00	4.33
	D.subrostrata	-	-	1.33	4.33
	B. syrtis	-	-	1.00	1.58
	Cumulative %		**88.8**		**91.14**

Table 3.8: Contribution (mean percent abundance) of each species collected for isotope analysis to the macro-invertebrate communities at each site and location (n=3). – indicates that species were not abundant

	Percent Abundance							
Species	**TU IN**	**TU OUT**	**TL IN**	**TL OUT**	**WP IN**	**WP OUT**	**RG IN**	**RG OUT**
A. stutchburyi	9.3	12.3	3.7	38.1	7.7	9.1	25.0	44.4
M. liliana	2.0	5.6	4.4	2.6	3.0	10.7	4.7	4.4
D. subrostrata	0.7	1.0	5.2	1.1	0.8	1.6	3.9	3.0
Z. lutulentus	2.3	8.4	3.3	4.1	2.1	2.4	3.9	2.2
H. crassa	-	-	2.2	-	0.8	0.8	3.1	3.3
A. oxycephala	-	-	2.2	-	-	2.0	6.2	4.4
A. aucklandica	10.3	22.8	16.0	7.8	12.8	8.6	12.5	26.7
G. americana	0.7	-	-	-	1.3	-	2.1	4.4
O. papillosa	-	-	2.2	2.2	0.8	1.2	1.6	2.2
A. macroura	0.7	-	-	-	-	0.8	-	-
Total	26	50	39	56	29	37	63	95

3.3 Stable Isotope Analyses

A total of 344 samples (including control sites) were analysed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios (Tables 3.9 and 3.10). To avoid confusion, abbreviations used for potential food sources and consumers in Figures 3.5-3.11 are as follows: *Z. muelleri* live blades (SG) and detritus (SGD), phytoplankton (PP), microphytobenthos (MPB), *A. stutchburyi* (AUS), *M. liliana* (MAC), *D. subrostrata* (DILO), *Z. lutulentus* (ZEA), *H. crassa* (HEL), *A. macroura* (A.mac), *A. oxycephala* (A.oxy), *A. aucklandica* (A.auck), *G. americana* (G.amer) and *O. papillosa* (O.pap).

Table 3.9: Mean $\delta^{15}N$ and $\delta^{13}C$ isotope values (±1SD) for *n* samples of each potential food source collected from all study sites. Standard deviations are in brackets.

Site	Species	*n*	Mean $\delta^{15}N$	Mean $\delta^{13}C$
TU	*Zostera muelleri*	4	5.4 (0.2)	-10.3 (0.0)
	Zostera muelleri detritus	4	5.4 (0.4)	-9.6 (0.1)
	Phytoplankton	4	7.7 (5.4)	-22.0 (0.6)
	Microphytobenthos	4	6.0 (1.5)	-17.6 (0.6)
TL	*Zostera muelleri*	4	5.4 (0.3)	-8.9 (0.1)
	Zostera muelleri detritus	4	6.0 (0.3)	-9.0 (0.1)
	Phytoplankton	4	4.5 (1.7)	-19.9 (0.6)
	Microphytobenthos	4	6.0 (0.7)	-16.1 (1.6)
WP	*Zostera muelleri*	4	5.9 (0.2)	-8.9 (0.1)
	Zostera muelleri detritus	4	6.1 (0.1)	-9.4 (0.1)
	Phytoplankton	4	5.2 (1.8)	-23.4 (0.6)
	Microphytobenthos	4	5.1 (2.7)	-17.3 (1.1)
RG	*Zostera muelleri*	4	6.6 (0.2)	-10.7 (0.1)
	Zostera muelleri detritus	4	7.9 (0.3)	-12.1 (0.3)
	Phytoplankton	4	9.5 (5.3)	-25.2 (0.3)
	Microphytobenthos	4	5.9 (0.5)	-21.4 (3.1)

Table 3.10: Mean $\delta^{15}N$ and $\delta^{13}C$ isotope values (±1SD) for *n* samples of consumers collected from all study sites and locations. – indicates unavailability of data.

Site	Species	*n*	IN		OUT	
			Mean $\delta^{15}N$	Mean $\delta^{13}C$	Mean $\delta^{15}N$	Mean $\delta^{13}C$
	Suspension Feeders					
TU	*Austrovenus stutchburyi*	4	8.4 (0.1)	-17.9 (0.0)	9.0 (0.1)	-17.3 (0.1)
TL		4	8.9 (0.3)	-15.4 (0.4)	8.6 (0.2)	-16.4 (0.0)
WP		4	9.0 (0.1)	-16.8 (0.0)	8.9 (0.1)	-16.7 (0.0)
RG		4	9.4 (0.1)	-18.4 (0.1)	9.6 (0.1)	-18.6 (0.1)
	Surface Deposit Feeders					
TU	*Macomona liliana*	4	10.1 (0.2)	-10.7 (0.1)	9.5 (0.1)	-9.9 (0.7)
TL		4	9.0 (0.2)	-10.0 (0.0)	8.8 (0.1)	-10.2 (0.1)
WP		4	8.0 (0.1)	-12.1 (0.2)	8.6 (0.0)	-9.1 (0.0)
RG		4	9.9 (0.1)	-13.5 (0.1)	9.2 (0.1)	-14.4 (0.1)
TU	*Diloma subrostrata*	4	9.0 (0.2)	-8.6 (0.0)	8.4 (0.1)	-8.9 (0.1)
TL		4	9.2 (0.0)	-8.7 (0.0)	9.0 (0.1)	-9.3 (0.1)
WP		4	8.4 (0.4)	-10.3 (0.2)	6.9 (0.2)	-8.0 (0.2)
RG		4	9.0 (0.6)	-11.1 (0.5)	8.8 (0.6)	-11.9 (0.6)
TU	*Zeacumantus lutulentus*	4	8.9 (0.2)	-9.6 (0.1)	8.5 (0.3)	-9.2 (0.2)
TL		4	9.3 (0.2)	-9.0 (0.2)	8.8 (0.2)	-9.2 (0.1)
WP		4	8.1 (0.3)	-10.6 (0.3)	7.3 (0.2)	-7.8 (0.1)
RG		4	9.4 (0.1)	-12.6 (0.6)	8.9 (0.2)	-12.6 (0.7)
TU	*Aquilaspio aucklandica*	4	7.9 (0.1)	-8.8 (0.1)	9.0 (0.0)	-11.0 (0.0)
TL		4	-	-	9.7 (0.1)	-10.2 (0.1)
WP		4	7.6 (0.0)	-11.3 (0.0)	-	-
RG		4	9.4 (0.0)	-14.0 (0.0)	-	-

Table 10: continued

Site	Species	*n*	IN		OUT	
			Mean $\delta^{15}N$	Mean $\delta^{13}C$	Mean $\delta^{15}N$	Mean $\delta^{13}C$
	Surface Deposit Feeders					
TU	*Orbinia papillosa*	4	-	-	9.1 (0.0)	-12.6 (0.0)
TL		4	7.8 (0.3)	-12.1 (0.2)	8.1 (0.3)	-11.7 (0.1)
WP		4	11.1 (0.1)	-12.7 (0.1)	8.7 (0.6)	-11.3 (0.1)
RG		4	10.8 (0.1)	-15.2 (0.0)	-	-
TU	*Helica crassa*	4	8.3 (0.5)	-10.7 (0.8)	8.1 (0.7)	-11.2 (1.3)
TL		4	8.4 (0.3)	-10.4 (0.1)	8.2 (0.1)	-11.3 (0.2)
WP		4	7.3 (0.2)	-12.2 (0.1)	7.1 (0.2)	-11.2 (0.1)
RG		4	7.6 (0.2)	-13.0 (0.3)	7.0 (0.5)	-13.6 (1.1)
	Predators					
TU	*Aonides oxycephala*	2	11.7 (0.1)	-10.8 (0.0)	11.4 (0.1)	-11.5 (0.0)
TL			-	-	-	-
WP		2	-	-	9.3 (0.0)	-11.2 (0.0)
RG			-	-	-	-
TU	*Aglaophamus macroura*	4	11.6 (0.6)	-10.9 (0.0)	11.2 (0.1)	-11.0 (0.1)
TL			-	-	-	-
WP			-	-	-	-
RG			-	-	-	-
TU	*Glycera americana*	4	11.9 (0.0)	-12.8 (0.0)	11.9 (0.0)	-12.2 (0.0)
TL			-	-	-	-
WP			-	-	-	-
RG		4	12.7 (0.1)	-14.2 (0.1)-	13.3 (1.2)	-14.6 (0.6)

3.3.1 Isotope signatures of potential food sources

Preliminary results gathered from TU in 1999 illustrates that seagrass and *Ulva lactuca* are more enriched in ^{13}C than other potential food sources (Figures 3.5 and 3.6). The large differences in the $\delta^{13}C$ values (-7 to -23‰) of potential food sources suggest that the likely food sources of invertebrates can be distinguished based on their $\delta^{13}C$ values. Terrestrially derived organic matter (*Avicennia marina* subsp. *australasica*, *Juncus maritimus australiensis* and *Leptocarpus simplex*) was more depleted in ^{13}C compared to *U. lactuca* and *Z. muelleri* that utilise marine HCO_3^- as their carbon source. *Z. muelleri* (live blades and detritus) are also distinct from the other sources of organic matter; however, there was no consistent visual difference between the live blades and detritus (+0.5‰ for N). Furthermore, *Z. muelleri* collected and analysed in 1999 indicate that there is little temporal variation within the six years between sample collections.

There was little difference between phytoplankton and microphytobenthos (-1.0‰ for N and +4.5‰ for C), suggesting that the species composition of microphytobenthos may be similar to phytoplankton. The $\delta^{15}N$ and $\delta^{13}C$ ratios of phytoplankton were variable between sites with values ranging from 9.5‰ at RG to 4.5‰ at TL and -20‰ at TL to -25‰ at RG (e.g. Figure 3.6). The variation in ^{15}N of phytoplankton was very large compared to ^{13}C. Phytoplankton $\delta^{13}C$ values varied significantly among sites ($P<0.05$) but $\delta^{15}N$ did not (Table 3.11). The $\delta^{13}C$ values of microphytobenthos at each site were very variable compared to $\delta^{15}N$ (Figure 3.6). Similarly to phytoplankton, $\delta^{13}C$ of microphytobenthos also varied among sites but $\delta^{15}N$ did not (Table 3.1).

Significant differences observed in $\delta^{13}C$ of phytoplankton and microphytobenthos was mainly due to the large difference at RG compared to other sites. In addition, phytoplankton and microphytobenthos $\delta^{13}C$ and $\delta^{15}N$ values were similar to those recorded in literature (Appendices: Figures 1-4). Graphical observation of the mean $\delta^{13}C$ values of *Z. muelleri* (live blades and detritus) shows that it is more enriched in ^{13}C compared to phytoplankton and MPB with values from all sites ranging from -8.0 to -12.1‰. The $\delta^{13}C$ and $\delta^{15}N$ values of *Z. muelleri* were also within the wide range values presented in literature (Appendices: Figures 5 and 6). The $\delta^{13}C$ and $\delta^{15}N$ of live blades and detritus was significantly different between sites (ANOVA; $P<0.05$; Figures 3.6-3.11 and Table 3.11). Post-hoc LSD tests suggest that the significant differences was driven by RG.

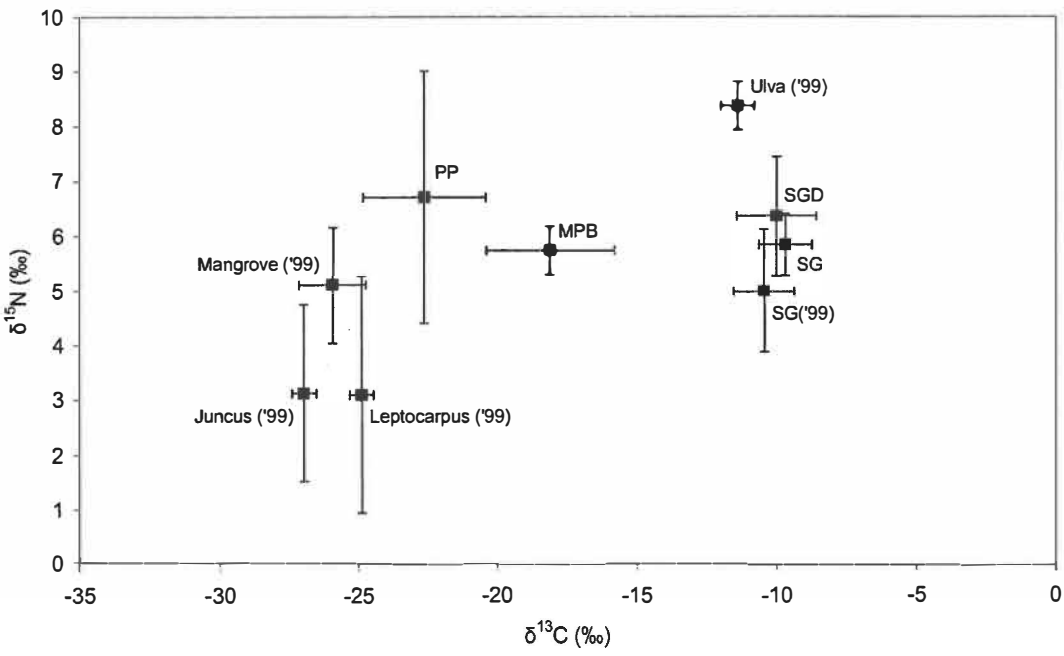

Figure 3.4: Mean carbon and nitrogen stable isotope ratios (±1SD) of the potential food sources (PP, MPB, SG and SGD) across study sites (n=16). Preliminary isotope results of *A. marina* subsp. *australasica* (Mangrove), *J. maritimus australiensis* (Juncus), *L. simplex* (Leptocarpus), *Z. muelleri* (SG) and *U. lactuca* (Ulva) collected from TU in 1999 are also plotted (n=6) (Hicks and Pilditch, 2004).

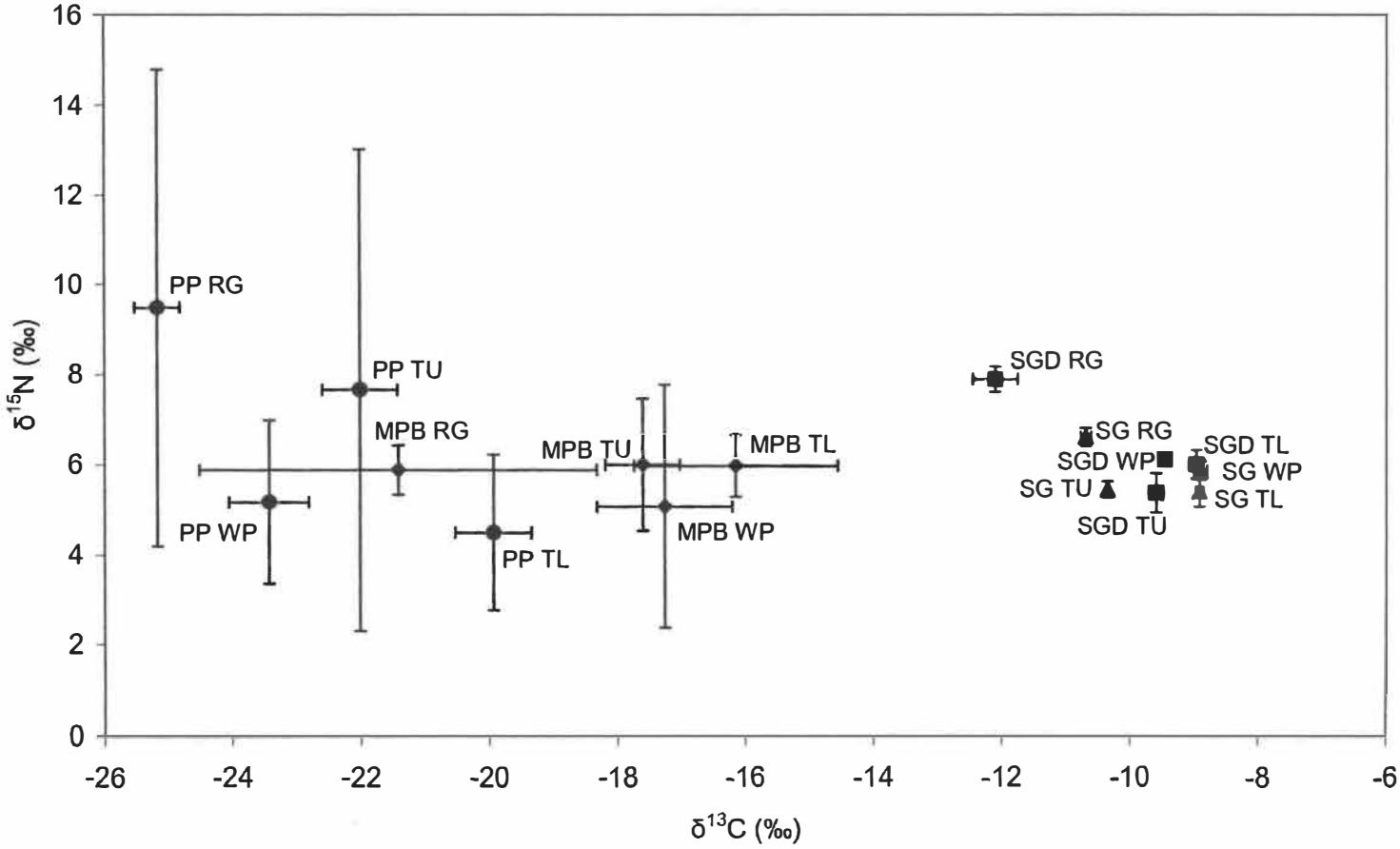

Figure 3.5: Mean stable $\delta^{15}N$ and $\delta^{13}C$ isotope signatures (±1SD) of potential food sources: phytoplankton (PP), microphytobenthos (MPB), live seagrass blades (SG) and seagrass detritus (SGD) from all study sites (n=4).

Table 3.11: One-way ANOVA and LSD post-hoc results of the effect of site on the mean $\delta^{15}N$ and $\delta^{13}C$ signatures of the potential food sources. Significant results are marked: *P <0.05.

Variable	Source of variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD Post-hoc test
Seagrass $\delta^{15}N$	Site	3	1.24	21.9	<0.001*	TU=TL<WP<RG
	Error	12	0.057			
Seagrass $\delta^{13}C$	Site	3	3.52	671	<0.001*	
	Error	12	0.005			TL=WP>TU>RG
Seagrass Detritus $\delta^{15}N$	Site	3	4.69	48.1	<0.001*	TU<TL=WP<RG
	Error	12	0.097			
Seagrass Detritus $\delta^{13}C$	Site	3	7.89	204	<0.001*	
	Error	12	0.039			TL>TU=WP>RG
Phytoplankton $\delta^{15}N$	Site	3	21.2	1.34	0.306	
	Error	12	15.8			
Phytoplankton $\delta^{13}C$	Site	3	19.6	65.2	<0.001*	
	Error	12	0.301			TL>TU>WP>RG
Microphytobenthos $\delta^{15}N$	Site	3	0.794	0.311	0.817	
	Error	12	2.55			
Microphytobenthos $\delta^{13}C$	Site	3	21.0	6.18	0.009*	
	Error	12	3.40			TU=TL=WP>RG

3.3.2 Isotope signatures of macro-invertebrate consumers

Dual isotope plots of the mean $\delta^{15}N$ and $\delta^{13}C$ signatures of macro-invertebrate consumers and their potential food sources sampled from each site and sampling location were plotted (Figures 3.7-3.10). Standard deviations for consumers are not plotted in figures to avoid confusion but are available in Table 3.10. The $\delta^{15}N$ and $\delta^{13}C$ values of macro-invertebrate consumers were variable between locations among sites and ranged between 6.9 to 13.3‰ and -18.6 to -7.8‰, respectively. It was expected that if seagrass is being assimilated by organisms inside the seagrass beds then the $\delta^{13}C$ of organisms will be more enriched relative to the $\delta^{13}C$ values of organisms on non-vegetated sediment.

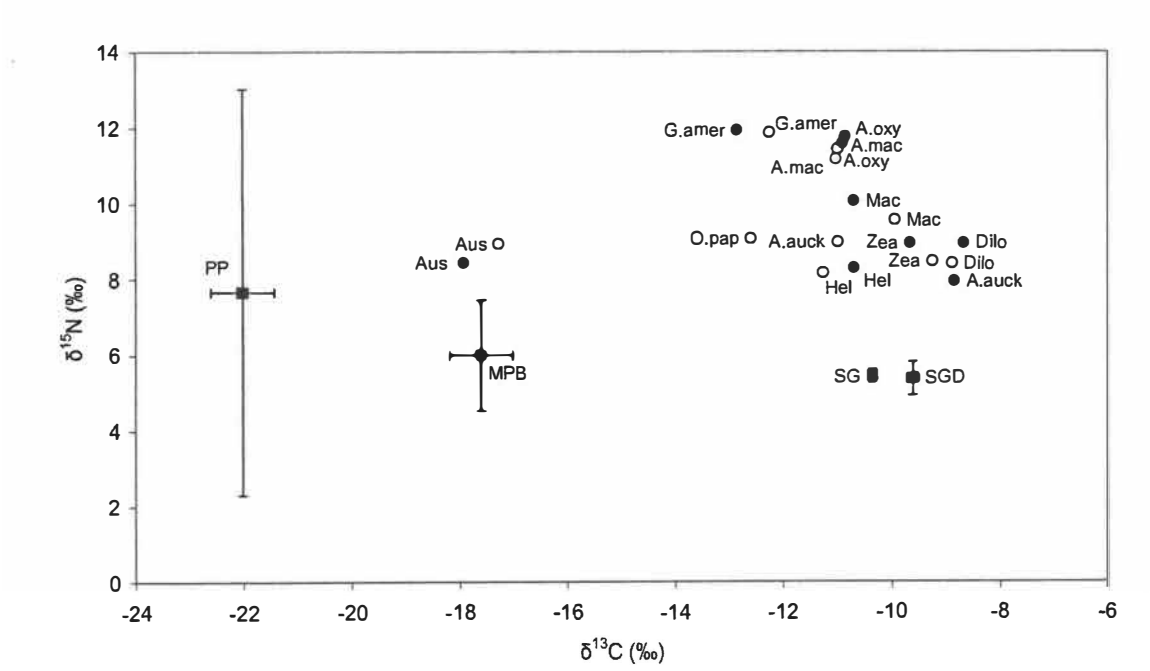

Figure 3.6: Tauranga Upper (TU). Mean stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios (±1SD) for potential food sources (■) and macro-invertebrate consumers collected IN (●) and OUT (○) of the seagrass bed (n=4).

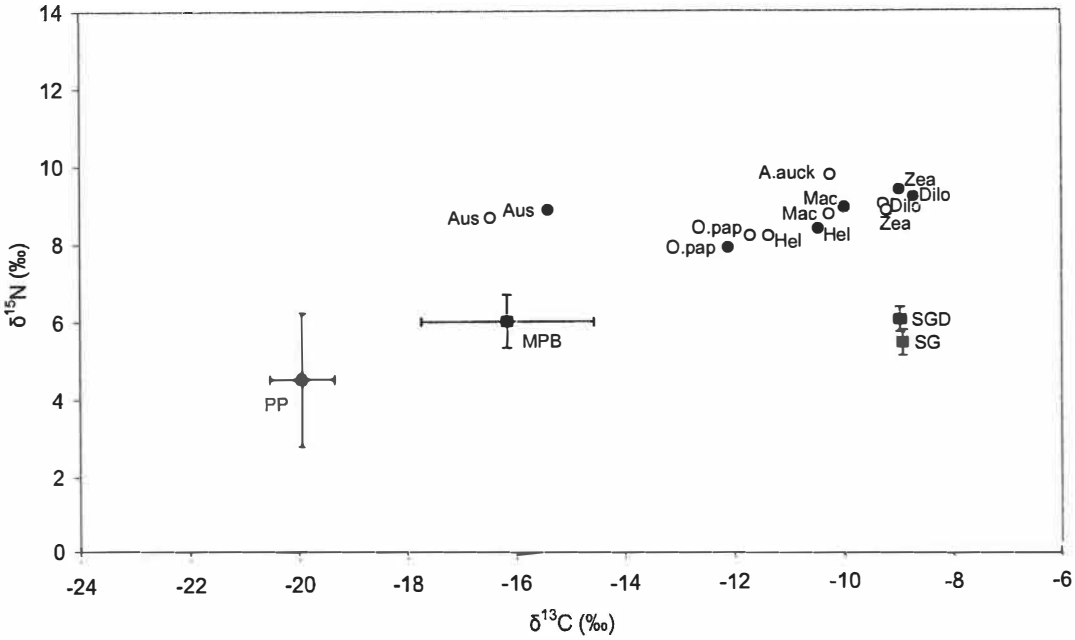

Figure 3.7: Tauranga Lower (TL). Mean stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios (±1SD) for potential food sources (■) and macro-invertebrate consumers collected IN (●) and OUT (○) of the seagrass bed at site (n=4).

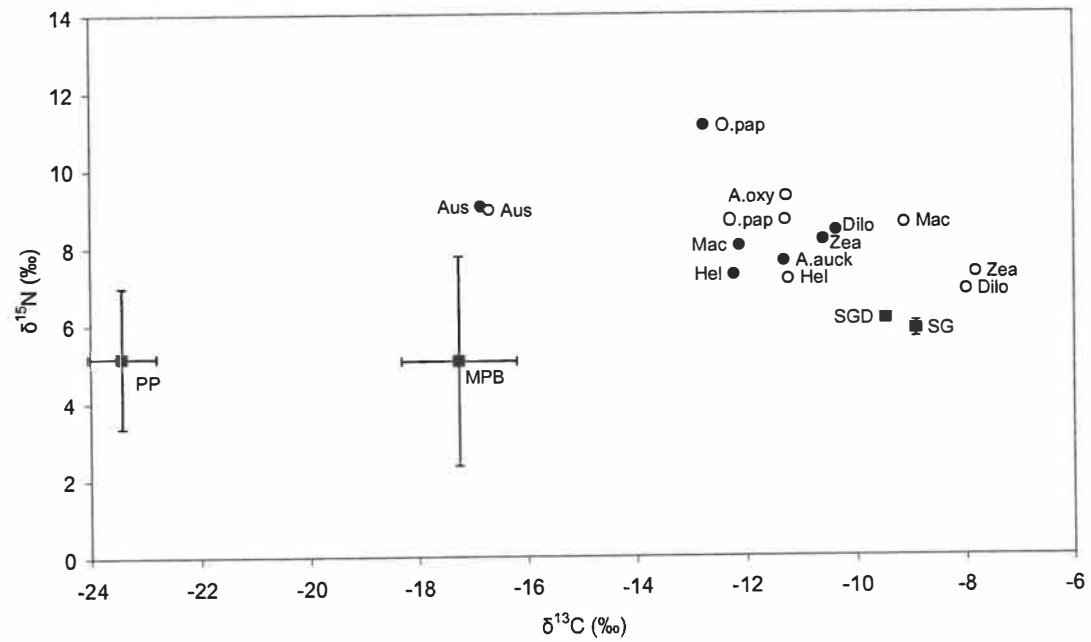

Figure 3.8: Whangapoua (WP). Mean stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios (±1SD) for potential food sources (■) and macro-invertebrate consumers collected IN (●) and OUT (○) of the seagrass bed at study site (n=4).

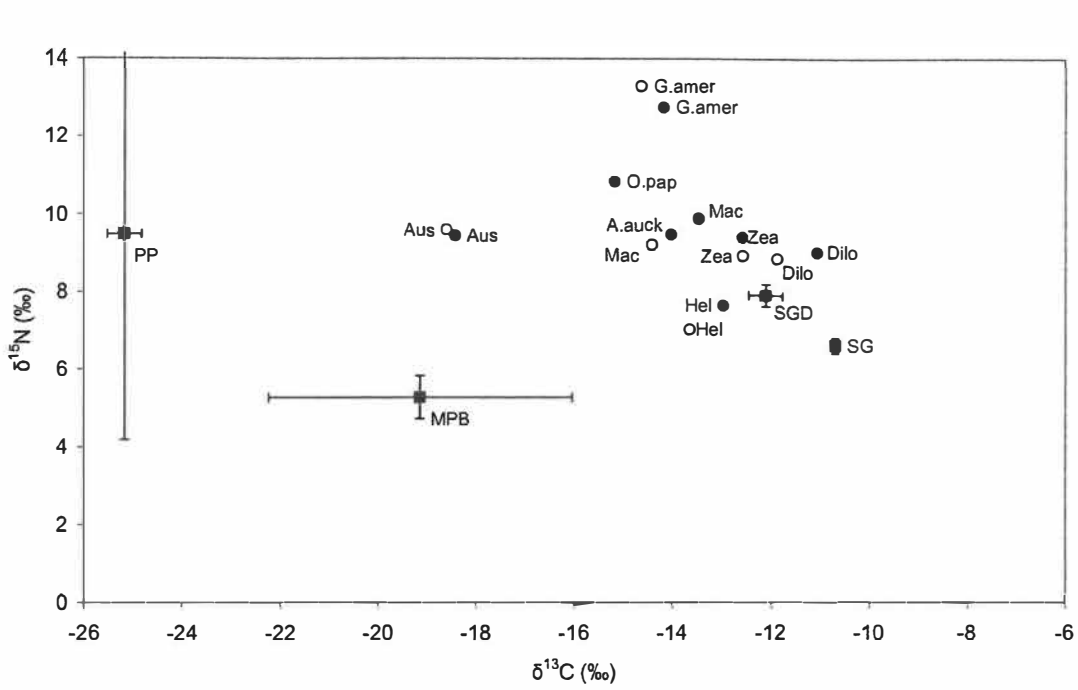

Figure 3.9: Raglan (RG). Mean stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios (±1SD) for potential food sources (■) and macro-invertebrate consumers collected IN (●) and OUT (○) of the seagrass bed at study site (n=4).

The $\delta^{13}C$ values of *A. stutchburyi* were consistently more depleted compared to the other consumers collected at all locations from all sites and were similar to values reported in literature for suspension-feeding bivalves (Appendices: Figures 7 and 8) (Table 3.10 and Figures 3.7-3.10). This suggests that *A. stutchburyi* is utilising a different food source or combination of food sources compared to the other consumers sampled. Subsequently, consistent among sites the $\delta^{13}C$ values of *A. stutchburyi* was similar to microphytobenthos and had a $\delta^{15}N$ fractionation of approximately 3-4‰ relative to microphytobenthos (Figures 3.7-3.10). The effect of site on the $\delta^{15}N$ and $\delta^{13}C$ of *A. stutchburyi* was significant (P<0.05) and LSD post-hoc test results inferred that the difference between RG and the east coast sites contributed to the significant difference among sites (Table 3.12). The nitrogen isotope signatures of *A. stutchburyi* at

sampling locations were similar; however, significant differences were observed in the $\delta^{13}C$ of *A. stutchburyi* at sampling locations among sites (ANOVA; $P<0.05$; Figure 3.7-3.10 and Table 3.12). *A. stutchburyi* was more depleted in ^{13}C at TU IN and WP IN and more enriched at TL IN and RG IN compared to OUT locations. Differences observed in consumer $\delta^{13}C$ values between locations among sites are indicative of the utilisation of varying proportions of the different food sources. Furthermore, there was no consistent variation in the $\delta^{13}C$ of *A. stutchburyi* between IN and OUT among study sites (ANOVA; $P<0.05$; Figures 3.7-3.10 and Table 3.12). It appears that all food webs at each site are driven mainly by benthic carbon rather than pelagic phytoplankton. However, it is recognised that this may change throughout the year with season.

Grouped with most of the other macro-invertebrates, *M. liliana* displayed enriched $\delta^{13}C$ values and was similar to that of *Z. muelleri* live blades and detritus (Figures 3.7-3.10 and Table 3.10). Due to the refractory nature of seagrass and the feeding mechanisms of *M. liliana*, the assimilation of seagrass carbon by *M. liliana* would have to be via a detrital pathway that has not been identified in the present study. The mean $\delta^{13}C$ and $\delta^{15}N$ values of *M. liliana* was significantly different between sites and ranged from to -13.9‰ at RG to -10.1‰ at TL and to 8.3‰ at WP to 9.8‰ at TU and was consistent with deposit-feeding bivalve values recorded in literature (Appendices: Figures 9 and 10) (ANOVA; $P<0.05$; Table 3.12). The differences in the $\delta^{15}N$ and $\delta^{13}C$ values of *M. liliana* between locations were also significantly different ($P<0.05$).

The mean $\delta^{15}N$ and $\delta^{13}C$ values of the top shell *D. subrostrata* were also enriched in ^{13}C and had an approximate fractionation of 2-3‰ relative to *Z. muelleri* at all sites and locations (Figures 3.7-3.10). The mean $\delta^{15}N$ and $\delta^{13}C$ values of *D. subrostrata* among sites (across locations) were significantly different and ranged between 7.6‰ at WP to 9.1‰ at TL and between -11.5‰ at RG to -8.8‰ at TU (ANOVA; $P<0.05$; Figures 3.7-3.10 and Table 3.12). Differences between locations were not significantly different for $\delta^{13}C$, however visual inspection of the data shows slight differences between IN and OUT that is positive at TL and negative at WP (Tables 3.12). Conversely, differences in the $\delta^{15}N$ of *D. subrostrata* between locations was significant ($P<0.05$) which may be an indication of environment anthropogenic conditions. At all study sites, the mean $\delta^{15}N$ and $\delta^{13}C$ values of *Z. lutulentus* was consistently similar to those values of *D. subrostrata* and was also grouped with the macro-invertebrate consumers enriched in ^{13}C (Figures 3.7-3.10). The similarity of $\delta^{13}C$ of *Z. lutulentus* to *D. subrostrata* may indicate a similarity in their respective diets. *Z. lutulentus* also had similar $\delta^{13}C$ values as *Z. muelleri* live blades and detritus at all locations among sites and had a fractionation of approximately 3-4‰ relative to *Z. muelleri*. The mean $\delta^{15}N$ and $\delta^{13}C$ signatures of *Z. lutulentus* for sampling locations (averaged across sites) inside the seagrass bed was 9.0 and -10.4‰ compared to 8.8 and -9.7‰ on non-vegetated sediment. The $\delta^{13}C$ and $\delta^{15}N$ values of both gastropods were within the range presented in literature for deposit feeding gastropods (Appendices: Figures 9 to 10). Differences existing between sites and locations for $\delta^{15}N$ and $\delta^{13}C$ values of *Z. lutulentus* were significant ($P<0.05$) (Table 3.12). Macro-invertebrate consumers, including *D.* subrostrata and *Z. lutulentus* collected from both inside and outside seagrass

display $\delta^{13}C$ values that are ^{13}C-enriched similar to *Z. muelleri* (Figures 3.7-3.10). However, *Z. muelleri* did not occur on non-vegetated sediments, inferring that these mobile grazers may move in and out of seagrass to feed. In addition, this result may indicate the exportation of seagrass to non-vegetated regions

Although the mud crab, *H. crassa*, was enriched in ^{13}C compared to phytoplankton and microphytobenthos it was consistently depleted at all location among sites relative to *Z. muelleri* (Figures 3.7-3.10). This suggests that *H. crassa* may utilise a number of food sources including seagrass and microphytobenthos but also a number of unidentified food sources. Both the $\delta^{15}N$ and $\delta^{13}C$ values of *H. crassa* displayed consistent significant differences between sites ($P<0.05$) but differences between locations was not significant (Table 3.12). The $\delta^{13}C$ and $\delta^{15}N$ values of *H. crassa* were within the range of $\delta^{13}C$ and $\delta^{15}N$ for crustaceans presented in literature (Appendices: Figures 11 and 12).

A number of species of polychaetes were collected to account for their inconsistent abundances at each site and location. *G. americana* was collected from both locations at TU and RG and mean $\delta^{15}N$ and $\delta^{13}C$ values were 12.4 and -13.5‰, respectively (Figure 3.7-3.10). Consistently, in both food webs the $\delta^{15}N$ and $\delta^{13}C$ values were enriched relative to potential food sources and consumers. The large enrichment in ^{15}N for *G. americana* is characteristic of a carnivorous predator and subsequently, the $\delta^{13}C$ values of this species are similar to consumers such as *O. papillosa* and *A. aucklandica* which may be potential food sources for *G. americana*. *A. oxycephala* and *A. macroura* were

collected from both locations at TU and were more enriched in ^{13}C but had similar $\delta^{15}N$ values compared to *G. americana* (Figure 3.7). These species may also be predators consuming both dead macro-invertebrate material and plant material (as the $\delta^{13}C$ values are in the same region as *Z. muelleri*). The $\delta^{15}N$ and $\delta^{13}C$ values of these species were very similar indicating that they may occupy the same trophic guild and consume similar diets. Polychaete species *O. papillosa* was collected from TU, TL and RG, whilst *A. aucklandica* was collected from TU, WP and RG (Figures 3.7-3.10). Both species fall into the aggregation of ^{13}C-enriched consumers but at all sites where *O. papillosa* was collected, it was slightly less enriched in ^{13}C compared to the other consumers. Visual observations imply that it may consume a number of food sources including microphytobenthos as it sits intermediate between microphytobenthos and *Z. muelleri* at TU, TL and RG (Figures 3.7, 3.8 and 3.10). The $\delta^{13}C$ and $\delta^{15}N$ values of polychaete species collected in this study may be compared to the $\delta^{13}C$ and $\delta^{15}N$ of similar polychaete species recorded in literature (Appendices: Figures 13 and 14).

Graphical observation of species collected at the control sites A and B at RG revealed no significant differences in $\delta^{13}C$ and $\delta^{15}N$, implying that the differences in the isotopic ratios of organisms IN and OUT is independent of distance (Figure 3.11). Consistent with each of the other study sites (Figures 3.7-3.10), *A. stutchburyi* has similar $\delta^{13}C$ values compared to microphytobenthos and *M. liliana*, *D. subrostrata* and *Z. lutulentus* are more ^{13}C-enriched and clustered together. Furthermore, and similarly to the other study sites TU, TL, WP and RG, polychaete species *G. americana* and *A.*

macroura are greatly ^{15}N-enriched with similar $\delta^{13}C$ values and a fractionation of approximately 2-3‰ relative to *M. liliana*, *D. subrostrata* and *Z. lutulentus*.

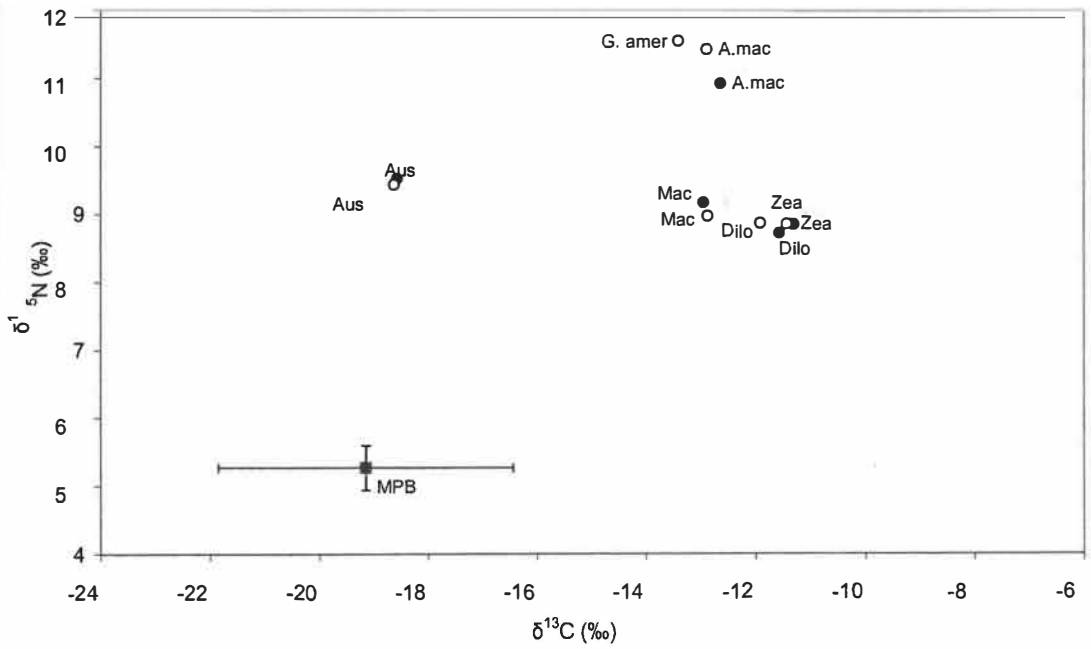

Figure 3.10: Raglan, control site. Mean stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios (±1SD) for MPB and macro-invertebrate consumers collected from control sites A (●) and B (○) situated 50 m apart on non-vegetated sediment (n=4).

Table 3.12: Two-way ANOVA and LSD post-hoc results of the effects of site and location on the mean $\delta^{15}N$ and $\delta^{13}C$ signatures of macro-invertebrate consumers. Significant results are marked: *P <0.05.

Variable	Source of variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD post-hoc test
Austrovenus stutchburyi $\delta^{15}N$	Site	3	1.11	46.8	<0.001*	
	Location	1	0.059	2.50	0.129	
	Site × Location	3	0.217	9.20	<0.001*	TU IN<TU OUT
	Error	24	0.024			TL IN> TL OUT
Austrovenus stutchburyi $\delta^{13}C$	Site	3	9.93	508	<0.001*	
	Location	1	0.088	4.50	0.044*	
	Site × Location	3	1.02	52.3	<0.001*	TU IN<TU OUT
	Error	24	0.02			TL IN>TL OUT
Macomona liliana $\delta^{15}N$	Site	3	3.66	177	<0.001*	
	Location	1	0.321	15.5	<0.001*	
	Site × Location	3	0.653	31.5	<0.001*	TU IN>TU OUT
	Error	24	0.021			WP IN<WP OUT
						RG IN> RG OUT
Macomona liliana $\delta^{13}C$	Site	3	26.1	411	<0.001*	
	Location	1	3.22	50.6	<0.001*	
	Site × Location	3	5.96	93.7	<0.001*	TU IN<TU OUT
	Error	24				WP IN<WP OUT
						RG IN>RG OUT
Diloma subrostrata $\delta^{15}N$	Site	3	3.29	23.5	<0.001*	
	Location	1	2.98	21.3	<0.001*	
	Site × Location	3	0.836	5.99	0.003*	TU IN>TU OUT
	Error	24	0.140			WP IN>WP OUT

Table 3.12: Continued...

Variable	Source of variability	*df*	Mean Square Error	*F* ratio	*P* value	Significant LSD Post-hoc test
Diloma subrostrata $\delta^{13}C$	Site	3	12.6	129	<0.001*	
	Location	1	0.315	3.22	0.085	
	Site × Location	3	4.32	44.3	<0.001*	TL IN>TL OUT
	Error	24	0.098			WP IN<WP OUT
						RG IN>RG OUT
Zeacumantus lutulentus $\delta^{15}N$	Site	3	3.44	68.2	<0.001*	RG=TL>TU>WP
	Location	1	2.68	53.2	<0.001*	IN>OUT
	Site × Location	3	0.064	1.27	0.307	
	Error	24	0.050			
Zeacumantus lutulentus $\delta^{13}C$	Site	3	22.3	186	<0.001*	
	Location	1	4.46	37.3	<0.001*	
	Site × Location	3	3.82	31.9	<0.001*	WP IN<WP OUT
	Error	24	0.120			
Helice crassa $\delta^{15}N$	Site	3	2.52	15.5	<0.001*	(TU=TL)>(WP=RG)
	Location	1	0.571	3.52	0.073	
	Site × Location	3	0.110	0.680	0.574	
	Error	24	0.162			
Helice crassa $\delta^{13}C$	Site	3	9.95	22.0	<0.001*	
	Location	1	0.641	1.42	0.245	
	Site × Location	3	1.48	3.28	0.038*	WP IN<WP OUT
	Error	24	0.452			

4 Discussion

The $\delta^{13}C$ and $\delta^{15}N$ values indicated that seagrass is an important food source for most of the macro-invertebrate species sampled, such as *Diloma subrostrata* and *Zeacumantus lutulentus*. Furthermore, organisms including *Helice crassa* and *Macomona liliana* had $\delta^{13}C$ values intermediate between seagrass and microphytobenthos, indicating the consumption of both sources of organic matter. The filter-feeding bivalve *Austrovenus stutchburyi* appeared to consume microphytobenthos rather than phytoplankton. Polychaetes *Glycera americana* and *Aglaophamus macroura* displayed $\delta^{15}N$ values greater than those of other consumers, suggesting that they were predominantly predators. Three trophic levels (potential food sources, suspension and herbivorous deposit feeders and predators) were identified at each study site. There was no significant difference in the isotopic compositions of macro-invertebrates between sampling locations, but a significant difference was observed between sites, where macro-invertebrate tissues tended to be more enriched in ^{15}N and depleted in ^{13}C at Raglan compared to the remaining sites on the east coast. Macro-invertebrate communities within seagrass at each site were generally more diverse and were dissimilar than those in adjacent non-vegetated sediment. However, the percent abundance of organisms sampled for stable isotope analysis was lower in seagrass beds than in non-vegetated sediment.

4.1 Stable Isotope Signatures of Food Sources

The $\delta^{13}C$ signatures of the potential food sources at all study sites ranged between -25 and -9‰ and were distinct from each other enabling the flow of carbon to be traced through the food web (Fry and Sherr 1984, Peterson 1999). Phytoplankton was the most ^{13}C-depleted food source and seagrass (fresh blades and detritus) was the most ^{13}C-enriched, with microphytobenthos intermediate (-23, -10 and -20‰, respectively). This observation is consistent with the finding of Fry and Sherr (1984), where phytoplankton and seagrass displayed mean $\delta^{13}C$ values of -21 and -10‰, respectively and the mean $\delta^{13}C$ value of benthic algae was intermediate.

4.1.1 Phytoplankton

Mean $\delta^{13}C$ and $\delta^{15}N$ isotopic values for phytoplankton across study sites were -23 and 7‰, respectively. These values were similar to those recorded by Leduc (2006) in Harwood and Waitati estuaries, Otago, New Zealand, and also similar to the average values -21‰ and 9‰ recorded by Peterson and Howarth (1987) and Fry and Sherr (1984) (Appendices: Figures 1 and 2). Furthermore, phytoplankton values in literature are often estimated from particulate organic matter (POM) in the water column which represents a variable mixture of living and dead phytoplankton and other components. Mean $\delta^{13}C$ POM values from literature were -21‰ (Vizzini et al. 2002), consistent with mean $\delta^{13}C$ phytoplankton signatures from literature and also in the present study (-23‰). Variation in the mean $\delta^{13}C$ values of phytoplankton was observed at all sites and has also been reported in literature (Peterson 1999). The variation, according to Fry et al. (1983), is possibly due to variations in the isotopic ratios among estuarine

phytoplankton species and thought to be caused by temporal and spatial environmental (differences in inorganic carbon inputs) (Wada et al. 1993) and physiological factors (ability to use different carbon and nitrogen sources; size of the algae) (Falkowski 1991, Peterson 1999, Vizzini et al. 2002). Consequently this variation can be great between study sites (McMillian et al 1980).

4.1.2 Microphytobenthos

Microphytobenthos has been reported by Behringer and Butler (2006) to be a significant source of organic matter for macro-invertebrate organisms in estuarine food webs. Mean isotopic signatures for microphytobenthos were 6 to 5‰ for $\delta^{15}N$ and -16 to -24‰ for $\delta^{13}C$. These values are intermediate between phytoplankton and seagrass at all study sites. The $\delta^{13}C$ signatures of microphytobenthos from the present study are similar to literature values reported for benthic algae, -8 to -27‰ (Fry and Sherr 1984) and are also consistent with the only other known recorded values in New Zealand by Leduc (2006) (Appendices: Figures 2 and 3). In addition, the $\delta^{13}C$ values of microphytobenthos at each site were approximately 8‰ less than seagrass which is similar to the findings of Smit et al. (2006).

4.1.3 Seagrass

Mean $\delta^{13}C$ values of seagrass across study sites was -11 to -9‰, within the wide range (-3 and -24‰) published by McMillan et al. (1980) and McMillan and Smith (1982) (Appendices: Figures 5 and 6). Seagrass is more enriched in ^{13}C than C_3 plants generally are ($\delta^{13}C$ approximately -28‰, O'Leary 1988). Mayberly (1990) reports that intertidal species such as *Ulva lactuca* and seagrass display increased

$\delta^{13}C$ values due their ability to use HCO_3^- as a carbon source aided by high levels of external carbonic anhydrase (Hurd 2000, Raven et al. 2002). The enrichment of seagrass tissue in ^{13}C is common among seagrass species and several investigations have recorded that seagrasses are enriched in ^{13}C relative to most other marine plants and organic matter (e.g. Fry et al. 1983, Fry and Sherr 1984, Vizzini et al. 2002). In the present study, the mean $\delta^{13}C$ and $\delta^{15}N$ values of fresh seagrass blades and detritus were similar across sites, -10 and 5‰ and -10 and 6‰, respectively. Hyndes and Lavery (2005), Vizzini et al. (2002) and Kurata et al. (2001) reported that there is a negligible change in the C and N isotope signatures of detritus relative to the starting materials (fresh seagrass). Conversely, Peterson (1999) reported that N isotope ratios can change during decomposition of detritus from seagrass, which is generally what was observed in this study with a mean change of <1‰ at all sites except for TU. Many studies have reported the flow of seagrass through food webs (e.g. Kharlamenko et al. 2001, Winning et al. 1999, Fry et al. 1983, Hyndes and Lavery 2005, Kitting et al. 1984, Vizzini et al. 2002). However, the way in which seagrass is utilised (e.g. fresh material or detritus) is generally uncertain. Researchers such as Jones et al. (2003), Smit et al. (2006) and Vizzini et al. (2002) state that although seagrass carbon may be utilised by consumers, only specific species are able to exploit the fresh photosynthetic leaves and largely it is not readily utilised by herbivores mainly due to its poor nutritional value. Seagrasses contain a high proportion of complex carbohydrates, such as cellulose and lignin therefore making this source of organic matter poorly digestible (Hyndes and Lavery 2005, McCutchan et al. 2003, Vizzini et al. 2002). Due to the refractory nature of seagrass, it is suggested that the main route of transfer for seagrass carbon entering the food chain is

through the detrital pathway where it is broken down by mechanical and biological means (Jones et al. 2003, Kharlamenko et al. 2001, Kurata et al. 2001, Vizzini et al. 2002).

4.2 Stable Isotope Signatures of Macro-Invertebrates and Spatial Variability

It is generally accepted that the $\delta^{13}C$ isotopic signatures of animals is +0.5-1‰ (McCutchan et al. 2003, Page and Lastra 2003, Peterson and Fry 1987), relative to their diet. If seagrass contributes to the diet of macro-invertebrates then the $\delta^{13}C$ values of animals should be ^{13}C-enriched and similar to the $\delta^{13}C$ value of seagrass. Alternatively, if animals are relying on algae for nutrition then the $\delta^{13}C$ values should be ^{13}C-depleted and closer to the values of food sources such as phytoplankton and microphytobenthos (approximately between -21 and -18‰) (Fry et al. 1983, Jones et al. 2003). The nitrogen isotope ratios of benthic consumers have an increase in $\delta^{15}N$ of approximately 2.4‰ per trophic transfer (Carmichael et al. 2004, McCutchan et al. 2003) and in the present study were used to provide trophic level information for benthic macro-invertebrates. Three trophic levels within the communities at each site were observed; potential food sources, deposit and suspension feeding and predatory organisms.

4.2.1 Herbivorous deposit feeders

The mean $\delta^{13}C$ value for *D. subrostrata* and *Z. lutulentus* were the same (-10‰) and were also similar to the $\delta^{13}C$ values of seagrass suggesting that both species utilise the same organic matter (seagrass) as a food source. $\delta^{13}C$ values of *D.*

subrostrata and *Z. lutulentus* were similar to other herbivorous grazing gastropod species recorded in literature, including *Littorina squalida* (-9.6‰) (Kharlamenko et al. 2001) (Appendices, Figures 9 and 10). The $\delta^{15}N$ values of *D. subrostrata* and *Z. lutulentus* were also similar (approximately 9‰) and they had an approximate $\delta^{15}N$ fractionation of +3‰ relative to the mean $\delta^{13}C$ value of seagrass (6‰). During sampling *D. subrostrata* was often observed on seagrass leaves and detritus inside seagrass beds rather than directly on the sediment. Therefore, observations during sampling and the stable isotope results suggest that these gastropods exclusively use *Z. muelleri* as a food source. Many studies support the findings of this study, including research conducted by Kharlamenko et al. (2001). Behringer and Butler (2006) found that seagrass carbon plays an important role as a food resource for *L. squalida* and *Lithopoma tectum* in the *Zostera marina* community.

The mean $\delta^{13}C$ and $\delta^{15}N$ values of the deposit feeding bivalve *M. liliana* ($\delta^{13}C$ = -11 and $\delta^{15}N$ = 9‰), crustacean *H. crassa* (-11 and 8‰) and polychaete species *Orbinia papillosa* (-12 and 9‰) and *Aquilaspio aucklandica* (-11 and 9‰) were clustered together at all sites, intermediate between the $\delta^{13}C$ values of seagrass and microphytobenthos. Compared to literature values of the relevant taxa the $\delta^{13}C$ and $\delta^{15}N$ isotope values obtained were within the ranges reported in literature (Appendices: Figures 7-14). The aggregation of these species suggests that a combination of benthic resources (seagrass and microphytobenthos) and potentially other unidentified sources such as *Ulva lactuca* are being utilised by these organisms. Unpublished preliminary data collected in 1999 reported $\delta^{13}C$ and $\delta^{15}N$ values of *U. lactuca* to be -11 and 8 ‰ (Hicks and Pilditch 2004,

Maberly 1990). Therefore, where *U. lactuca* is present, species such as *M. liliana* and *H. crassa* may be assimilating it as a food source. The $\delta^{13}C$ values of *M. liliana* were more similar to *D. subrostrata* and *Z. lutulentus* at TU and RG but were largely intermediate between *Z. muelleri* and microphytobenthos. These findings confirm that *M. liliana* is a deposit feeding bivalve in contrast to the suspension feeding bivalve *A. stutchburyi*. It is commonly recognised that the crustacean *H. crassa* is an omnivore (Gill 1998, McLay 1988), however at all sites, the mean $\delta^{15}N$ values are either similar to or are less enriched in ^{15}N compared to the other consumers sampled. Subsequently, it appears that *H. crassa* is more herbivorous than omnivorous. The results this study and other research shows that grazing represents an important conduit for the transport of nutrients from primary producers to consumers of higher trophic levels (Heck and Valentine 2006)

4.2.2 Suspension feeder: *Austrovenus stutchburyi*

A. stutchburyi was depleted in ^{13}C and has an isotopic composition similar to microphytobenthos rather than phytoplankton ($\delta^{13}C$ = -17 and $\delta^{15}N$ = 9‰). The mean $\delta^{13}C$ and $\delta^{15}N$ values of *A. stutchburyi* were consistent with those published for other filter-feeding bivalves feeding on an algal diet, including *Macoma balthica* ($\delta^{13}C$ = -16) (Herman et al. 2000, Riera et al. 1999) (Appendices: Figures 7 and 8). The results of this study suggest that microphytobenthos is being resuspended into the water column from the benthos and contributing organic matter to the diet of this bivalve. This finding is supported by research conducted by de Jonge and van Beusekom (1995) and Delgado et al (1991) in the Ems estuary where they reported that under natural estuarine conditions, sediment

including microphytobenthos is rapidly exchanged between tidal flats and the overlying water (Lucas et al. 2000). *A. stutchburyi* have short siphons that extend just above the sediment-water interface (Crowe 1999, Gill 1998, Lundquist et al. 2004, Morton and Miller 1973) and therefore it is physiologically possible that resuspended microphytobenthos from the sediment is assimilated by *A. stutchburyi* as a food source. The importance of microphytobenthos as a food source for suspension feeding bivalves has been reported by Machas et al. (2003) and Sauriau and Kang (2000). Herman et al. (2000) and Middleburg et al. (2000) also demonstrated the assimilation of benthic algae by suspension feeders using laboratory experiments. Conversely, Hackney and Haines (1980) reported that filter-feeding bivalves tend to reflect the $\delta^{13}C$ signature of phytoplankton in the water column. Phytoplankton seemed to play an insignificant role as a food source to *A. stutchburyi* at the study sites, however this maybe due a decreased abundance of phytoplankton during winter when the sampling was conducted. The abundance of phytoplankton during the summer months may be more significant with increased water temperature and light levels. In summer, suspension feeding bivalves displayed low $\delta^{13}C$ values indicative of the assimilation of phytoplankton (Page and Lastra 2003 and Machas et al. 2003).

4.2.3 Predatory polychaetes

The tissues of *G. americana*, *Aonides oxycephala* and *A. macroura* ($\delta^{15}N$ values ranging from 9 to 13‰) were consistently enriched in ^{15}N, indicative of a higher trophic level and the $\delta^{13}C$ values of these polychaetes overlapped many of the herbivorous macro-invertebrates (for example, *O. papillosa*, *A. aucklandica*), suggesting that they are possibly a food source for these organisms. Due to the

greater $\delta^{15}N$ values and similar $\delta^{13}C$ values of *G. americana*, *A. oxycephala* and *A. macroura* compared to those of the herbivorous deposit feeders previously mentioned, it suggests that these polychaetes are predators. In addition, the assimilation of seagrass by herbivorous grazers, which are themselves in turn consumed by these predatory polychaetes demonstrates that seagrass organic matter enters the food chain and the $\delta^{13}C$ values of seagrass is echoed through the trophic levels of organisms within the communities.

4.2.4 Spatial variability in the isotope signatures of macro-invertebrates between sampling locations and study sites

Prior to sampling it was hypothesised that organisms collected from non-vegetated sediment would display $\delta^{13}C$ values that were depleted in ^{13}C suggesting dependence on microphytobenthos or phytoplankton. In addition, organisms collected from within the seagrass were hypothesised to display $\delta^{13}C$ values that were more enriched in ^{13}C similar to their seagrass habitat. Fry and Sherr (1984) support these hypotheses, however results of the present study suggested that organisms of the same species had similar diets between seagrass and non-vegetated sediments were not significant across study sites. This suggests that organisms sampled from non-vegetated sediment also had a dependence on seagrass organic matter. Hackney and Haines (1980) sampled estuarine invertebrates from two neighbouring areas dominated by *Spartina* and the other, *Juncus*. Their findings indicated that the detritivores and omnivores did not show preference for either macrophyte species, rather both sources of organic matter were being utilised. Also, Behringer and Butler (2006) reported that seagrass may contribute to food webs of unvegetated habitats. Samples were collected in winter

and with increased storm activity and wave action, seagrass detritus may be removed from seagrass beds and transported to non-vegetated sites where there is no obvious presence of seagrass (Dauby 1995). Therefore, it may be hypothesised that the isotope signatures of macro-invertebrate consumers during the winter become more enriched with $\delta^{13}C$ values close to or similar to seagrass due to the increased transportation of detritus to non-vegetated areas.

Control sampling locations at Raglan showed no difference in the isotopic signatures of macro-invertebrates between locations and therefore differences in the values of macro-invertebrates at TU, TL, WP and RG were not due to the distance between sampling locations. The $\delta^{13}C$ values of *D. subrostrata* and *Z. lutulentus* at the control site were not as enriched in ^{13}C as those species from the sites associated with seagrass. This suggests that in areas away from seagrass, macro-invertebrate isotopic signatures do not reflect the assimilation of seagrass but may reflect another source of organic matter enriched in ^{13}C. Raven et al. (2002) reported that algae such as some red and green species can exhibit enriched $\delta^{13}C$ values similar to seagrass (approximately -10‰) which is characteristic of their ability to use HCO_3^-. Furthermore Maybery (1990) reported that *Ulva lactuca* also displays enriched $\delta^{13}C$ values similar to seagrass. Therefore, the enriched $\delta^{13}C$ signatures of the herbivorous gastropods at the control study site and also potentially at the other study sites maybe due to the consumption of macro-algae enriched in ^{13}C.

Significant differences existed in the isotopic signatures of food sources and benthic macro-invertebrates sampled between east (TU, TL, WP) and west coast

(RG) study sites were observed. The isotopic values of food sources and consumers sampled at RG were generally more enriched in $\delta^{15}N$ and depleted in $\delta^{13}C$ compared to the other study sites. This suggests that there are differences in the inorganic nutrient inputs fuelling the productivity of these systems. One possible explanation and documented by McClelland et al. (1997) is that elevated $\delta^{15}N$ signatures of phytoplankton and macroalgae maybe linked to terrestrial nitrogen inputs from adjacent catchments. Supporting the findings of McClelland et al. (1997), Cabana and Rasmussen (1996) and Carmichael et al. (2004) also found that as wastewater inputs into Pleasant Bay, Cape Cod increased the $\delta^{15}N$ isotopic signatures of suspended and benthic organic matter and consequently the tissues of *Mercenaria mercenaria* had elevated $\delta^{15}N$ values. Furthermore, the $\delta^{15}N$ values of water that are affected by anthropogenic nitrogen input and that of those pristine habitats are approximately 11.0 and 3.3‰, respectively (Cabana and Rasmussen 1996).

4.3 Macro-Invertebrate Community Compositions

The macro-invertebrate community composition within seagrass compared to adjacent non-vegetated sediment was distinct across all study sites. Seagrass is known to alter community structure (Smit et al. 2006) and positive correlations between macrofaunal biomass, diversity and abundance within seagrass beds (Alfaro 2006, Heck and Wetstone, 1977), compared to adjacent non-vegetated sediment have been recorded (Hackney and Durako 2004, Heck et al. 1989, van Houte-Howes et al. 2004, Vizzini et al. 2002). Differences in macro-invertebrate community

compositions between locations may be due to a myriad of factors, including habitat complexity and productivity.

Seagrass provides a three-dimensional habitat structure, whereby micro- and macro-fauna are able live within the root-rhizome system, on the blades, or within the interstitial space created between the sediment and blades (Heck and Valentine 2006, Inglis 2003). This three-dimensional structure of seagrass provides a number of habitats, increasing macro-invertebrate abundance and diversity and also provides a nursery for commercially important fish and shellfish species which are not present in non-vegetated sediments (Heck and Valentine 2006, Klumpp and Nichols 1983, Stoner 1980).

Seagrass in itself is an area of high productivity compared to adjacent non-vegetated sediment. However, the productivity of seagrass beds is enhanced by the presence of epiphytes (Kitting et al 1984, Loneragan et al. 1997, Nichols et al. 1985, Smit et al. 2006) and also by the sedimentation of organic matter from the over lying water column caused by low current velocities (Inglis 2003, Howard 1982, Heiss et al. 2000).

4.4 Research Limitations

Although this study provides important evidence of the use of seagrass biomass as a food source by consumers it is important to consider the possibility that other sources of organic matter may be contributing to the isotope results observed.

The assumed dependence on *Z. muelleri* by macro-invertebrates may not be accurate if other potential food sources also enriched in ^{13}C are present. Some species of algae present in estuaries (Raven et al. 2002) and *Ulva lactuca* (Maberly 1990), can exhibit enriched $\delta^{13}C$ values similar to seagrass (approximately -10‰) which is characteristic of their ability to use HCO_3^-. Therefore, the enriched $\delta^{13}C$ signatures of organisms may not be due to the seagrass but maybe to another source of organic matter. Moreover, the interpretation of the diets of consumers is limited knowledge of the various factors that may cause variation in isotope signatures and also the types of tissues analysed and their respective turnover rates.

A further research limitation existed in the isotopic variability of the food sources phytoplankton and microphytobenthos sampled. Isosource mixing models (Phillips and Gregg 2001) were going to be used however, the variability in $\delta^{13}C$ for microphytobenthos and in $\delta^{15}N$ for phytoplankton at all sites was too large and therefore mixing models were not used. Calculating the relative proportions of the food sources selected based on the above problem would be invalid because the model is severely dependent on exact $\delta^{13}C$ values of the food sources (Fry and Sherr 1984, Peterson 1999). Furthermore, in most instances, consumers did not fall within the source polygons and therefore the use of mixing models would have been ineffective.

4.5 Further Research

The analysis of different tissues with varying turnover rates would provide an indication of the short term (analysis of fast turnover tissues) and long term (analysis of short turnover tissues) diets of consumers (Fry and Sherr, 1984). The isotope analysis of various tissues is an indication of the organisms assimilated diet over a period of time (days to years) and therefore changes in the diet of a consumer may not necessarily be seen depending on the turnover rates of the tissues analysed. Therefore, the analysis of various types of tissue coupled with gut contents analysis would reveal animals assimilated long-term diet and immediate diet and this combination of techniques would ensure more robust conclusions.

In this study it has been shown that stable isotope analysis can be used to asses the linkages between potential food sources and consumers. Complementary studies identifying whether any isotopic differences occur between fresh seagrass and detritus with controlled feeding experiments would also be beneficial. In addition, investigation of a number of other potential food sources including the role of epiphytes on seagrass blades would clarify the contributions of various organic matter sources to estuarine food webs. Many studies have identified the importance of epiphytes to seagrass food webs (e.g. Kitting et al 1984, Nichols et al. 1995, Smit et al. 2006), however in the present study the presence of epiphytes on the blades of seagrass and on detritus was erratic and consequently were not sampled due to logistic and time constraints. In addition, it would also be

advantageous to sample more species that also are of importance (in terms of abundance) (Appendices: Tables 1 and 2), such as *Nucula hartvigiana*.

It is known that differences in the isotopic signatures of consumers are due to their in part to their assimilated diets and also fractionation. In primary producers (for example, phytoplankton compared to seagrass), the isotopic signatures are mainly due to differences in the source of and their uptake of carbon. Gannes et al. (1997) proposed that patterns of stable isotope ratios observed in consumers may be a result of an interaction between ecological, physiological and biochemical processes. Thus, individuals with similar diets may exhibit differing isotopic ratios due to internal physiological processes such as nutrient cycling (Hobson et al., 1995). Controlled laboratory experiments involving a number of different species being fed the same food would aid understanding of the variability of consumer stable isotope signatures. Furthermore, the interpretation and reconstruction of estuarine food webs would be more accurate.

Replicated sampling of potential food sources such as phytoplankton and microphytobenthos would be beneficial to reduce stable isotope variability Furthermore, mixing models may be then used to provide an estimation of the proportions of food sources to the diet of a consumer. The utilisation of mixing models would be beneficial in providing clarity in the flow of nitrogen and carbon to the food webs.

4.6 Conclusions: The Role of *Z. muelleri* in Estuarine Food Webs

Macro-invertebrate community analysis confirmed that species sampled for stable isotope analysis had mean abundances between 26 - 63% and 37 - 95% of the macro-invertebrate communities in seagrass and non-vegetated sediment, respectively. The analysis of C and N isotopic signatures of macro-invertebrates collected at Tauranga, Whangapoua and Raglan estuaries suggested that secondary production was fuelled by benthic organic matter (seagrass and microphytobenthos) rather than phytoplankton in the water column. *Z. muelleri* appeared to be the food source used by gastropods *D. subrostrata* and *Z. lutulentus*, and it also contributed to the diets of *M. liliana*, *H. crassa*, *O. papillosa* and *A. aucklandica*. The refractory nature of seagrass does not appear to influence its role in the food web, because the similar $\delta^{13}C$ signatures of macro-invertebrate consumers between sampling locations suggests that seagrass detritus is being transported from seagrass beds and used by consumers in non-vegetated areas. The contribution of *Z. muelleri* as a food source supports a high abundance of consumers and at present this seems sustainable as the biomass of seagrass within these estuaries is as high as 52% of the intertidal area at Tauranga Harbour for example. Microphytobenthos also played a role as a food source for *A. stutchburyi* and also for invertebrate species such as *M. liliana* whose diet appeared to consist of a combination of microphytobenthos and seagrass. Microphytobenthos was made available for assimilation by *A. stutchburyi* through resuspension into the overlying water column, illustrating that benthic production can be made available to suspension feeding organisms as well as deposit feeders.

The quantification of food web structure has important implications for the management of estuaries. Results of this study indicate that seagrass is an important C and N source for macro-invertebrate communities. This demonstrates the importance of seagrass in our estuaries and why the need to mitigate further loss is imperative.

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Appendices

Table 1: Species list and mean abundances of macro-invertebrates at each site (TU, TL, WP and RG) and location (IN and OUT)

Taxa	Species	TU		TL	
		IN	OUT	IN	OUT
Bivalve	*Austrovenus stutchburyi*	12	12	3	34
	Macomona liliana	3	5	4	2
	Paphies australis	0	1	1	0
	Nucula hartvigiana	40	17	12	26
Gastropod	*Diloma subrostrata*	1	1	5	1
	Cominella glandiformis	1	2	3	2
	Notoacmea helmsi	3	1	1	1
	Zeacumantus lutulentus	3	8	3	4
	Melagraphia aethiops	2	0	5	0
	Eatoniella sp.	4	1	3	0
	Haminoea zealandiae	0	0	1	0
Crustacean	*Eliminus modestus*	1	2	0	0
	Helice crassa	0	0	2	0
	Hemigrapsus crenulatus	1	0	0	0
	Halicarcinus whitei	0	1	4	0
	Pagurus novaezelandiae	1	0	0	0
	Isopoda	0	0	0	1
	Ostrocod	32	4	12	0
	Colurostylis lemurum	2	0	1	2
	Gammeridae sp.	5	8	18	2
	Paracalliope sp.	1	0	16	0
Annelid	*Nereidae* sp.	3	3	2	4
	Boccardia syrtis	2	1	0	0
	Aonides oxycephala	0	0	2	0
	Aquilaspio aucklandica	14	22	14	7
	Scolecolepides benhami	0	0	0	5
	Glycera americana	1	0	0	0
	Magelona dakini	2	5	0	0
	Oligochaetes	4	0	3	1
	Nemertean	3	3	0	7
	Macroclymenella stewartensis	0	1	0	0
	Scoloplos cylindrifer	0	3	2	0
	Orbinia papillosa	0	0	2	2
	Armandia sp.	4	2	2	2
	Aglaophamus macroura	1	0	0	0
	Goniada sp.	0	1	2	0
	Pectinarid sp.	1	0	2	1
	Pomatoceros sp.	0	0	0	1
Other	*Stylochoplana* sp.	0	0	0	0
	Grahamina nigripenne	0	0	0	0
	Amourochiton maorianus	1	2	0	1
	Patiriella regularis	3	0	1	0
	Anthropleura aureoradiata	0	3	0	0

Table 1: continued

Taxa	Species	WP		RG	
		IN	OUT	IN	OUT
Bivalve	*Austrovenus stutchburyi*	9	11	16	20
	Macomona liliana	4	13	3	2
	Paphies australis	0	0	0	0
	Nucula hartvigiana	14	24	12	9
Gastropod	*Diloma subrostrata*	1	2	3	1
	Cominella glandiformis	2	4	7	2
	Notoacmea helmsi	3	1	3	2
	Zeacumantus lutulentus	3	3	3	1
	Melagraphia aethiops	0	2	2	0
	Eatoniella sp.	1	0	0	0
	Haminoea zealandiae	2	1	0	0
Crustacean	*Eliminus modestus*	0	11	0	0
	Helice crassa	1	1	2	2
	Hemigrapsus crenulatus	0	0	0	0
	Halicarcinus whitei	0	1	0	0
	Pagurus novaezelandiae	0	0	0	0
	Isopoda	0	0	0	0
	Ostrocod	9	8	0	0
	Colurostylis lemurum	2	4	0	3
	Gammeridae sp.	34	9	0	0
	Paracalliope sp.	21	2	1	0
Annelid	*Nereidae* sp.	3	6	4	5
	Boccardia syrtis	2	0	2	2
	Aonides oxycephala	0	3	4	2
	Aquilaspio aucklandica	15	11	8	12
	Scolecolepides benhami	5	3	6	0
	Glycera americana	2	0	1	2
	Magelona dakini	0	0	0	0
	Oligochaetes	0	0	3	0
	Nemertean	2	2	4	3
	Macroclymenella stewartensis	1	3	0	0
	Scoloplos cylindrifer	0	0	0	0
	Orbinia papillosa	1	2	1	1
	Armandia sp.	2	0	1	2
	Aglaophamus macroura	0	1	0	0
	Goniada sp.	0	1	1	0
	Pectinarid sp.	1	0	1	0
	Pomatoceros sp.	0	1	0	0
Other	*Stylochoplana* sp.	0	1	0	0
	Grahamina nigripenne	1	1	0	0
	Amourochiton maorianus	0	0	0	0
	Patiriella regularis	2	0	0	0
	Anthropleura aureoradiata	0	21	0	1

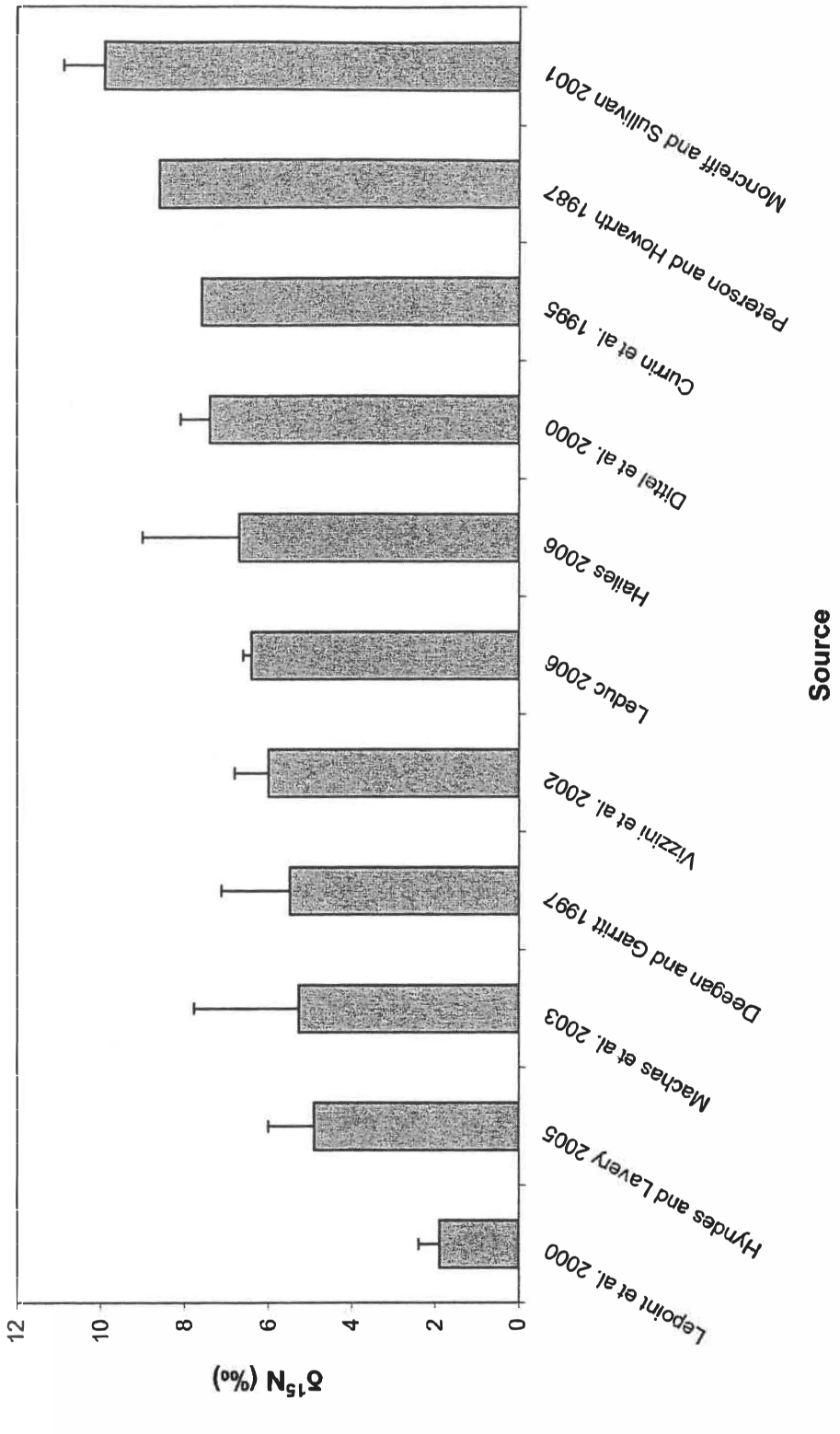

Figure 1: Mean $\delta^{15}N$ values of phytoplankton (±1SD) collected from literature; phytoplankton (2006) is data from the present study.

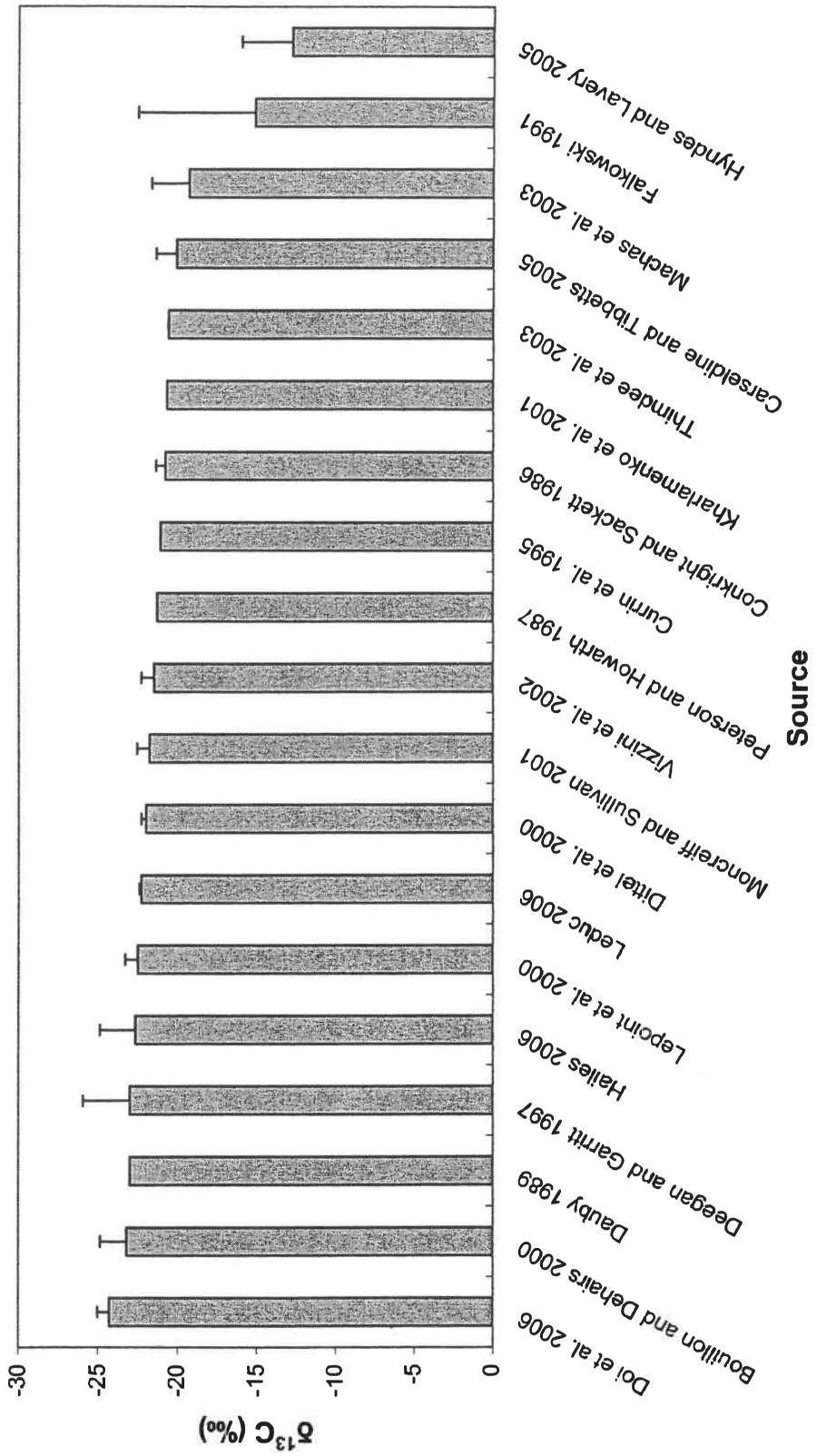

Figure 2: Mean $\delta^{13}C$ values of phytoplankton (±1SD) collected from literature; phytoplankton (2006) is data from the present study.

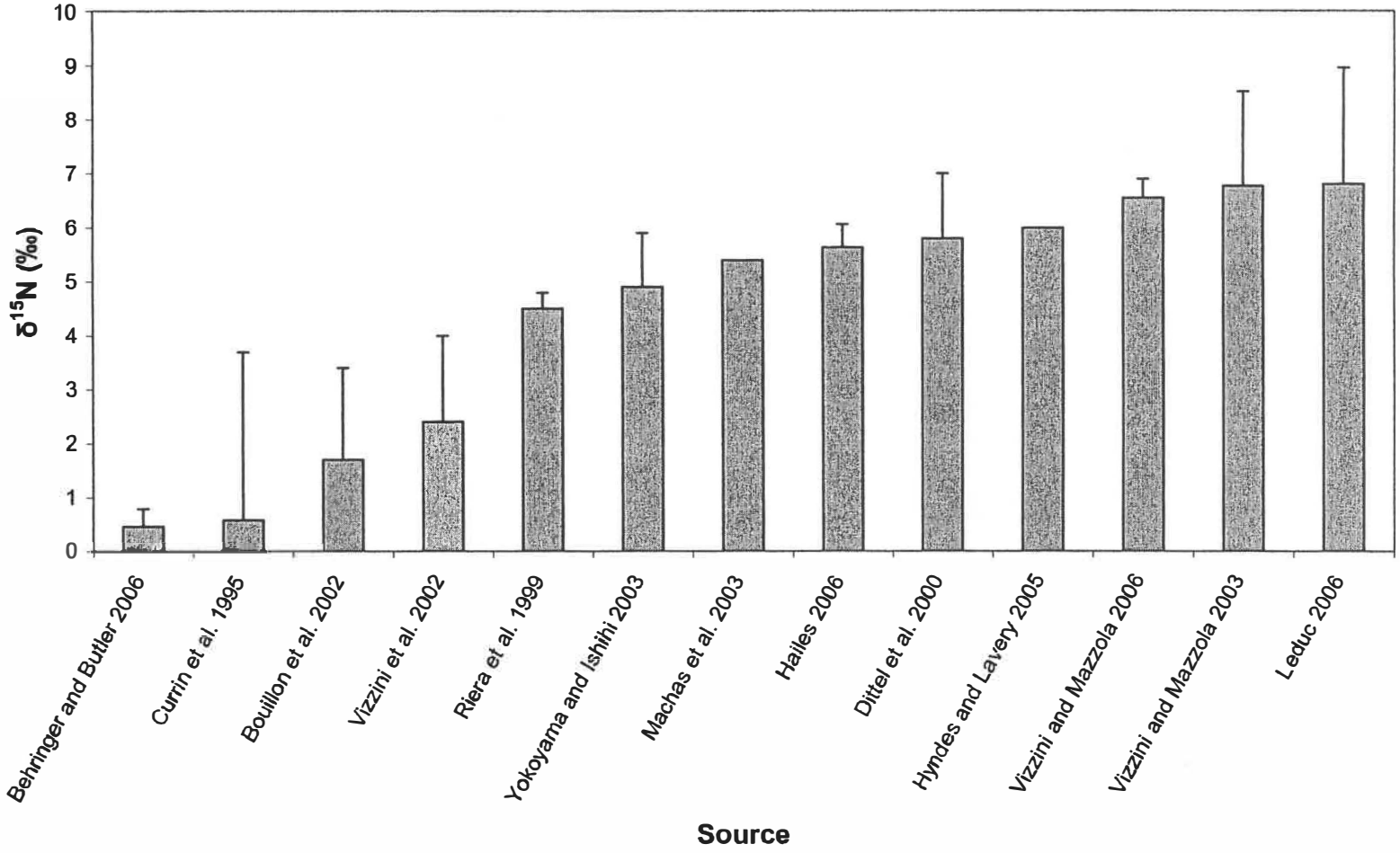

Figure 3: Mean $\delta^{15}N$ values of microphytobenthos (±1SD) collected from literature; microphytobenthos (2006) is data from the present study.

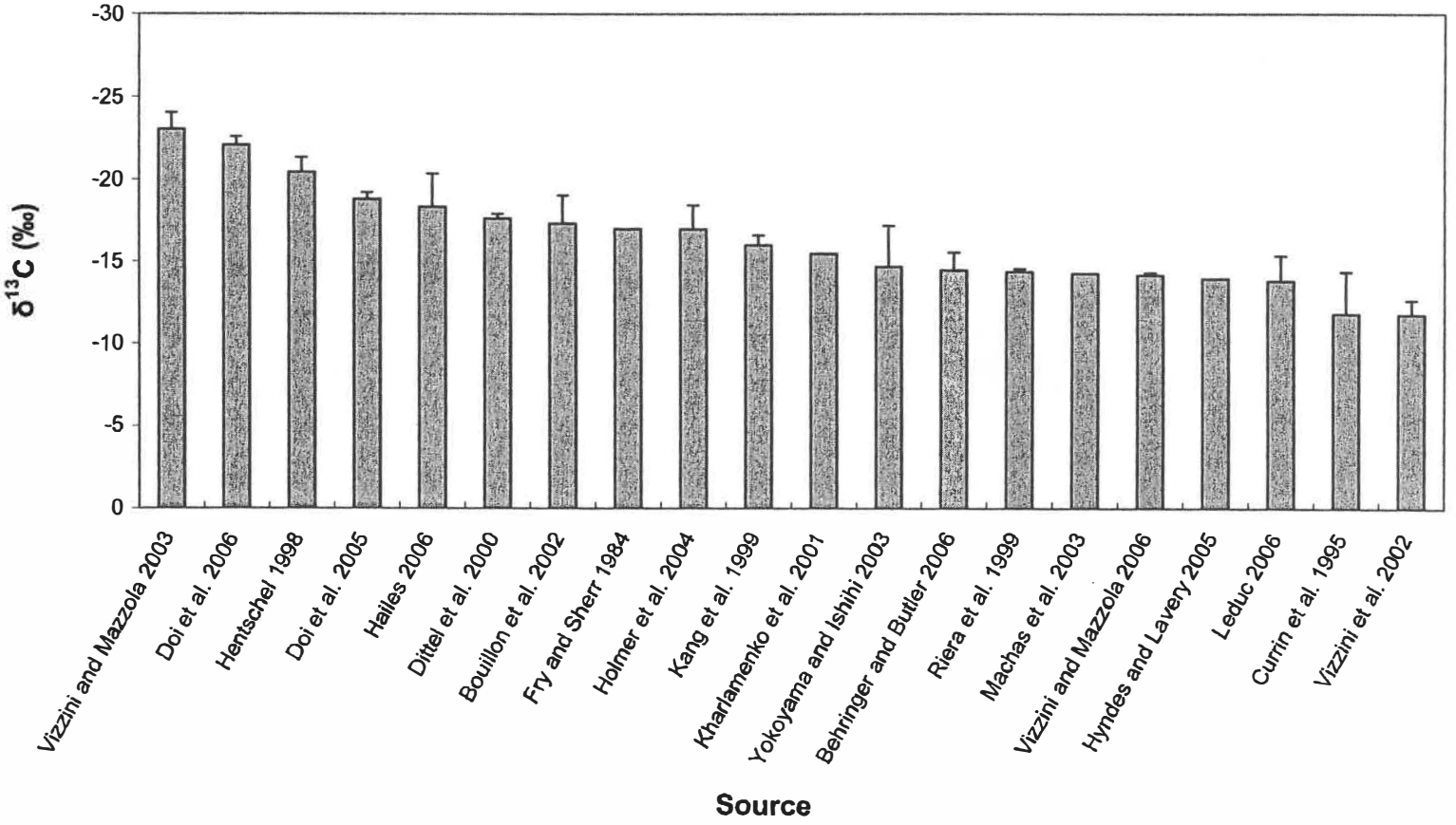

Figure 4: Mean $\delta^{13}C$ values of microphytobenthos (±1SD) collected from literature; microphytobenthos (2006) is data from the present study.

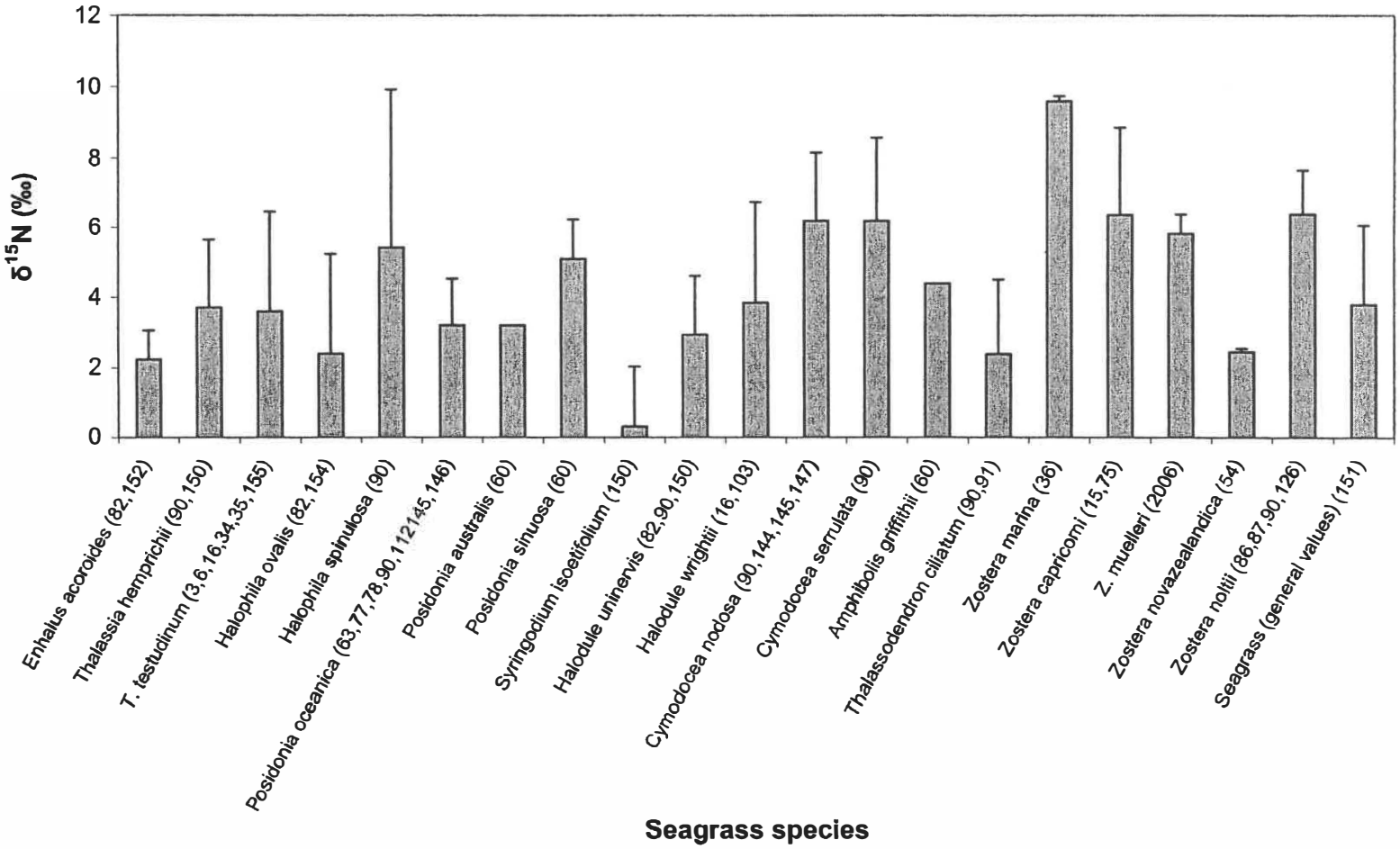

Figure 5: Mean $\delta^{15}N$ values of seagrass species (±1SD) collected from literature; *Z. muelleri* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

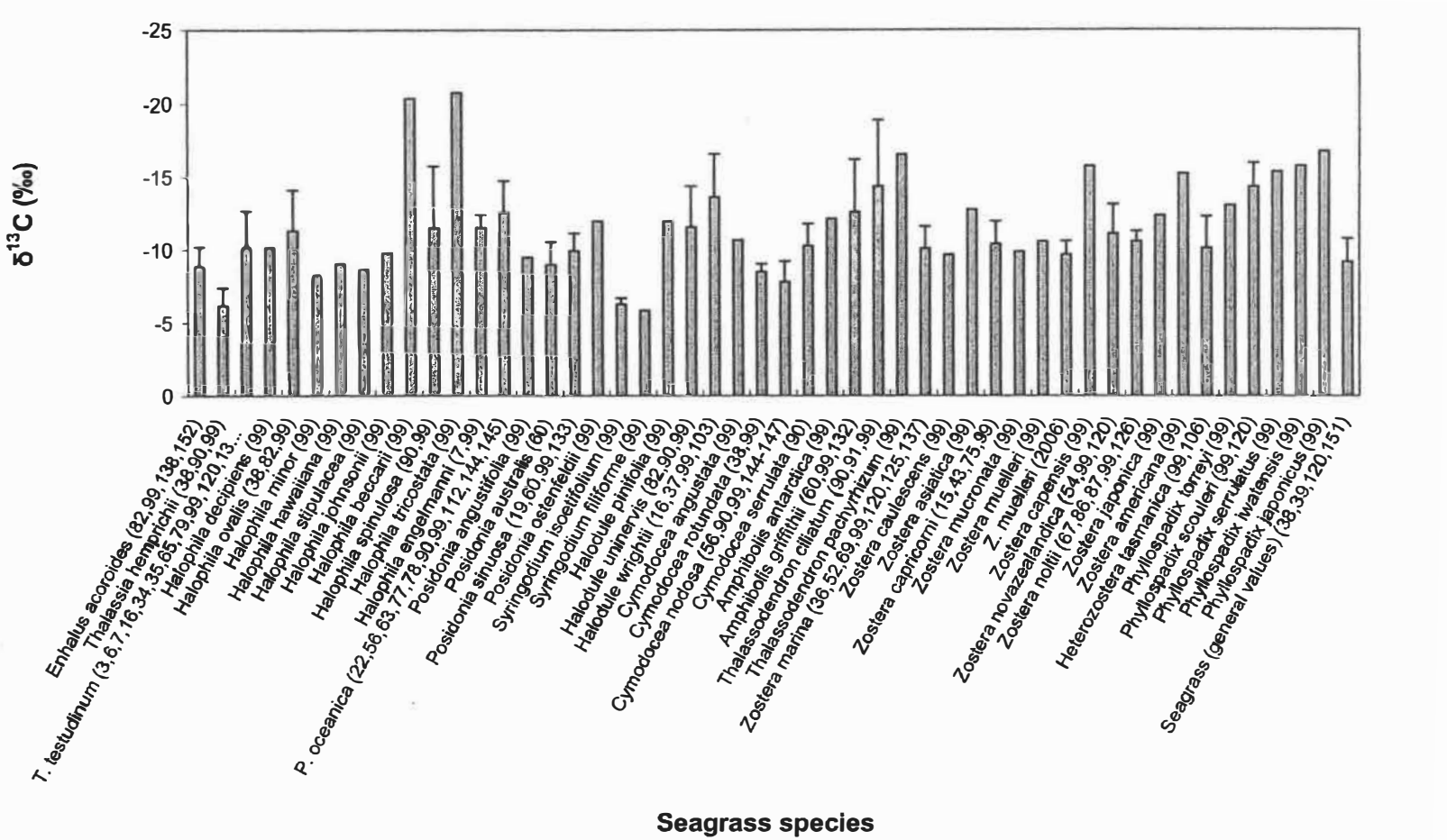

Figure 6: Mean $\delta^{13}C$ values of seagrass species (±1SD) collected from literature; *Z. muelleri* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

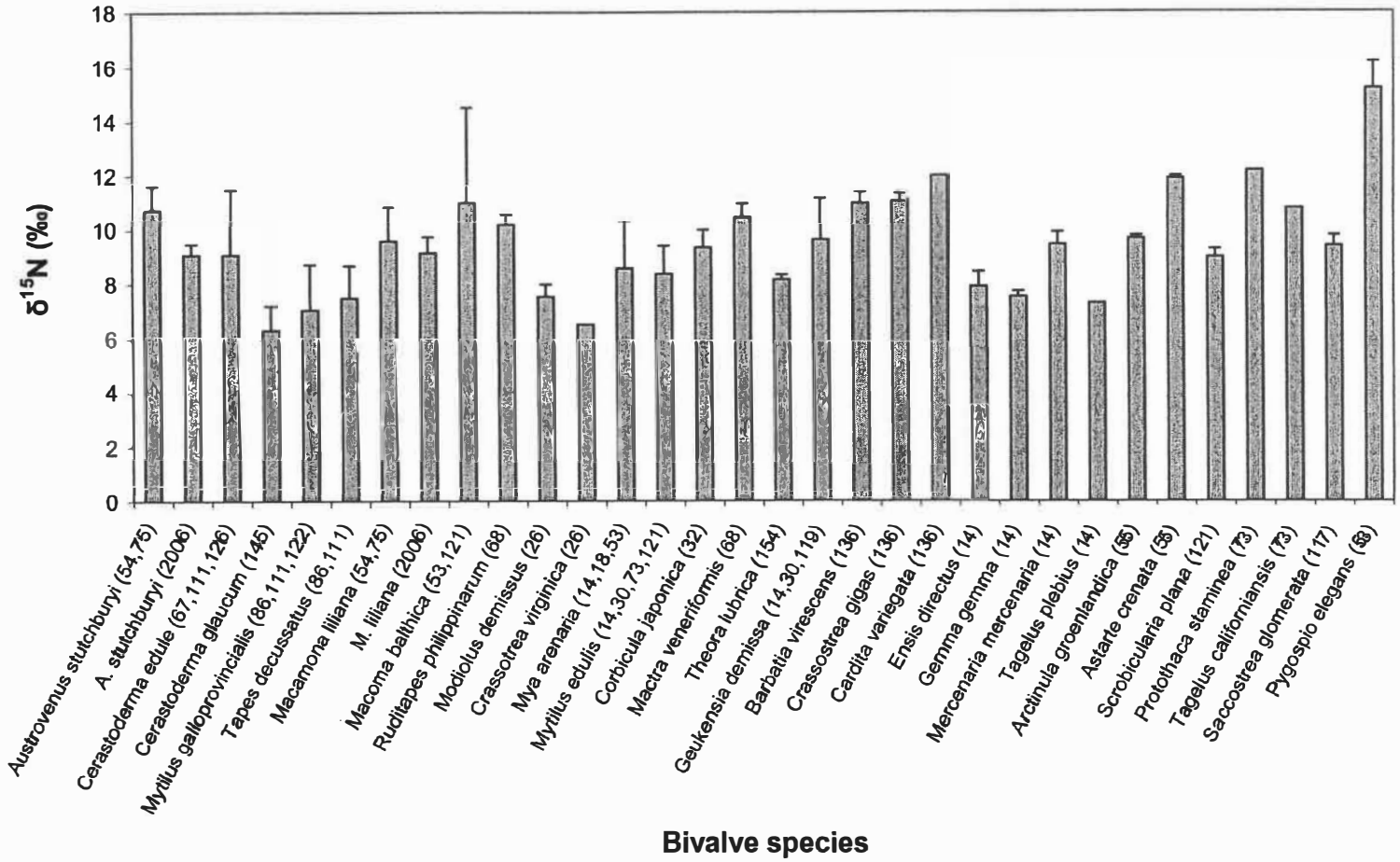

Figure 7: Mean $\delta^{15}N$ values of bivalves (±1SD) collected from literature; *A. stutchburyi*- and *M. liliana* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

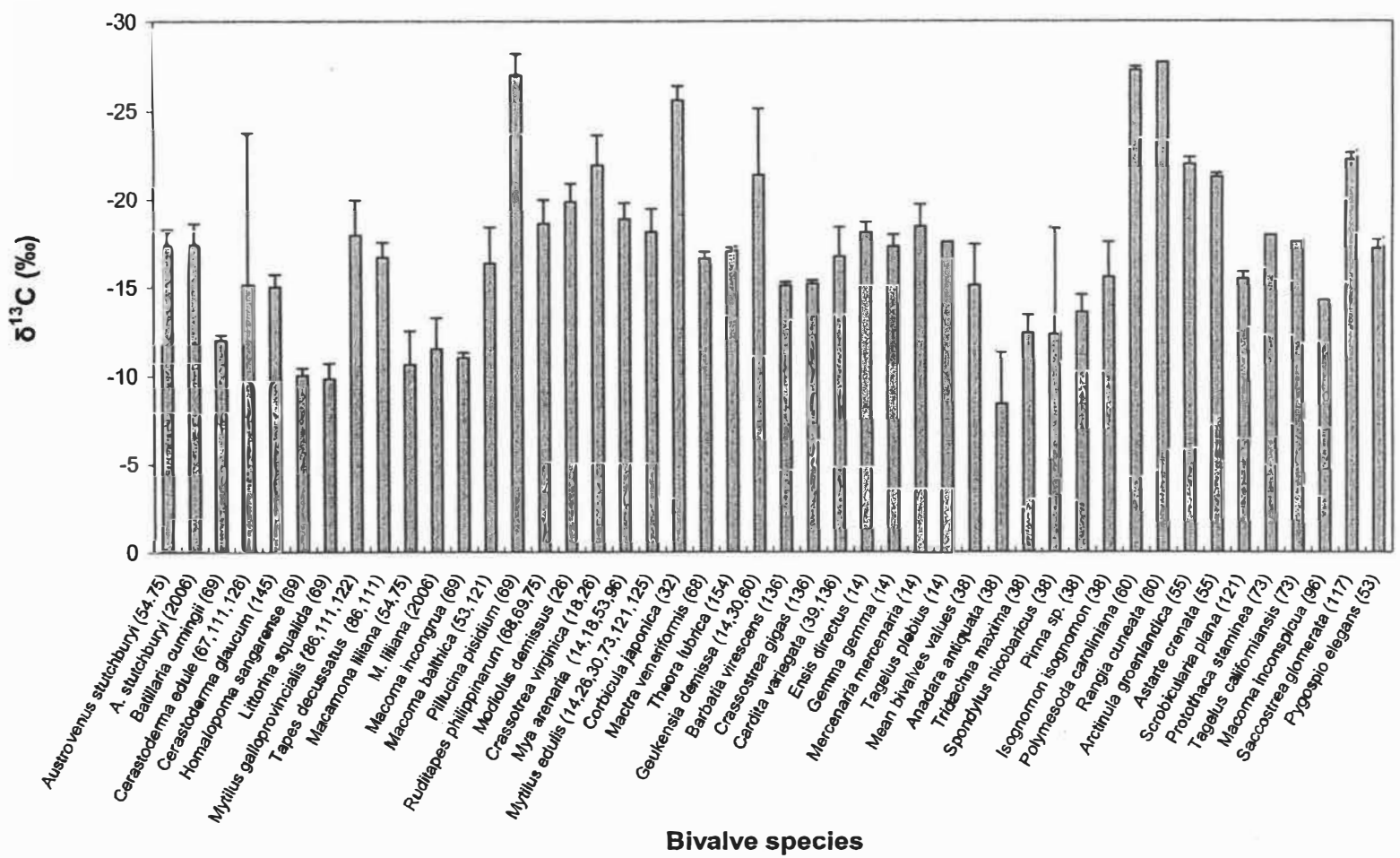

Figure 8: Mean $\delta^{13}C$ values of bivalves (±1SD) collected from literature; *A. stutchburyi*- and *M. liliana* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

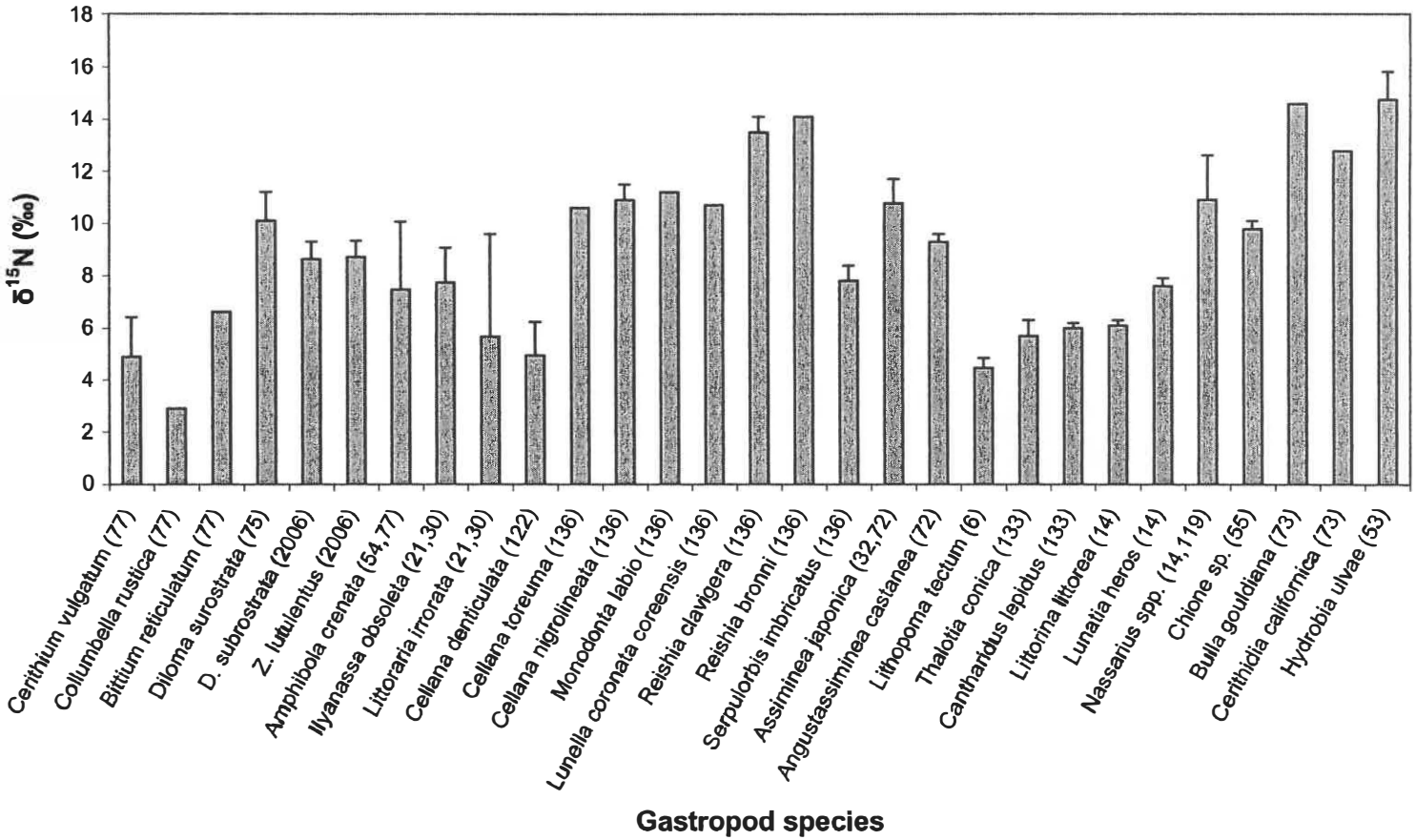

Figure 9: Mean $\delta^{15}N$ values of gastropods (±1SD) collected from literature; *D. subrostrata* and *Z. lutulentus* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

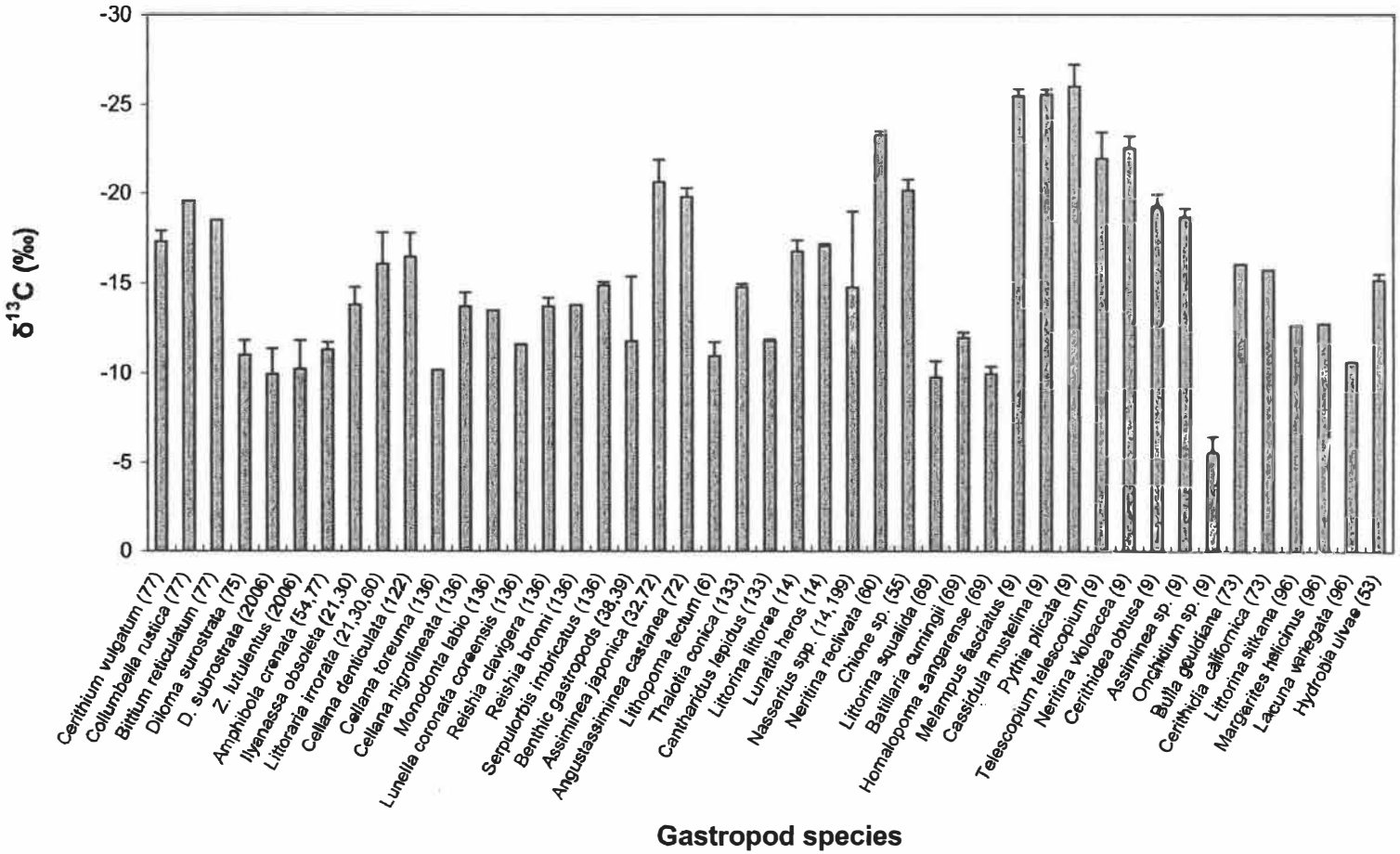

Figure 10: Mean $\delta^{13}C$ values of gastropods (±1SD) collected from literature; *D. subrostrata* and *Z. lutulentus* (2006) is data from the present study. Values in brackets indicate the source of the data (see Reference List)

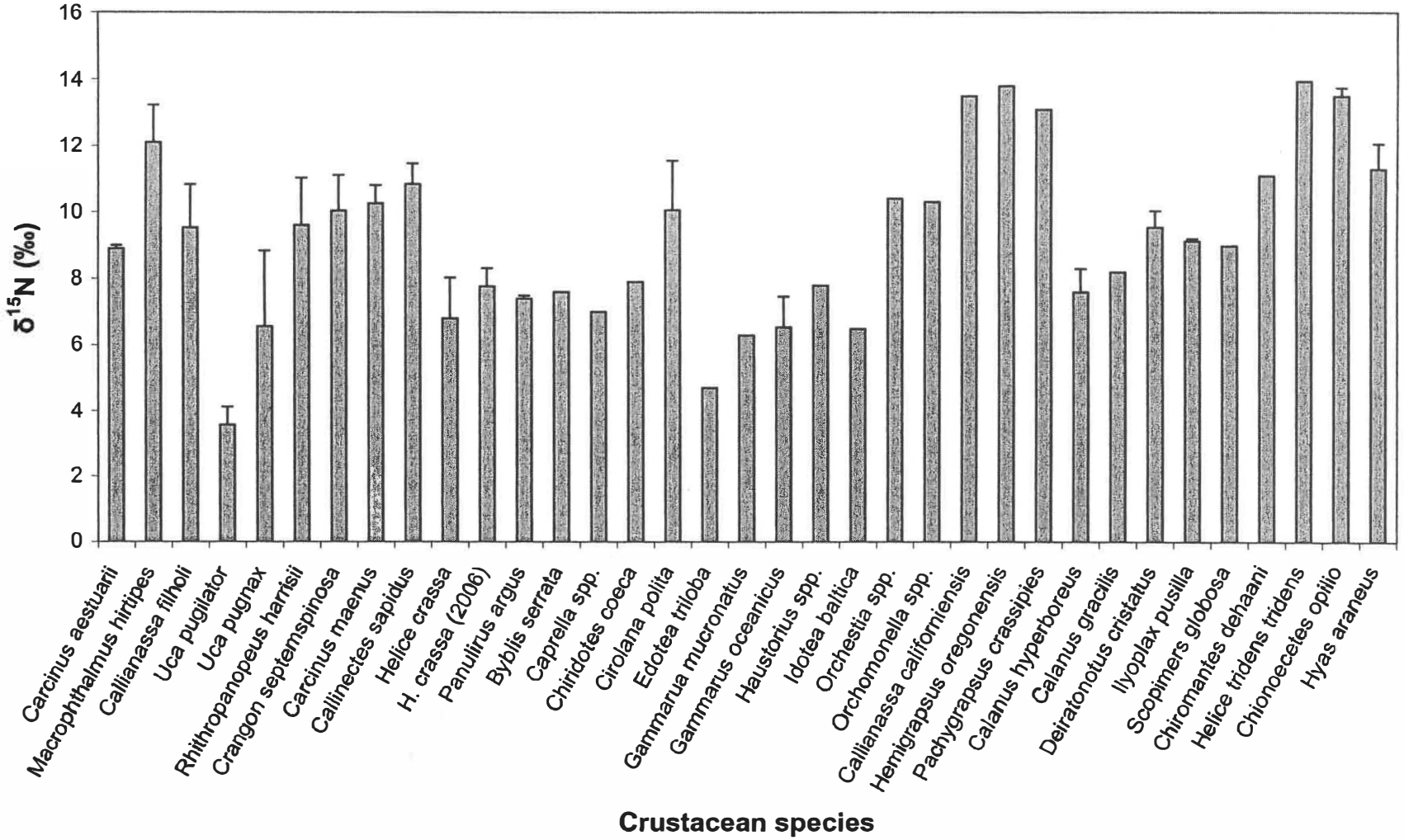

Figure 11: Mean $\delta^{15}N$ values of crustaceans (±1SD) collected from literature; *H. crassa* (2006) is data from the present study

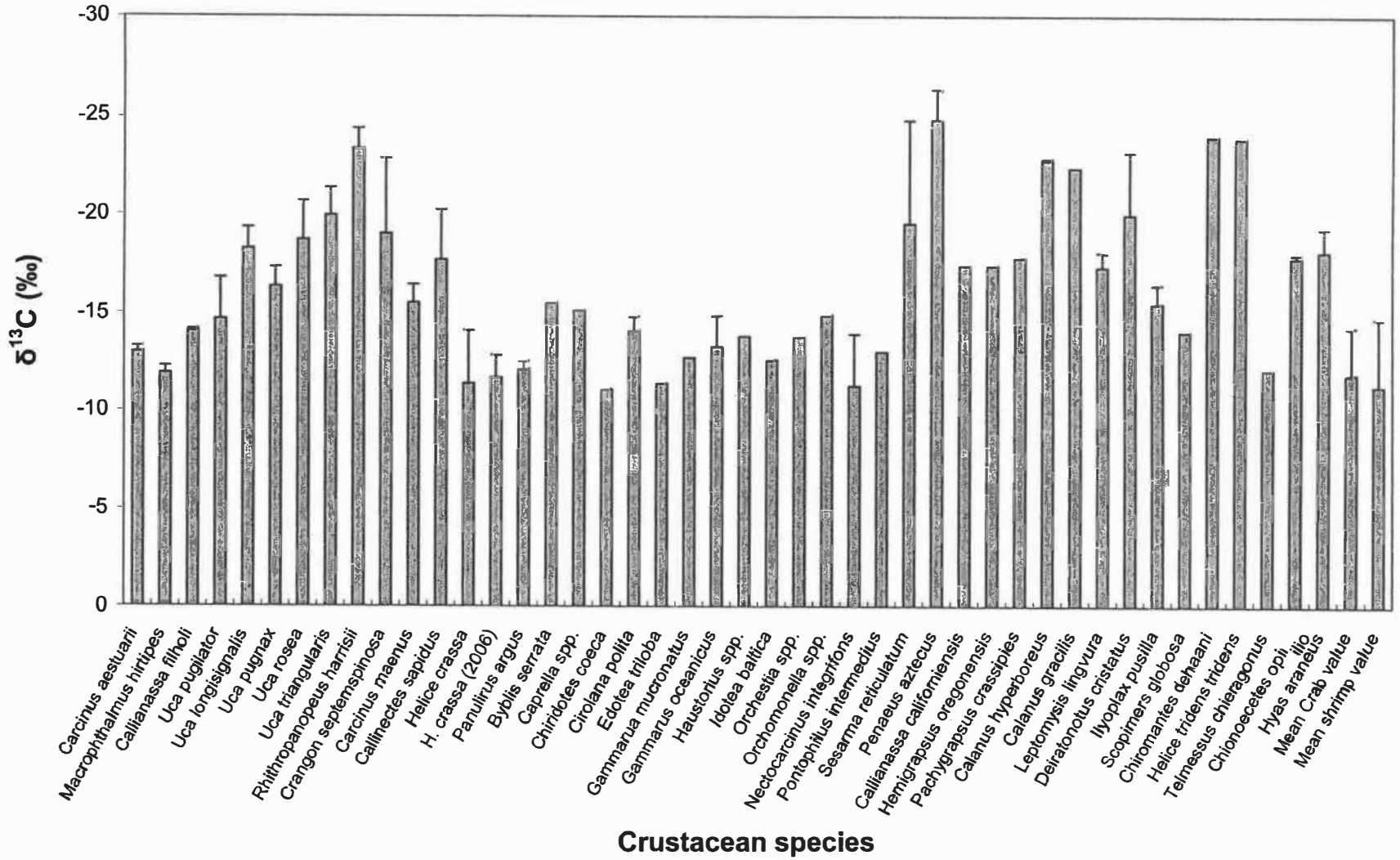

Figure 12: Mean $\delta^{13}C$ values of crustaceans (±1SD) collected from literature; *H. crassa* (2006) is data from the present study

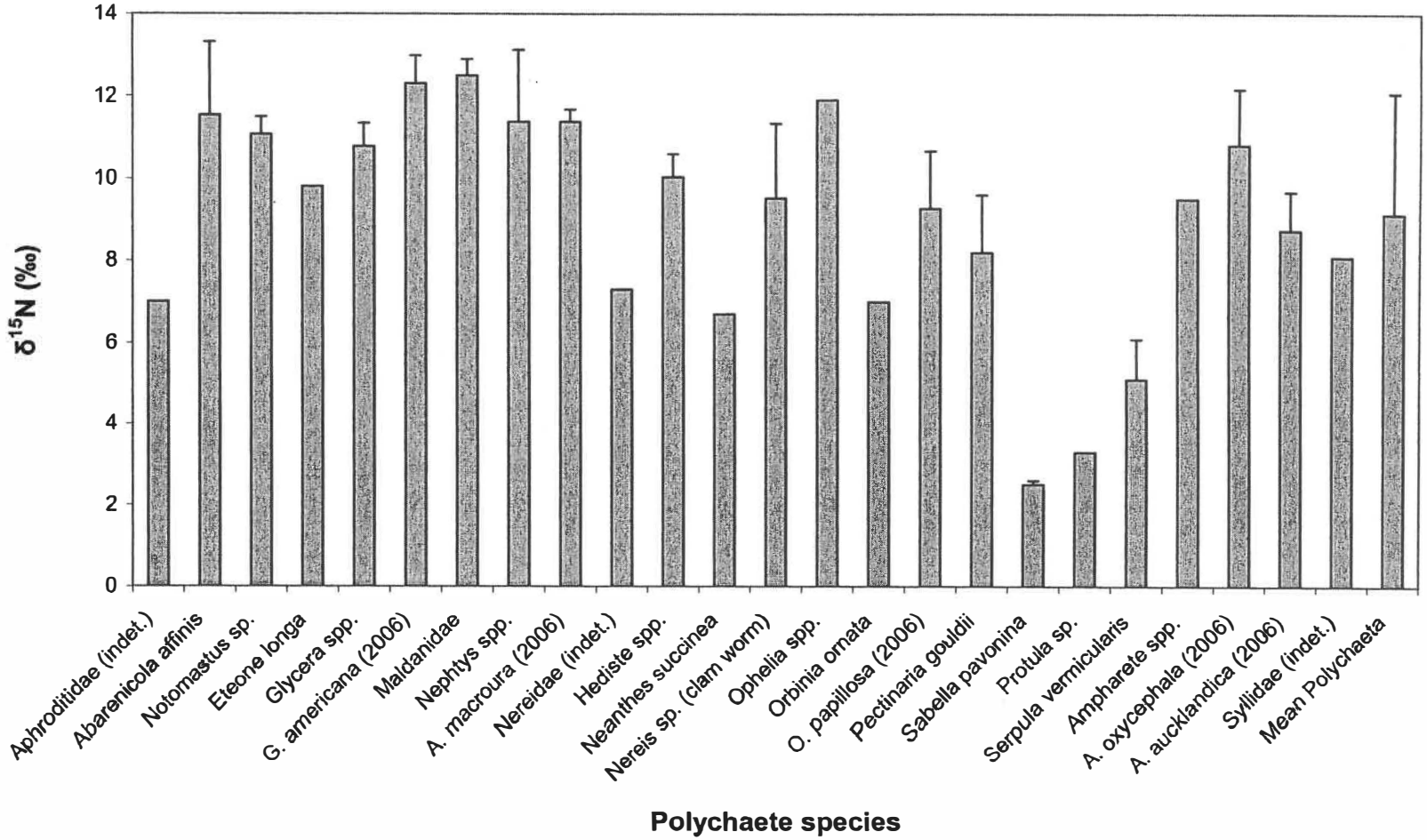

Figure 13: Mean $\delta^{15}N$ values of annelids (±1SD) collected from literature; *A. macroura*-, *A. aucklandica*-, *A. oxycephala*-, *G. americana*- and *O. papillosa* (2006) are data from the present study

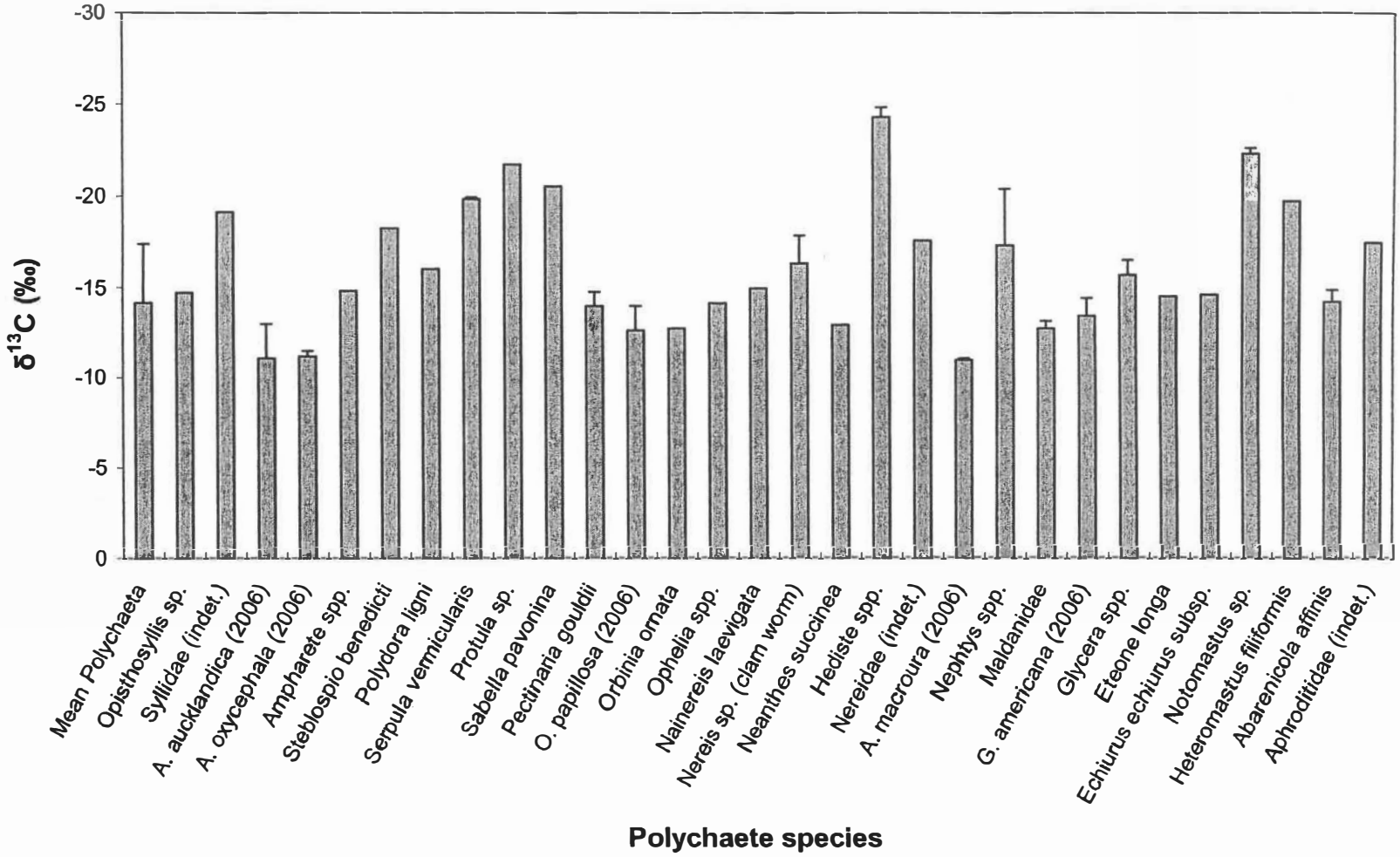

Figure 14: Mean $\delta^{13}C$ values of annelids (±1SD) collected from literature; *A. macroura*-, *A. aucklandica*-, *A. oxycephala*-, *G. americana*- and *O. papillosa* (2006) are data from the present study