Sedimentary Nitrogen Uptake & Assimilation in the Temperate Zooxanthellate Anemone *Anthopleura aureoradiata*

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by

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A thesis submitted to the Victoria University of Wellington in partial fulfilment of the requirements for the degree of Masters of Science in Marine Biology

TE WHARE WĀNANGA O TE ŪPOKO O TE IKA A MĀUI
VICTORIA
UNIVERSITY OF WELLINGTON

Victoria University of Wellington
School of Biological Sciences
2008

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr Joanne Davy & Milena Palka, for providing me with field dissolved nutrient data; Dr Geoff Chambers for allowing me to utilise the School of Biological Sciences radioactive facilities; Dr Matt Mills for his expertise and willingness to answer questions regarding his work; Dr Ken Ryan, for equipment and experimental advice; Dr Sarah Bury and the staff at the Delta^{Plus} Mass Spectrometry lab at National Institute of Water and Atmospheric Research, Wellington for sample analyses, but more importantly for taking the time to help someone new to isotopes; My good friends, Dmitri and Glenn for giving up their holiday time to help with preliminary fieldwork; "Professor" Christopher Gibbons for his help and friendship throughout the grad-school ordeal. A big thanks also to technician Joanne Long for prompt provisions with equipment and helpful advice whenever needed.

An additional thanks to Drs Clayton Cook and Ken Ryan for grading the initial copy of this thesis and providing useful comments for preparation of this final version, as well as a subsequent manuscript.

To the greatest supervisor on this planet Dr Simon Davy, which without, none of this would have been possible – thank you so very much for giving me the opportunity to be one of your students. It's been a pleasure and I have learned so much from you.

Lastly, a huge thanks to my family, especially my Mum & Dad for their support, but most of all, for putting up with me these past few years.

It's now time for me to do you all proud….

ABSTRACT

This study investigated the potential for, and efficiency of, particulate nitrogen uptake from the sediment and subsequent consequences of this on the nutrient status of endosymbiotic dinoflagellates (zooxanthellae) in the temperate zooxanthellate anemone *Anthopleura aureoradiata*. Sediment was collected from a mudflat and labelled with $(^{15}NH_4)_2SO_4$ before being provided to *A. aureoradiata* at low (5 g dry weight) and high sediment (20 g dry weight) loads for 6 hours. While no discernible change in the isotopic content of the sediment could be detected, analysis of the host and algal symbionts revealed that ^{15}N had been taken up. Uptake by the host was similar at both high and low sediment loads, but the algal symbionts acquired more nitrogen at the lower load (1.13 versus 0.93 atom % ^{15}N in the low and high loads, respectively). Evaluation of this particulate nitrogen uptake from the sediment was further examined by measuring the nitrogen status of the zooxanthellae. This was determined by measuring the extent to which ammonium (40 μM NH_4^+) enhanced the rate of zooxanthellar dark carbon fixation above that seen in filtered seawater (FSW) alone; the enhancement ratio was expressed as [dark NH_4^+ rate/dark FSW rate]. $V_{D'}/V_L$, a further index of nitrogen status, was also calculated where $V_{D'}$ = [dark NH_4^+ rate - dark FSW rate] and V_L = rate of carbon fixation in the light. When anemones were starved for 2-8 weeks, zooxanthellar nitrogen deficiency became apparent at ≥ 4 weeks, with NH_4^+/FSW and $V_{D'}/V_L$ averaging up to 2.90 and 0.11, respectively. In comparison, when anemones were fed 5 times per week for 8 weeks the addition of ammonium had little effect, indicating nitrogen sufficiency; NH_4^+/FSW and $V_{D'}/V_L$ values were 1.03 and -1.0×10^{-3}, respectively. The nitrogen status of zooxanthellae from anemones starved and incubated with and without sediment was examined with no apparent difference between sediment and no sediment treatments; zooxanthellar nitrogen deficiency became apparent at ≥ 4 weeks in both treatments, with NH_4^+/FSW and $V_{D'}/V_L$ averaging up to 3.73 and 0.17 for the sediment treatment and 2.74 and 0.15 for the no sediment treatment, respectively. The nitrogen status of zooxanthellae from anemones found on a mudflat (Pauatahanui Inlet) and a rocky intertidal site (Kau Bay) was different. Zooxanthellae from mudflat anemones were nitrogen sufficient with NH_4^+/FSW and $V_{D'}/V_L$ values averaging up to 1.26 and -6.0×10^{-3}, respectively. Nitrogen deficient zooxanthellae were present in anemones from the rocky intertidal. Anemones from tide

pools in the upper littoral zone had NH_4^+/FSW and $V_{D'}/V_L$ values of 2.99 and 0.11, respectively, while anemones from the mid littoral zone had NH_4^+/FSW and $V_{D'}/V_L$ of 2.90 and 0.13, respectively; there was no significant difference in nitrogen status between zooxanthellae from high shore tide pool anemones and aerially exposed mid-littoral anemones. These results suggest that while particulate nitrogen can be taken up from the sediment by this species, dissolved inorganic nitrogen such as ammonium in the seawater, and especially the interstitial water surrounding infaunal anemones on mudflats, may be a more important source of nitrogen in the field.

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Methods

$$Atom\ \%\ ^{15}N = \frac{^{15}N}{^{14}N + ^{15}N} \times 100$$

$$APE = atom\ \%\ ^{15}N_{final} - atom\ \%\ ^{15}N_{initial}$$

$$Ammonium\ enhancement\ ratio = \frac{dark\ NH_4^+\ C\ fixation\ rate}{dark\ FSW\ C\ fixation\ rate}$$

$$V_D/V_L = \frac{dark\ NH_4^+\ C\ fixation\ rate - dark\ FSW\ C\ fixation\ rate}{C\ fixation\ in\ the\ light}$$

1

INTRODUCTION

1.1 Symbiosis

The word symbiosis can be used to describe various degrees of close relationship between organisms of different species (Douglas 1994). The term was first used in 1879 by the German mycologist, Heinrich Anton de Bary, who defined it as "the living together of two unlike organisms" (Wilkerson 2001). There is no single universally agreed upon definition of symbiosis (Wilkerson 2001), however these biological interactions can commonly be categorised in one of three different ways. The first is mutualism, where both organisms benefit from the relationship. Second is parasitism, where one organism benefits and the other is harmed (but does not necessarily die). Lastly, is commensalism, whereby one benefits and the other neither benefits or is disadvantaged (Smith & Douglas 1987; Douglas 1994; Wilkerson 2001). Despite this categorisation, mutualism, commensalism and parasitism form parts of a continuum of interactions where an interaction is never exclusively one or the other (Davies 1992). Symbiotic relationships may involve an organism living on another (ectosymbiosis) or inside another (endosymbiosis). Endosymbioses generally involve one organism or species, usually the smaller one (known as the symbiont), living inside a much larger 'host' organism (Douglas 1994). Finally, symbiotic relationships, may be either obligate, meaning necessary for survival of at least one organism, or facultative, where the relationship is useful but not vital (Smith & Douglas 1987; Douglas 1994; Moran 2006).

1.2 Cnidarian-Dinoflagellate Symbioses

1.2.1 Zooxanthellae

Since Karl Brandt (1881) first described an algal-invertebrate symbiotic system in the marine environment, many marine hosts have been found to harbour unicellular phototrophs as intra- or intercellular endosymbionts (Battey 1992). These photosynthetic microbes include cyanobacteria (blue-green algae), rhodophytes, chlorophytes and diatoms, but the majority are dinoflagellates (Smith & Douglas 1987). Most symbiotic dinoflagellates belong to the genus *Symbiodinium* in the family Gymnodiniidae and are often called "zooxanthellae" because of their yellow to brownish colouration (Douglas 1994). For many years, it was thought that all zooxanthellae were a single pandemic species *Symbiodinium microadriaticum*, but detailed examination of zooxanthella cultures, zooxanthella ultrastructure and advances in molecular phylogenetics suggest more of a multi-species complex (Smith & Douglas 1987; Trench & Blank 1987; Rowan & Powers 1991; Douglas 1994). At present the *Symbiodinium* genus is divided into at least eight highly divergent lineages: clades A through to H (LaJeunesse 2004).

Zooxanthellae have a remarkable geographical and host distribution. Their known biogeographical distribution in the Pacific Ocean extends from Alaska (60°N) to New Zealand (45°S) (Buddemeier & Fautin 1996) and throughout the Atlantic, from the tropics as far north as Scotland and the east coast of North America (Roberts ***et al.*** 1999b). Relationships with zooxanthellae are also widespread in the Indo-Pacific. Meanwhile, zooxanthellae have been discovered in a wide assortment of hosts including representatives of the cnidarian classes Anthozoa (including anemones, scleractinian corals, zoanthids, corallimorphs, blue corals, alcyonacean corals and sea fans), Hydrozoa (including milleporine fire corals) and Scyphozoa (including rhizostome and coronate jellyfish) and the molluscan classes Gastropoda and Bivalvia (including tridacnid clams, heart cockles, and possibly, conch) as well as sponges, large miliolid Foraminifera (sub family – Soritinae) and a giant heterotrich ciliate (Trench 1993; Baker 2003). Almost all zooxanthellae in invertebrate hosts are intracellular meaning they reside within host cells where they are largely restricted to the host's gastrodermal

layer (Muscatine & Lenhoff 1963; Glider *et al.* 1980). They are separated from the host cytoplasm by an animal vacuolar membrane, providing the boundary across which all cell-to-cell communication and nutrient transfer must occur (Wakefield & Kempf 2001).

1.2.2 The Evolution & Onset of Cnidarian-Algal Symbioses

Although the exact origins of algal-invertebrate symbiotic relationships are unknown, there are some suggestions as to how they arose (Schnepf 1992). It is proposed that zooxanthellar symbioses originated via the phagocytosis of the autotrophic cell by heterotrophic cells; delayed or what is sometimes known as "retarded" digestion allowed some of the captured cells to continue photosynthesising with any excess photosynthate exuded into the cytoplasm of the host cell – beneficial grounds for the retainment of such algae by the host cell (Schnepf 1992; Ruppert *et al.* 2004).

The onset of symbiosis can occur at a variety of stages during the life of the host, depending on the host species (Barneah *et al.* 2004). Zooxanthellae can be transmitted horizontally, where the host's sexual offspring acquire algae from the surrounding environment (open system), or vertically, being passed directly from host parent to offspring (closed system); the latter mode is also known as maternal inheritance (Trench 1987, Douglas 1994; Davy & Turner 2003; Barneah *et al.* 2004). Horizontal transmission offers the host the opportunity to recombine with different zooxanthellar types that may be differentially adapted to the existing environmental conditions which newly produced offspring enter. The risk, however, is that the host may fail to establish a symbiosis, leaving it with severely reduced growth and fitness (Weis *et al.* 2001; LaJeunesse *et al.* 2004; Pasternak *et al.* 2006; Baird *et al.* 2007). Approximately, eighty five percent of symbiotic cnidarians, mainly broadcast spawning species, acquire their symbionts in this manner (Weis *et al.* 2001; Baird *et al.* 2007). This is because many produce asymbiotic (lack zooxanthellae) gametes, which are fertilised in the water column. As a result, the planula larvae are also asymbiotic and must obtain a new complement of zooxanthellae at some stage during their ontogeny (Weis *et al.* 2001). In contrast, vertical transmission guarantees that a host is provided with a complement of symbionts (Smith & Douglas 1987). This is the norm during asexual reproduction, where daughter polyps automatically receive some algae from the

parent (Schwarz *et al.* 2001). Zooxanthellae inherited from the parent may also be placed in the egg cytoplasm immediately prior to fertilization and subsequent release (Davy & Turner 2003), a process more common among cnidarian species which "brood" young (Schwarz *et al.* 2001).

Regardless of the mode of symbiont transmission, the host and the algal symbiont possess the ability to separate from the symbiosis or reform the symbiosis after disbanding. Expulsion of zooxanthellae from cnidarian hosts takes place either as a result of stress (temperature, osmotic, sedimentation, solar irradiance, host starvation or host disease) or through predation on the host (Muller-Parker 1984; Rogers 1990; Lesser 1996; Hoegh-Guldberg 1999). It may also occur under 'normal' conditions via host regulation of the symbiont population (Muscatine & Pool 1979; Jones & Yellowlees 1997). Many zooxanthellae extruded either in the mucus of the host or the faeces of the predator are still viable (having passed intact through the digestive tract), and capable of reinfecting other hosts (Douglas 1994). Zooxanthella cells have been isolated from the interstitial water of benthic sediment samples, but are generally thought not to lead a permanent free-living existence in nature (Carlos *et al.* 1999; Coffroth *et al.* 2006).

Free-living zooxanthella cells are incorporated into the cnidarian gastrodermis following phagocytosis by the gastrodermal cells (Battey 1992). Cells may come into contact with the host after attraction to chemical stimuli, or by host ingestion either of fish faeces containing zooxanthellae, zooplankton that have recently fed on zooxanthellae or the egested mucus of other symbiotic cnidarians (Battey 1992). However, not all strains of *Symbiodinium* will enter into a stable symbiosis with a particular host species. Many studies show that *Symbiodinium* strains vary in their ability to infect different hosts (Schoenberg & Trench 1980; Davy *et al.* 1997; Coffroth *et al.* 2001; Weis *et al.* 2001). Using aposymbiotic (individuals with zooxanthellae eliminated) adult, juvenile or larval stage cnidarians, these authors all found that the zooxanthella strain found naturally in the experimental host grew more efficiently and attained the highest stable density in the host compared to other strains. These results indicate that there is a degree of specificity in cnidarian-dinoflagellate associations. Although the mechanism by which this occurs is still unclear, it has been speculated that it could be related to cell surface recognition before and after phagocytosis of the

zooxanthella cell (Meints & Pardy 1980; McAuley & Smith 1982). Indeed, evidence indicates that zooxanthella surface glycoproteins may be responsible, with a reduction in infection rates of the aposymbiotic anemone *Aiptasia pulchella* following the concealment of the glycoprotein sites on the zooxanthellae (Lin *et al.* 2000; Wood-Charlson *et al.* 2006).

1.3 Carbon & Energy Flux Between Symbiotic Partners

Zooxanthellae make a major contribution to the energy budget of their cnidarian hosts (Douglas 1994). Zooxanthellae are photoautotrophs, meaning they are able to convert the Sun's energy into food via photosynthesis (Muscatine 1990). More than 100 years ago, Brandt recognised that zooxanthellae could supply photosynthetically fixed carbon to their hosts (Battey 1992). Carbon is mainly translocated by zooxanthellae as glycerol, glucose, amino acids and organic acids (Muscatine 1967; Davy & Cook 2001) but also as lipid (Battey & Patton 1987), and can support host basal metabolism, growth and reproduction, as well aid host mucus production (Davies 1984; Battey 1992; McCloskey 1994; Engebretson & Muller-Parker 1999). Additionally, organic carbon is utilised as an energy source by hermatypic (reef building) corals for calcification, providing synthesis of the organic matrix of the skeleton (Muscatine & Cernichiari 1969; Young *et al.* 1971); this may in part contribute to light enhanced calcification in these corals (Barnes & Chalker 1990; Moya *et al.* 2006). In some symbiotic associations, the zooxanthellae can provide all of the carbon required for the metabolic requirements of the host, however this amount is likely dictated by environmental conditions such as irradiance, temperature, and nutrients, and consequently may differ between individual hosts of the same species, as well as between different species and geographic regions (e.g. tropical and temperate regions) (Battey 1992; Hoegh-Guldberg 1999; Muller-Parker & Davy 2001).

1.4 Nitrogen Flux Between Symbiotic Partners

The algal symbionts of cnidarians take up host waste nitrogen and metabolise it into a form which can be utilised by the host, thereby retaining the essential nutrient within the symbiosis (Muscatine & Porter 1977; Hoegh-Guldberg & Williamson 1999). Symbiotic

Cnidaria are known to produce large amounts of nitrogenous waste (mostly ammonium produced via deamination of amino acids used as respiratory substrates; see Shick, 199l) and when incubated in darkness or treated to eliminate its symbiotic zooxanthellae, the

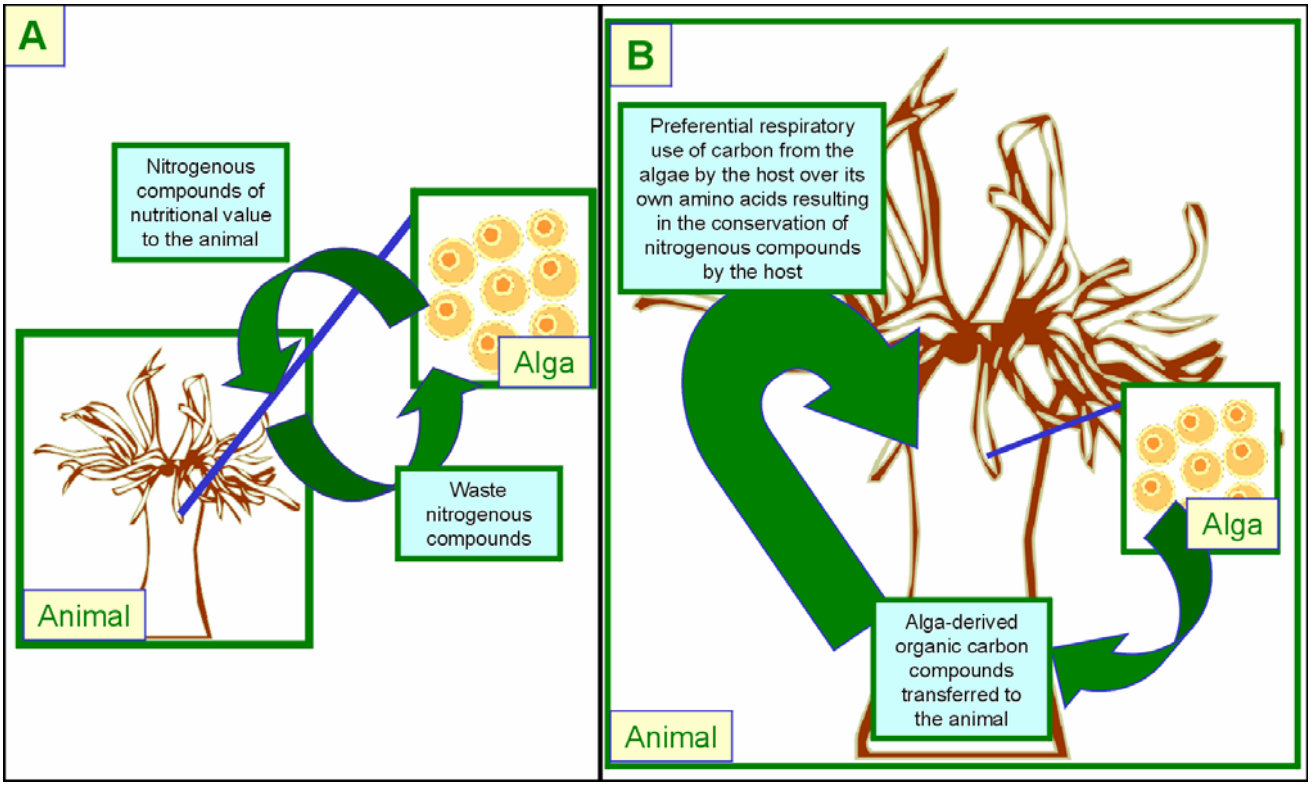

***Fig 1**. Current hypotheses of nitrogen relationships in alga–invertebrate symbioses. (A) Nitrogen Recycling: algal recycling of animal-derived nitrogenous waste compounds, e.g. ammonium, to organic nitrogen compounds, e.g. essential amino acids, which are translocated to the animal cells. (B) Nitrogen Conservation: The preferential use of photosynthetic derived carbon from zooxanthellae by the host for basal respiration instead of its own amino acids. The subsequent conservation of amino acids increases the activity of the ammonium-assimilating enzyme glutamine synthetase in host tissues allowing the animal to convert its own waste ammonium into beneficial nitrogenous compounds. These compounds are then either used in growth or stored for later use by the animal. Figure based Wang & Douglas (1998).*

host excretes large amounts of this ammonium into the surrounding seawater (Wilkerson & Muscatine 1984; Rees 1986; Wang & Douglas 1998). Two hypotheses exist explaining why this occurs: The nitrogen recycling hypothesis and the nitrogen conservation hypothesis (Figure 1) (Rees 1986). Nitrogen recycling involves the bidirectional flux of nitrogen between symbiotic partners while nitrogen conservation involves the preferential use of photosynthetic derived carbon from zooxanthellae by the host for basal respiration over its own amino acids (Domoter & D'Elia 1984; Rees; 1986; Rees & Ellard 1989; Wang & Douglas 1998).

1.4.1 The Nitrogen Recycling Hypothesis

Nitrogen recycling involves the bidirectional flux of nitrogen (Figure 1). Firstly, the transfer of the animal's waste nitrogen compounds to the algal cells, which assimilate the nitrogen via the glutamine synthetase – glutamate synthase [GS/GOGAT] pathway into compounds of nutritional value to the animal (i.e. amino acids such as alanine) (Domotor & D'Elia 1984; Wilkerson & Muscatine 1984; Trench 1993). Secondly, the translocation of these latter compounds back to the animal (Markell & Trench 1993; Wang & Douglas 1999). Thus, in aposymbiotic individuals with zooxanthellae eliminated, waste nitrogen is no longer taken up by the algae and as a result is expelled into the surrounding medium, suggesting that the algae are a large sink for this waste nitrogen.

1.4.2 The Nitrogen Conservation Hypothesis

In nitrogen conservation, the subsequent preservation of amino acids in preference to photosynthetically derived carbon increases the activity of the ammonium-assimilating enzyme glutamine synthetase in host tissues (Rees 1986; Rees & Ellard 1989; Lipschultz & Cook 2002). Therefore, in cases when the algae are eliminated from the symbiosis, the lack of photosynthate from the zooxanthellae results in decreased activity of host glutamine synthetase and a consequent increase in ammonium efflux (Wang & Douglas 1998). Evidence for both nitrogen recycling (Domotor & D'Elia 1984; Wilkerson & Muscatine 1984; Rahav *et al.* 1989; Sutton & Hoegh-Guldberg 1990; Markell & Trench 1993; Trench 1993; Roberts *et al.* 1999a; Wang & Douglas 1999) and nitrogen conservation (Rees 1986; Rees & Ellard 1989; Wang & Douglas 1998; Lipschultz & Cook 2002) exists and as a result, the dominant mechanism may be host specific (Rees 1986; Wang & Douglas 1998; Wang & Douglas 1999) or may depend on the availability of photosynthate within the host itself (Lipschultz & Cook 2002).

1.5 Nutritional Interactions: Tropical VS Temperate Symbioses

Zooxanthella-cnidarian associations are particularly abundant in tropical waters and are often attributed as the reason why these organisms are so successful in these nutritionally poor environments (Fitt *et al.* 1982). Temperate cnidarian hosts also harbour zooxanthellae in high numbers but face different environmental conditions than do their tropical counterparts (Muller-Parker & Davy 2001). Tropical seas are characterized by high irradiance, high temperature and low nutrient and plankton supplies, all of which remain fairly constant throughout the seasons, while temperate seas undergo pronounced seasonal cycles in irradiance, temperature, nutrient levels and plankton abundance (Table 1).

1.5.1 Photosynthesis

Solar irradiance is the single most important environmental factor for autotrophy because it determines the amount of photosynthetic carbon fixed by zooxanthellae. Both the daily and maximum irradiance levels measured at the sea surface, are similar for the two temperate and tropical sites highlighted in Table 1, however, seasonal variation is more prominent at higher latitudes. Additionally, light attenuation or penetration through the seawater show a similar trend seasonally at temperate and tropical latitudes (Muller-Parker & Davy 2001; Nybakken & Bertness 2005). In tropical regions, the angle and height of the Sun over the horizon gives maximum light penetration into the water column, changing very little seasonally. Conversely, in temperate regions, the angle of the sun above the horizon changes between summer and winter meaning that in temperate regions, the amount of light penetrating the sea surface and available for zooxanthellar productivity is far lower in the winter than in the summer (Nybakken & Bertness 2005). The portion of light entering the water column in temperate regions is further reduced in winter due to storm-induced mixing and increased runoff (Farrant *et al.* 1987; Muller-Parker & Davy 2001).

The ability of zooxanthellae to fix carbon via photosynthesis means they are an important source of nutrition to their hosts (Muscatine 1981). No clear difference is evident with respect to photosynthetic parameters such as the maximum rate of

photosynthesis, photosynthetic efficiency and compensation irradiance between tropical and temperate zooxanthellate anemones (reviewed by Muller Parker & Davy 2001). However, some tropical anemones possess specialised photosynthetic "auxiliary" vesicles containing high densities of zooxanthellae not present in their temperate counterparts (Day 1994). The lack of these structures in temperate anemones reflect a lower requirement for light harvesting in food rich temperate seas compared to the tropics (Muller-Parker & Davy 2001). Additionally, the motility of zooxanthellate anemones means they are able to modify their light environment by moving to positions with an optimal light regime. Positive and negative phototactic behaviour has been observed in anemones from both latitudes; behaviour attributed to the presence of zooxanthellae. However, why is it that the temperate zooxanthellate anemones *Cereus pedunculatus*, *Anthopleura ballii* (Davy *et al.* 1997), *Anthopleura artemisia* (Anderson 2000) and *Anthopleura aureoradiata* (S. K. Phillips, MSc Thesis, Victoria University of Wellington, 2006) actively bury themselves in the sediment? These results indicate a reduced requirement for light harvesting and autotrophy at high latitudes (Muller-Parker & Davy 2001).

Calculated proportions of photosynthate transferred to the host can vary greatly between tropical and temperate regions (Muller-Parker & Davy 2001). Due to zooxanthella productivity being poorer in temperate latitudes than in the tropics, it has been proposed that more carbon is available to tropical hosts than to temperate hosts (Tsuchida & Potts 1994). The most common technique for assessing the importance of translocated carbon to the host is to calculate the potential contribution of zooxanthellae to the animal's respiratory carbon requirements (CZAR) (Muscatine *et al.* 1981). CZAR percentage values for tropical corals (Davies 1984; Muscatine *et al.* 1984; Edmunds & Davies 1986; Davies 1991) and tropical jellyfish (Kremer *et al.* 1990; McCloskey *et al.* 1994) typically peak well in excess of 100% as do most tropical anemones (Battey & Patton 1987; Smith 1986; Day 1994) the only exception is the anemone *Bunodeopsis globulifera,* which has a peak of 92% (Day 1994). On the other hand, the temperate anemones *C. pedunculatus* and *A. ballii* (Davy *et al.* 1996; Davy *et al.* 1997) have CZAR peaks below 75% while the temperate octocoral *Capnella gaboensis* has a maximum CZAR of only 34% (Farrant *et al.* 1987). Comparisons of these values indicate that the potential for autotrophy is far greater in tropical regions than in temperate regions (Muller-Parker & Davy 2001).

Table 1. *Environmental parameters for a representative temperate site (Anacortes, WA) and tropical site (Discovery Bay, Jamaica) during winter and summer. All values for Anacortes are monthly means for January and July 1991-1999 (SPMC: unpublished data courtesy of Shannon Point Marine Centre water quality database), except for irradiance data for January (1992-1997) and July (1991-1996; PB NERR: unpublished data courtesy of Padilla Bay national Estuarine Research Reserve database). Values for Discovery Bay are for the months of January and July, as noted. Table redrawn from Muller-Parker & Davy 2001.*

	Temperate site	Tropical site	Reference	
	Anacortes	Discovery Bay	Anacortes	Discovery Bay
Latitude; Longitude	48°30'N; 122°41'W	18°28'N; 77°24'W		
Daily integrated surface irradiance ($mol \cdot m^{-1} \cdot L^{-1}$)			PB NERR	Porter 1985
winter	7	31[a]	PB NERR	Porter 1985
summer	45	41[a]		
Maximum surface irradiance ($\mu mol \cdot m^{-1} \cdot L^{-1}$)				
winter	548	2100[b]	PB NERR	Day 1994
summer	1891	2015[c]	PB NERR	Edmunds & Davies 1986
Temperature (°C)				
winter	7.5	26.5[d]	SPMC	Gates 1990
summer	11.7	29[d]	SPMC	Gates 1990
Inorganic nutrients (µM)				
Nitrate + Nitrite				
winter	32		SPMC	
summer	16.7	0.39[e]	SPMC	D'Elia 1988
Ammonium				
winter	0.9		SPMC	
summer	2.6	0.2[e]	SPMC	D'Elia 1988
Phosphate				
winter	3.1		SPMC	
summer	2.3	0.2[e]	SPMC	D'Elia 1988
Chlorophyll *a* ($\mu g \cdot L^{-1}$)				
winter	0.29	0.08[f]	SPMC	Webber & Roff 1995
summer	4.34	0.12[f]	SPMC	Webber & Roff 1995

[a] Monthly means for 1984

[b] Single day in February 1990

[c] Calculated from data for a single day in June 1985

[d] Monthly means for 1985-1987

[e] Mean values for August 1985-1987

[f] Winter: January 1991; summer: mean of July 1989 and 1990

1.5.2 Dissolved Nutrients

Inorganic nutrient concentrations are higher in temperate waters than they are in tropical waters (Muller-Parker & Davy 2001). While nutrient levels remain continuously low in the tropics, the general trend in temperate seas is for a peak in nutrient levels during winter, when wind-driven mixing and increased terrestrial runoff carry nutrients into surface waters. These nutrients result in rapid plankton growth in spring when solar irradiance increases, but become depleted progressively until nutrients reach their lowest in summer. Increased wind-driven mixing and increased terrestrial runoff in autumn then boost nutrient supplies for plankton growth before being restricted by declines in solar irradiance during winter (Marty *et al.* 2002; Greenan *et al.* 2004; MacKenzie & Adamson 2004; Davy *et al.* 2006). For example, in summer, ammonium and nitrate/nitrite concentrations off the Pacific Northwest coast of the USA can be 13 and 43 times greater, respectively, than those seen around tropical reefs in Jamaica during the same period (Table 1).

While autotrophic carbon productivity satisfies the carbon requirements of the zooxanthellae, it does not provide the nitrogen necessary for zooxanthellar growth and proliferation (Rees 1991). Heterotrophic feeding by corals and other cnidarian hosts provides zooxanthellae with nitrogen, presumably derived from host catabolism or the digestion of zooplanktonic prey (nitrogen recycling) (D'Elia & Webb 1977). This excretory nitrogen influences the nitrogen status of the zooxanthellae and as a result, the feeding history of the host is directly reflected in the nitrogen health of the zooxanthellae (Cook *et al.* 1988; Cook *et al.* 1992; Cook *et al.* 1994; Davy *et al.* 2006). Cessation of host feeding often results in nutrient deficient zooxanthellae that have reduced growth rates (Cook *et al.* 1988; McAuley & Cook 1994), reduced photosynthetic rates per cell (Falkowski *et al.* 1989), increased carbon/nitrogen ratios (Cook *et al.* 1988), increased nutrient uptake rates (D'Elia & Cook 1988; Muller-Parker *et al.* 1988; Grover *et al.* 2002), increased lipid volumes (Muller-Parker *et al.* 1996), decreased chloroplast numbers (Muller-Parker *et al.* 1996), decreased chlorophyll *a* levels (Cook *et al.* 1988; Dubinsky *et al.* 1990) and reduced zooxanthellar densities (Berner & Izhaki 1994; Muller-Parker *et al.* 1996). Furthermore, nitrogen may originate from the direct absorption of dissolved inorganic nitrogen (DIN; ammonium - D'Elia *et al.* 1983 & nitrite/nitrate - Marubini & Davies 1996) and dissolved organic nitrogen

(DON; urea - Grover *et al.* 2006; & amino acids - Ferrier 1991; Hoegh-Guldberg & Williamson 1999) from the seawater surrounding the cnidarian.

Despite being able to utilise an array of nitrogenous sources (i.e. zooplankton, organic particles, DIN and DON), zooxanthellae in tropical invertebrate hosts are widely thought to be nitrogen limited (Rees 1991), as a result of both low levels of dissolved nitrogen, limited food supplies for the host and competition resulting from high densities of zooxanthellae (Cook & D'Elia 1987; Rees 1991; Muller-Parker & Davy 2001). Confirmation of nitrogen deficiency in tropical zooxanthellae comes from research showing the addition of dissolved nitrogen to reef corals increases zooxanthellar nitrogen levels (Muller-Parker *et al.* 1994), population densities (Muscatine *et al.* 1989; Muller-Parker *et al.* 1994; Stambler *et al.* 1994) and photosynthetic rates (Ambariyanto & Hoegh-Guldberg 1999). Perhaps the best evidence for nitrogen deficiency in tropical zooxanthellae comes from the enhancement of dark carbon fixation in freshly isolated zooxanthellae after the addition of ammonium (Cook *et al.* 1992; Cook *et al.* 1994; Davy *et al.* 2006). Ammonium is required to stimulate amination of Krebs Cycle acids and the condensation of CO_2 to replace the aminated acids (Cook *et al.* 1992) and has the advantage that enhancement increases the more a cell becomes nitrogen deficient (with no enhancement when cells are nitrogen sufficient) (Flynn 1990; Davy *et al.* 2006). Indeed, Cook *et al.* (1992) and Cook *et al.* (1994) established that zooxanthellae in field populations of the sea anemone *Aiptasia pallida* and Bermudan corals *Montastrea annularis* and *Madracis mirabilis* are nitrogen deficient, and nitrogen sufficient *A. pallida* and *M. mirabilis* became nitrogen deficient after just one week of starvation, using the ammonium enhancement method. Additionally, this method has been used to determine nitrogen deficiency in free-living phytoplankton (Morris *et al.* 1971; Goldman & Dennett 1986; Flynn 1990), as well as symbiotic macroalgae (Davy *et al.* 2002), and for that reason, is an ideal tool for measuring the impacts of particulate matter or sedimentary nitrogen sources on zooxanthellae.

In contrast to the tropics, elevated nutrient levels and greater supplies of food indicate there is far greater potential for nitrogen sufficiency in temperate zooxanthellae and indeed the existing data point towards this (Davy *et al.* 2006). Glutamine/glutamate ratios of zooxanthellae from the temperate sea anemone *Anemonia viridis* are elevated

(McAuley 1994; Roberts *et al.* 2001a), while field populations of another temperate anemone, *Anthopleura elegantissima*, excrete ammonium into the surrounding seawater rather than retaining it (Jensen & Muller-Parker 1994). More recently, direct measurements of nitrogen status by Davy *et al.* (2006) indicate zooxanthellae in the temperate coral *Plesiastrea versipora* are nitrogen sufficient (or approach nitrogen sufficiency) in the temperate waters of southeastern Australia. Nevertheless, zooxanthellae of *P. versipora* became nitrogen deficient after two weeks of starvation illustrating the importance of host feeding on zooxanthellar nitrogen status (Davy *et al.* 2006).

1.5.3 Particulate Food

The abundance of planktonic prey in temperate waters is an order of magnitude higher than it is in tropical waters (Table 1). Elevated nutrient concentrations increase chlorophyll-*a* levels (presumably indicating phytoplankton) which are 36 times greater in the Pacific Northwest than around Jamaica, and ultimately increase the abundance of zooplanktonic food for cnidarian hosts (Muller-Parker & Davy 2001; Davy *et al.* 2006). The presence of zooxanthellae in cnidarians makes the symbiotic association polytrophic (many nutritional sources), allowing the acquisition of nutrition from both algal photosynthesis and host feeding (Muscatine & Porter 1977). In addition to the direct absorption of dissolved nutrients from the surrounding seawater, cnidarian-algal associations may also obtain nutrition via the catabolism of particulate food captured by the animal host. This can consist of phytoplankton (Fabricius *et al.* 1995; Fabricius *et al.* 1998; Ferrier-Pages *et al.* 1998; Ribes *et al.* 1998; Widdig & Schlichter 2001), zooplankton (Porter 1974; Porter 1977; Sebens *et al.* 1996; Ferrier-Pages *et al.* 1998), pelagic bacteria (Sorokin 1973; Bak *et al.* 1998; Ferrier-Pages *et al.* 1998), suspended or deposited particulate matter (Anthony 1999a; Anthony 2000; Mills 2000) and sediment (Rosenfeld *et al.* 1999; Mills & Sebens 2004). The uptake of the latter source is the primary focus of this thesis.

1.5.3.1 Planktonic Food

In many tropical cnidarians, the zooxanthellae alone can meet the host's respiratory, growth and reproductive needs, especially in well-lit tropical waters (Muscatine 1990).

In contrast, the lower levels of contribution from temperate zooxanthellae to the nutritional requirements of the host suggest that temperate associations are more likely to rely on heterotrophy than are their counterparts in the tropics (Muller-Parker & Davy 2001). Indeed, the temperate anemone *A. elegantissima* can acquire >2.5 mg carbon per day via zooplankton capture (Shick, Zamer, pers. comms. cited in Verde & McCloskey 1996) while no significant change in the body weight of the temperate anemones *A. ballii* and *A. viridis* was detected when field populations were caged in darkness with access to planktonic food (Davy *et al.* 1997). These results suggest that temperate zooxanthellate anemones can survive through heterotrophy alone (Muller-Parker & Davy 2001). Despite this, zooxanthella-cnidarian symbioses still persist in temperate regions, suggesting that over the long-term, zooxanthellae may provide a competitive advantage, enhancing host reproduction, and supplementing the host's metabolic needs during periods of food deprivation (Davy *et al.* 1997).

1.5.3.2 Particulate Matter or Sediment

In deep water or turbid environments, reduced light levels disrupt photosynthesis and thus decrease the magnitude of the photosynthate translocated to the host (Anthony & Hoegh-Guldberg 2003). The dependence on heterotrophy in symbiotic cnidarians must therefore increase (Dubinsky & Jokiel 1994). There has been much research into the effects of sediments on corals, predominantly the effects of increased turbidity and the ability of corals to reject sediments deposited onto their surfaces. Sediment hinders coral growth and calcification rates (Dodge & Vaisnys 1977; Kendall & Powell 1985; Tomascik & Sander 1985; Hubbard 1986), interferes with respiration, feeding and photosynthesis (Abdul-Salem & Porter 1988), abrades coral tissues (Loya 1976), amplifies energy dissipation (Abdul-Salem & Porter 1988; Riegl & Branch 1995), reduces fecundity and impedes settlement processes (Dodge & Vaisnys 1977; Hodgeson 1990; Babcock & Davies 1991; Hunte & Wittenberg 1992). The upshot of these has led to the widespread perception that sedimentation is disturbing to symbiotic cnidarians (reviewed by Rogers 1990). Meanwhile, several studies have demonstrated the negative effects on corals by displaying the extent to which corals have developed various defence mechanisms to sedimentation. Corals can reject sediment to a certain degree by means of morphological adaptations, by adopting sediment resistant growth forms (e.g. more multi-lobed, knobby, or rounded growth forms, columnar formations, fusion of adjacent coral colonies – Rogers 1990) and by directed behaviour such as hydrostatic

pumping, ciliary action and mucus production (Hubbard & Pocock 1972; Bak & Elgershuizen 1976; Lewis 1977; Lasker 1980; Simmons 1979; Logan 1988; Stafford-Smith & Ormond 1992; Stafford-Smith 1993). Despite these adverse consequences, Abelson *et al.* (1993) and Abelson & Loya (1995) speculated that due to patterns of coral distribution and coral morphological characteristics, some corals seemed to increase the amount of sediment encountered rather than avoiding it. Moreover, many studies have recognized the ability of corals to survive and proliferate at sites of high sediment loads (Loya 1976; Lewis 1977; Simmons 1979; Dollar & Grigg 1981; Rogers 1982; Hubbard 1986; Rice & Hunter 1992; Riegl & Branch 1995; Riegl *et al.* 1996).

Deposit feeding animals acquire food by swallowing large volumes of sediment. Associated with the sediment are colonising microbes (bacteria, microalgae, Protozoa & fungi), meiofauna and non-living organic matter, which can be a source of nutrients (carbon, nitrogen and/or phosphorus) to deposit feeders (Lopez & Levinton 1987). While several studies have hypothesised that some corals possessed the ability to utilise sediment as a supplementary food source (Logan 1988; Stafford-Smith & Ormond 1992; Abelson & Loya 1995), it had never been experimentally demonstrated. Only in the past decade, have several investigations determined that some tropical coral species are able to ingest and benefit nutritionally from sedimenting particles suspended in the water column or accumulated on their surfaces (Mills & Sebens 1997; Anthony 1999a; Anthony 1999b; Rosenfeld *et al.* 1999; Anthony 2000; Anthony & Fabricius 2000; Fabricius & Dommisse 2000; Mills 2000; Mills *et al.* 2004; Mills & Sebens 2004). The first direct evidence for the ability of a coral to consume sediment came from Rosenfeld *et al.* (1999). Experimentation with fluoroscein-isothiocyanate, which labels organic compounds in the sediment including proteins, peptides and amino acids, showed the consumption and transfer of labelled organic matter from the sediment into the cells of the solitary reef coral, *Fungia horrida.* Similarly, Anthony (1999a; 1999b; 2000) Anthony & Fabricius (2000) and Fabricius & Dommisse (2000) showed that various coral species from the Great Barrier Reef differed in their capacities to use suspended particulate matter (SPM) as a nutritional source. Using SPM labelled with the radioisotope ^{14}C, Anthony (1999a; 1999b; 2000) found that the corals *Pocillopora damicornis*, *Montipora digitata* and *Acropora millepora* ingest carbon from SPM provided to them. Additionally, Anthony (1999a) and Anthony & Fabricius (2000) proposed that SPM-associated carbon could provide corals which utilise it with half of

the carbon required for tissue growth and also compensate for decreased zooxanthellar productivity resulting from turbidity-induced lower light levels.

Suspended or downward-fluxing particulate matter as well as surficial or resuspended benthic sediment can also be a potential source of nitrogen to corals (Anthony 1999a; Anthony 1999b; Anthony 2000; Mills 2000; Mills & Sebens 2004; Mills *et al.* 2004). Although dissolved nitrogen is an important source of nitrogen to tropical corals, the waters surrounding coral reefs can have very low concentrations of DIN and DON (Wilkerson & Kremer 1992; Muller-Parker & Davy 2001). Similarly, while zooplankton is commonly considered the most important heterotrophically-obtained nitrogen resource, their abundance on reefs is highly variable and some zooplankton can actively avoid and escape coral tentacles (Heidelberg *et al.* 1997). In contrast, suspended or downward-fluxing particulate matter as well as surficial or resuspended benthic sediment is present all the time (Mills 2000). Mills *et al.* (2004) investigated the ability of four scleractinian coral species to acquire associated nitrogen from either suspended or deposited particulate matter (PM). Again, the nitrogenous organic component in the particulate matter was labelled, this time with the stable isotopic tracer ^{15}N before being provided to the corals *Siderastrea radians*, *Montastrea franksi*, *Diploria strigosa* and *M. mirabilis.* Three of the coral species *S. radians*, *M. franksi*, and *D. strigosa* were successfully able to obtain nitrogen through feeding on both the suspended and deposited particulate matter (DPM). Meanwhile, Mills & Sebens (2004) measured a decline in nitrogen content of benthic sediments (< 100µm particle size) following exposure to coral surfaces. Both Mills & Sebens (2004) & Mills *et al.* (2004) conclude that SPM, DPM and benthic sediments that become resuspended and land on coral surfaces are an important supply of nitrogen to many corals species that thrive in highly turbid tropical waters.

Corals are passive suspension feeding organisms (Douglas 1994). Rosenfeld *et al.* (1999), Anthony (1999a) and Anthony & Fabricius (2000) speculated that the role of sediment and particulate matter varied within and among species, depending on the type, loads, organic fractions and particle size of the sediment as well as the speed of water flow, the coral's morphology and the amount that the coral encounters highly turbid waters. Regardless of the nutritional properties of sediment, high sediment loads may cause most of the coral polyps to stop feeding and reject sediment altogether (Mills

2000; Mills & Sebens 1997). Anthony (1999a) measured decreasing particulate organic carbon assimilation efficiencies with increasing concentrations of SPM while Mills & Sebens (1997) found the sorting of more nutritional particles from the less nutritional particles by corals became less efficient with increasing particle load. Likewise, Mills & Sebens (2004) demonstrated as sediment loads increased, selective ingestion by corals of particles with high nutritional quality decreased or ceased altogether. Meanwhile, corals from regions where sedimentation was common were more likely to utilise sediment as a food source (Abelson *et al.* 1993; Anthony 2000). Fabricius & Dommisse (2000) discovered detritus and other small suspended sediment particles (<10 μm particle size) are an important food source for alcyoniid coral-dominated reef communities in high turbidity regimes. Likewise, Anthony (2000) showed that the coral species, *Pocillopora damicornis* and *Acropora millepora* from inshore, turbid reefs in the GBR lagoon have developed a greater capacity (10-20 times) to feed on SPM than conspecifics from less turbid nearshore and offshore (midshelf) reefs, suggesting that corals from more turbid environments were better adapted to utilising SPM. Similarly, Mills (2000) and Mills *et al.* (2004) found up to 20 times better ingestion rates for nitrogen associated with SPM on reefs in Bermuda when concentrations of SPM were nearly doubled.

Turbidity results in reduced light levels, which in turn disrupts zooxanthellar photosynthesis, impacting negatively on zooxanthellae (Anthony & Hoegh-Guldberg 2003). In spite of all the research indicating that the coral host profits nutritionally from particulate matter and sedimentary sources, the effects of such ingestion on zooxanthellae have largely been ignored. Most of the research thus far indicates that nutrients obtained from SPM and sediment are largely reserved for coral host growth. Anthony (1999a) found that relatively little of the carbon assimilated from ingested SPM was respired (13-34%) by the coral, with the majority used for tissue growth. Mills *et al.* (2004) detected no ^{15}N labelled nitrogen in zooxanthellar fractions following host uptake of ^{15}N labelled sediment. This lack of any ^{15}N labelled nitrogen prompted Mills *et al.* (2004) to suggest that nitrogen recycling in the corals *S. radians*, *M. franksi* and *D. strigosa* did not occur or was minor, and that nitrogen acquired by the host through ingestion was conserved for coral growth (in agreement with the nitrogen conservation hypothesis). However, nitrogen in particular is equally important for the growth of the symbiont (Rahav *et al.* 1989) and studies thus far that have detected

particulate matter or sedimentary consumption by the coral have not directly measured the nutritional outcomes of this intake on zooxanthellae. Perhaps, the effects of particulate matter or sediment host uptake on the zooxanthellae could be examined using the ammonium enhancement-nitrogen status method described above (see section 1.5.2).

All work thus far assessing sedimentary or particulate matter ingestion has been conducted in tropical coral species which inhabit turbid, but otherwise, food poor, low nutrient waters. Such conditions promote the utilisation of particulate matter or sediment as a source of nutrition (Anthony 1999a; Anthony & Fabricius 2000). Nonetheless, turbidity may be even more extreme in temperate seas, where several temperate zooxanthellate sea anemones (*C. pedunculatus*, *A. ballii* and *A. aureoradiata* – Davy *et al.* 1997; S. K. Phillips, MSc Thesis, Victoria University of Wellington, 2006) actively bury themselves in the sediment of coastal mudflat systems. These soft-substrate intertidal habitats are primarily comprised of unstable mud or silt and can therefore become tremendously turbid environments. The unusual habit exhibited by these anemones would have traditionally been thought to have had a negative impact on anemone growth and photosynthetic productivity through decreased light levels and reduced planktonic feeding. However, according to the research that uptake of particulate matter or sediment can compensate for decreased zooxanthellar productivity in turbid tropical waters (Anthony 1999a), mudflat sediment could potentially be an important source of nutrition to the zooxanthellate anemones that bury themselves in it. Indeed, intertidal mudflats are inhabited by numerous deposit feeding organisms (polychaetes, bivalves, gastropods) that utilise the sediment as a source of carbon and nitrogen (Lopez & Levinton 1987). Also, sediment particles can easily be stirred up or resuspended on mudflats and suspension-feeding organisms such as anemones will inevitably take up various amounts of these particles in addition to planktonic organisms.

1.6 Aims & Objectives

This study aimed to determine the potential for, and efficiency of, particulate nitrogen uptake from the sediment in *A. aureoradiata,* and the consequences of this on the nitrogen status of the zooxanthellae.

In particular, this study:

1. Determined whether *A. aureoradiata* could take up particulate nitrogen from the sediment and if so, whether this nitrogen was assimilated by the host, algal symbiont or both.
2. Compared and contrasted the nitrogen status of zooxanthellae in *A. aureoradiata* living on a turbid mudflat versus a non-turbid rocky shore.
3. Established whether the zooxanthellar nitrogen status in *A. aureoradiata* is enhanced by the availability of sediment and whether this could explain any differences seen between anemones living on mudflats versus those on the rocky shore.

Ultimately this work will contribute to the limited data on the nutritional benefits of particulate matter or sedimentary food sources to cnidarian-algal symbioses and is the first such study for a temperate association.

2

METHODS

2.1 The Anemone & its Habitat

Anthopleura aureoradiata (Figure 2), more commonly known as the mudflat anemone, is a common intertidal sea anemone found throughout New Zealand. On the mudflats, *A. aureoradiata* is typically found attached to the hard shells of the burrowing cockle *Austrovenus stutchburyi* that live just beneath the surface of the mud in the mid-intertidal zone (Figure 3 & 4C). Despite the presence of other similar sized, burrowing bivalves (*Paphies subtriangulata* and *Paphies australis*) in the area, this study found anemones predominantly attached to live cockles or to cockle shell debris (S. Morar, pers. obs.). The anemones are usually attached next to one another within the aerobic layer of the sediment, with generally an even demographic spread. They were not observed in the deeper anoxic mud, possibly due to lack of oxygen in that layer. Adults typically reach 2-3 cm in length when fully extended. The pedal disc of the animal is attached below the surface of the sediment approximately 0.5 cm, allowing withdrawal into the sediment (Figure 3). Anemones were sometimes observed to have their tentacles extended over the surface if covered in water as a result of the tide or the formation of shallow pools (Figure 3A & 4B). However, at other times, anemones remained retracted beneath the surface even when water was present above; perhaps as a result of sun exposure, turbidity or windy conditions (Figure 3B). The mudflat field site used in this study was Pauatahanui Inlet, Wellington, New Zealand (Figure 4 & 5C).

Meanwhile in the rocky intertidal (Figure 6), anemones were found aggregated in cracks and crevices attached to rocks (Figure 6D) in both tide pools in the upper-littoral zone (Figure 6B) and throughout the mid-littoral zone (Figure 6C). Tide pool anemones remained submerged in seawater while those lower down in the mid-littoral zone were exposed to tide, sometimes becoming uncovered to the air for long periods during low tide (depending on the height of the tide) but always submerged at high tide. No anemones were observed in the lower-littoral or sub-littoral zones (S. Morar, pers. obs.).

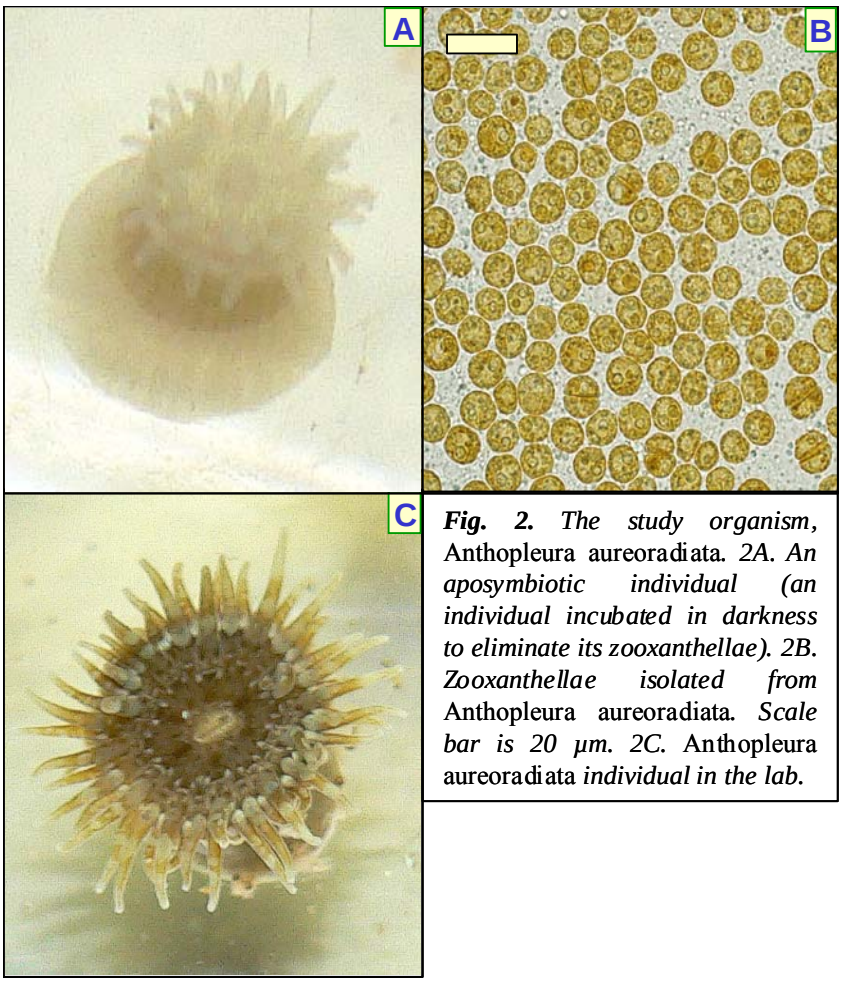

Fig. 2. *The study organism,* Anthopleura aureoradiata. *2A. An aposymbiotic individual (an individual incubated in darkness to eliminate its zooxanthellae). 2B. Zooxanthellae isolated from* Anthopleura aureoradiata. *Scale bar is 20 µm. 2C.* Anthopleura aureoradiata *individual in the lab.*

Although of the same species, anemones from the rocky-intertidal were visibly darker (browner) in colour in relation to their mudflat counterparts, possibly due to increased densities of zooxanthellae or photosynthetic pigment. The rocky intertidal site used in this study was Kau Bay, Wellington, New Zealand (Figure 5D & 6).

A. aureoradiata broods its young for an unknown period of time, releasing one to many offspring a couple of millimetres in length (S. Morar, pers. obs.).

2.2 Anemone Collection & Maintenance

A. aureoradiata was collected at low tide from Pauatahanui Inlet and brought back to the lab; the number collected is clarified in the sections relevant. These were housed in a large glass dish along with 1-µm FSW and maintained in an incubator with a 12h light/12h dark cycle (light irradiance levels ranging from 120-170 µmol photons m^{-2} s^{-1}) and a constant temperature of 16 ± 1°C. Unless stated, anemones were collected and maintained in the same way (same incubator) for the all the following experiments.

2.3 Sediment Collection

Sediment was collected from Pauatahanui Inlet at low tide. Using a tablespoon, the top 1-5 mm of sediment was collected and filtered through a 1 mm mesh size sieve into a 2-litre collecting jar. The sediment was brought back to the lab where it was allowed to settle, before the overlying water was decanted. 1000 ml 0.22-µm FSW was then added to the sediment, the sediment thoroughly stirred and allowed to settle, before the overlying water was again decanted. This rinsing process was repeated 4 times with the purpose of removing any remains of the original interstitial water from the sediment. Unless stated, sediment was collected rinsed and maintained in the same way for the all the following experiments, however the amount collected will be clarified in the relevant sections.

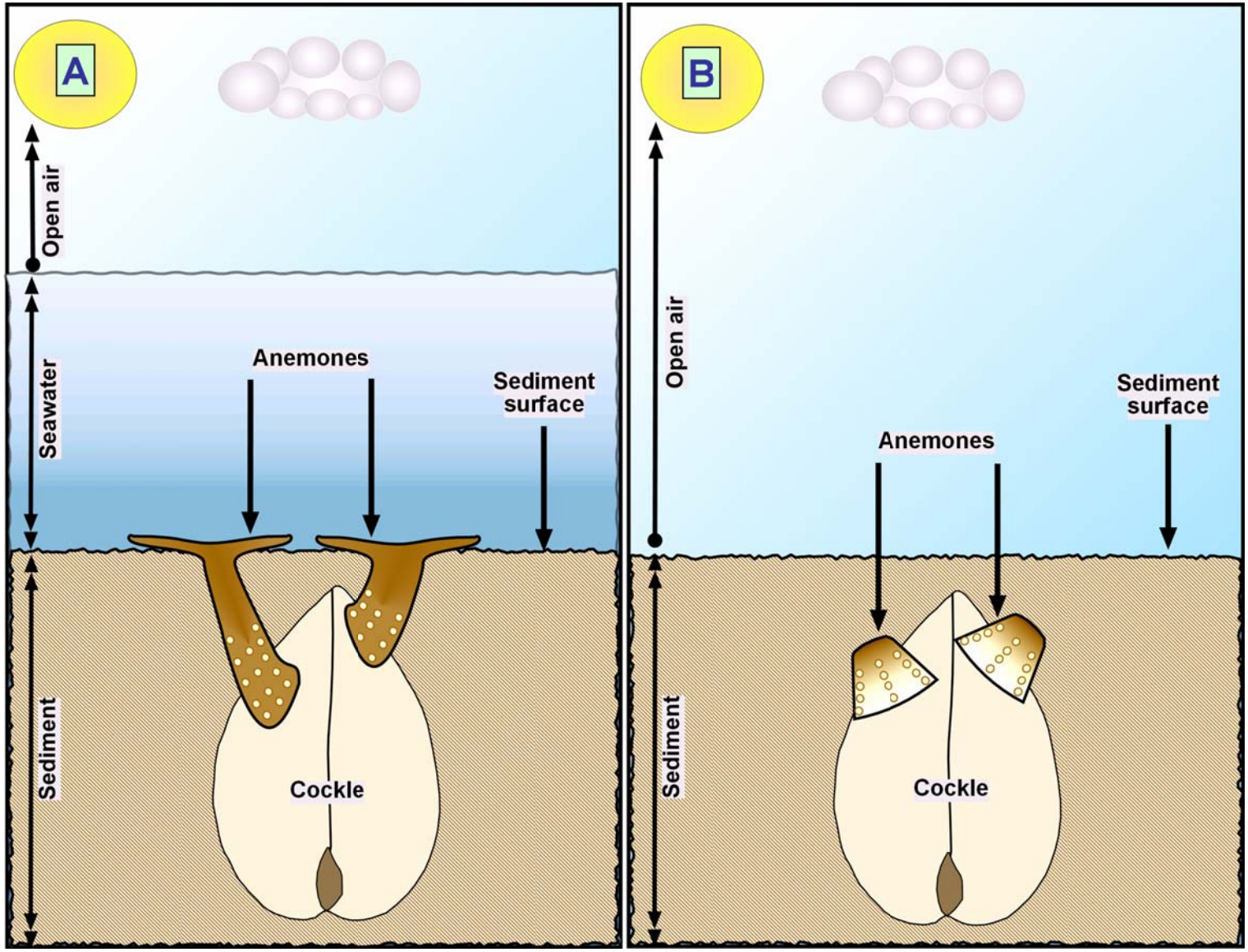

Fig. 3. *Cross section diagram of* Anthopleura aureoradiata *on a mudflat. The shells of cockles (*Austrovenus stutchburyi*) are the only hard substrate for attachment in this environment. 3A. Anemones extend their columns so the tentacles and oral discs reach the surface. Observations indicate this is common during periods when surface seawater is present (either at high tide or low tide) however sun exposure, wind and turbidity of the water also seem to influence whether anemones come to the surface or not (S. Morar, pers. obs.). 3B. Anemones remain buried beneath the surface (<15 mm) with their tentacles withdrawn at low tide. Anemones may spend long periods beneath the surface depending on the low tidal height.*

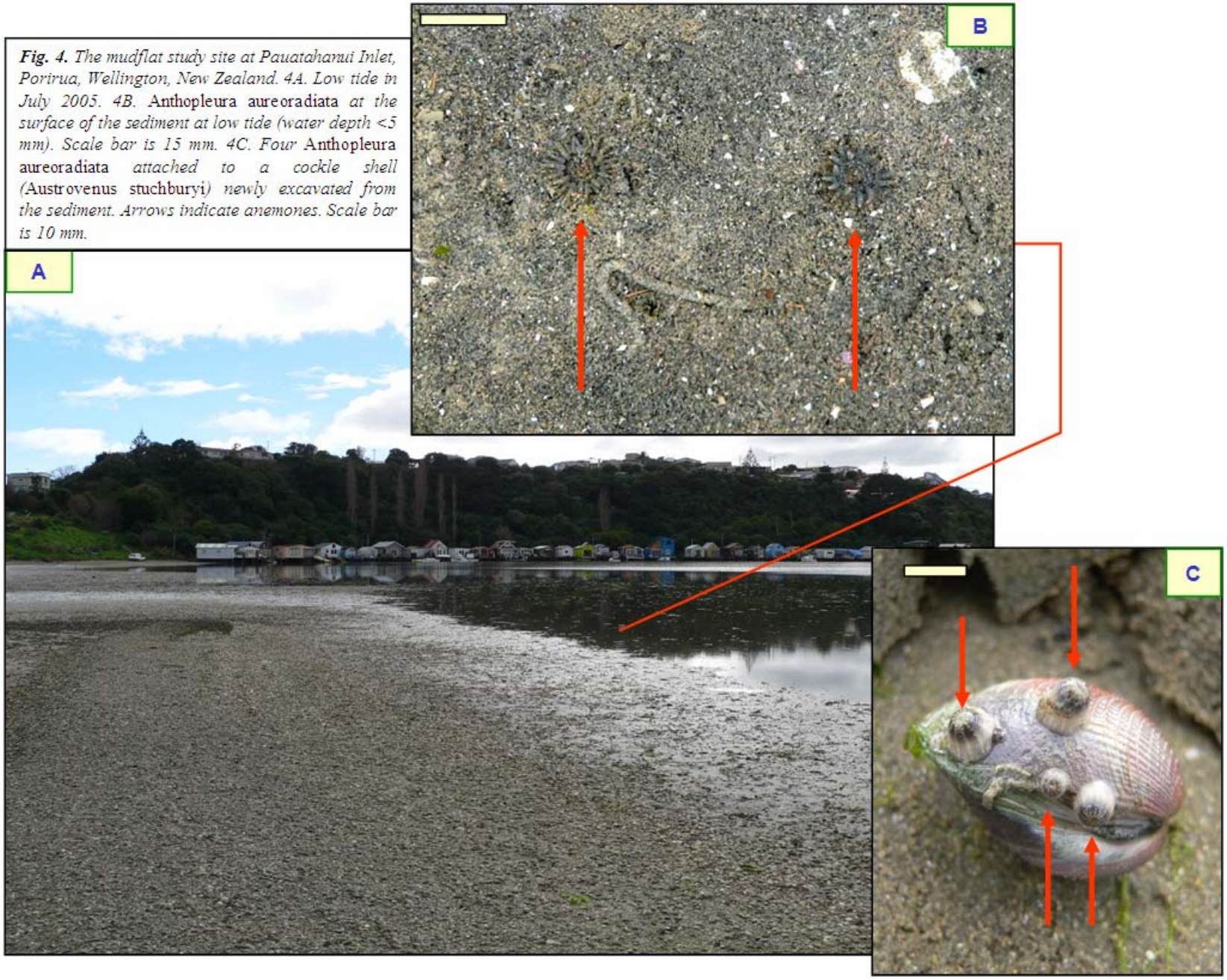

Fig. 4. *The mudflat study site at Pauatahanui Inlet, Porirua, Wellington, New Zealand. 4A. Low tide in July 2005. 4B.* Anthopleura aureoradiata *at the surface of the sediment at low tide (water depth <5 mm). Scale bar is 15 mm. 4C. Four* Anthopleura aureoradiata *attached to a cockle shell (*Austrovenus stuchburyi*) newly excavated from the sediment. Arrows indicate anemones. Scale bar is 10 mm.*

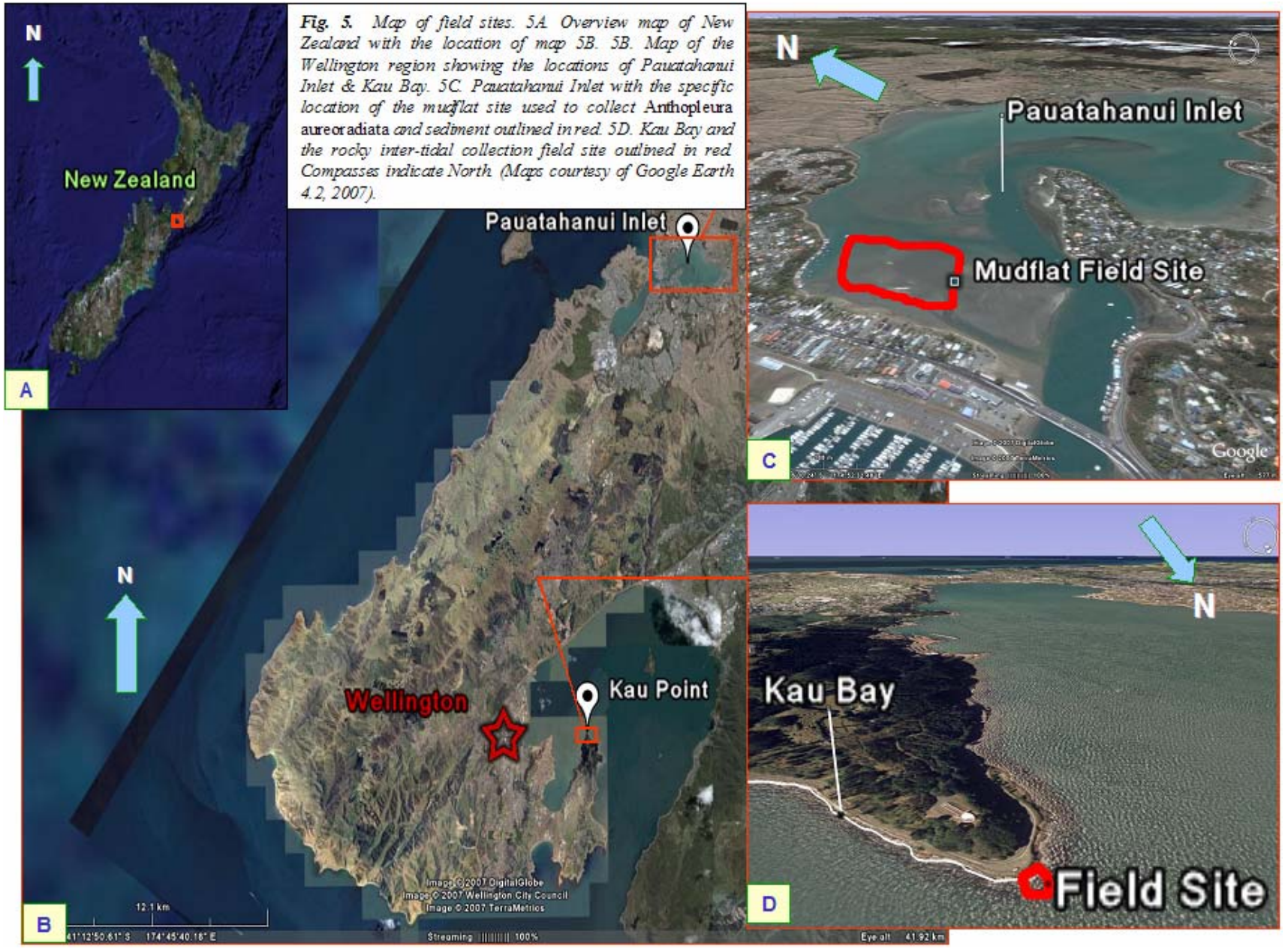

Fig. 5. Map of field sites. 5A. Overview map of New Zealand with the location of map 5B. 5B. Map of the Wellington region showing the locations of Pauatahanui Inlet & Kau Bay. 5C. Pauatahanui Inlet with the specific location of the mudflat site used to collect Anthopleura aureoradiata *and sediment outlined in red. 5D. Kau Bay and the rocky inter-tidal collection field site outlined in red. Compasses indicate North. (Maps courtesy of Google Earth 4.2, 2007).*

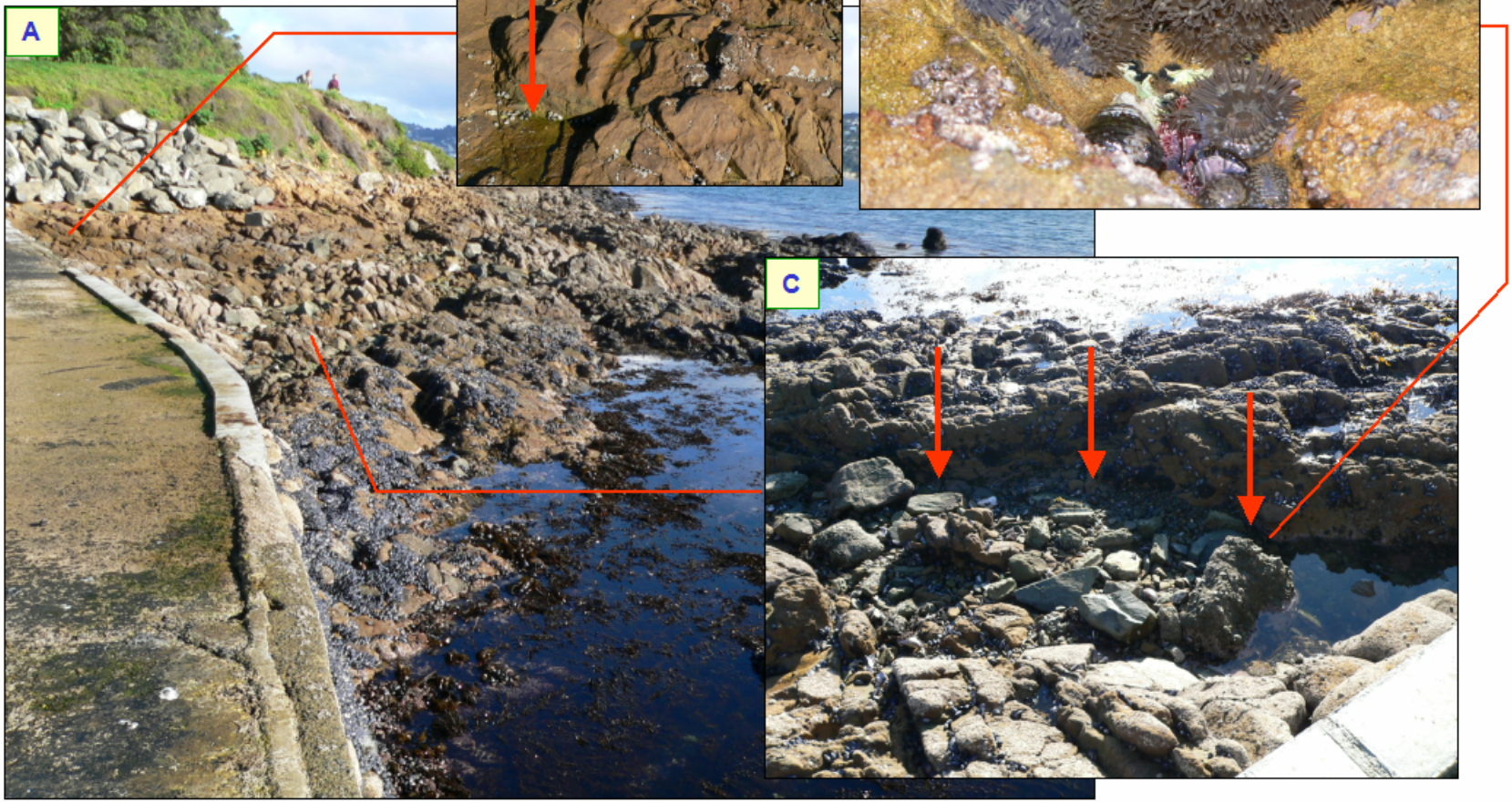

Fig. 6. *The rocky inter-tidal study site at Kau Bay, Wellington, New Zealand. 6A. Just after low tide in August 2007. 6B. The high shore height tide pools. Arrows indicate tidepools in the upper-littoral zone where* Anthopleura aureoradiata *was collected. 6C. The mid-littoral zone site where* Anthopleura aureoradiata *is exposed to the air at low tide. Arrows show where the low shore anemones were collected from. 6D. Aggregation of* Anthopleura aureoradiata *individuals attached to rocks in the mid-littoral zone. Scale bar is 10 mm.*

2.4 Sediment ^{15}N Enrichment

The sediment enrichment experiment was designed to assess which component of the sediment (biological/chemical/physical) was taking up the ^{15}N label. Sediment (1 kg) was collected on the 7th of June 2006 and prepared as per section 2.3. Sub-samples of the sediment were then centrifuged (4500 × g) and rinsed three times with 0.22-µm FSW to further remove any remains of the original interstitial water, ahead of being exposed to $(^{15}NH_4)_2SO_4$.

Uptake of $^{15}NH_4^+$ by the sediment was examined in three separate 200 ml glass dishes with sediment alone + 50 ml 0.22-µm FSW. To each dish, 50 µl of 20 mM 98% $(^{15}NH_4)_2SO_4$ (Sigma) dissolved in 0.22-µm FSW was added along with 5 g (blotted paper wet weight) of sediment (final conc. 2.68 µM 98% $[^{15}NH_4]_2SO_4$), before each dish was placed onto a magnetic stirrer; the speed of the magnetic stirrer was enough to keep all the sediment in suspension but not so fast that sediment splashed out of the dish. The sediment was sampled in duplicate (250 µl) over 48h (t = 0, 24 and 48h), then filtered onto pre-combusted (500°C for 4h) 2.5 cm diameter Whatman GF/F glass fibre filters under low vacuum (<10 mm Hg), and rinsed with 5 ml 0.22-µm FSW. The samples were dried (55-65°C) until reaching a constant weight, then fumed in a desiccator containing an open bowl of concentrated HCl for 24h to remove carbonates. Samples were again dried until constant weight prior to being packed into Nalgene 6-well plastic plates, sealed (to prevent hydration of samples) and placed into a box plastic box with desiccant packs, ready to be sent for ^{15}N analysis. All ^{15}N isotopic analysis was completed by Sarah Bury, National Institute of Water & Atmospheric Research (NIWA), Wellington.

One kilogram of sediment was again collected from on 15th June 2006 and prepared as above. Uptake of ^{15}N was further examined with another two treatments: 50 ml 0.22-µm FSW + ampicillin (50 µg/ml), and 50 ml 0.22-µm FSW + formalin (3% final concentration); these treatments tested for uptake via biological means. To both dishes 50 µl of 20 mM 98% $(^{15}NH_4)_2SO_4$ was added along with 5 g as above. Samples were taken and prepared for ^{15}N analysis as per above except that findings from the first

experiment led to sediment being sampled in duplicate over 24h (t = 0 and 24h), not 48h.

2.5 Particulate ^{15}N Uptake from the Sediment

This experiment was designed to evaluate the potential for, and efficiency of, particulate nitrogen uptake from the sediment of *A. aureoradiata*.

2.5.1 Sediment Collection & Labelling

Approximately 1 kg wet weight sediment was collected on 24th October 2006 and prepared as per section 2.3. The sediment was then patted dry with blotting paper before being made up to 2500 ml with 0.22-µm filtered seawater FSW and placed in a specially made stirrer (Figure 7) designed to keep all of the sediment in suspension while being exposed to $(^{15}NH_4)_2SO_4$ (final conc. 530.30 µM 98% $[^{15}NH_4]_2SO_4$). Once a day for 4 days, 35 ml of 10 mM 98% $(^{15}NH_4)_2SO_4$ dissolved in 0.22-µm FSW was added to the sediment suspension. At the end of the 4 days, the sediment was allowed to settle and rinsed a further 3 times as above with 0.22-µm FSW; but this time to remove any unincorporated ^{15}N label. The overlying water was then decanted and the labelled

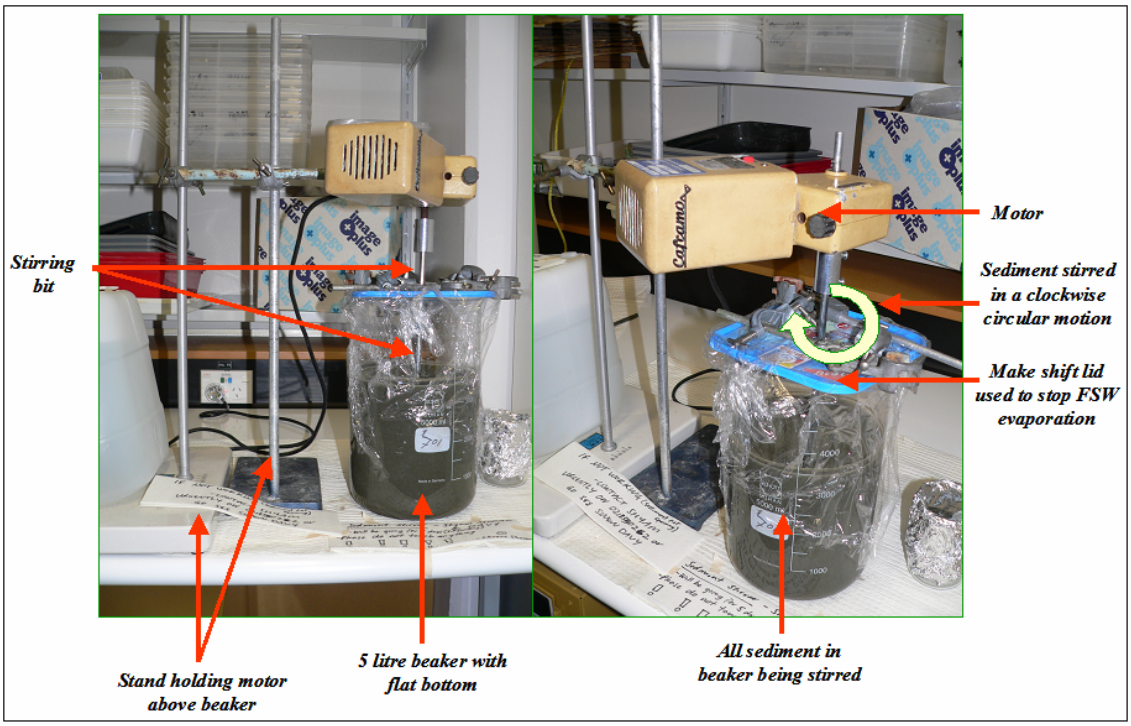

Fig. 7. *The stirrer which kept sediment in suspension while being enriched with $(^{15}NH_4)_2SO_4$. Motor speed was enough that all of the sediment (approx. 1 kg blotted dry, wet weight) remained suspended throughout the 4-day labelling period. This allowed all sediment used in the feeding experiments to be labelled with $(^{15}NH_4)_2SO_4$ at the same time.*

sediment placed in several acid-washed and pre-combusted (500°C for 4h) plexiglass dishes ahead of being freeze-dried for 48h and stored in a desiccator. The dried sediment allowed similar samples to be used for all replicates in the sedimentary particulate nitrogen uptake experiment.

2.5.2 Anemone Collection & Maintenance

Approximately 100 anemones were collected 17th October 2006 and maintained as per section 2.2. During this period, anemones were fed 5 times a week with *Artemia* sp. nauplii for two weeks and monitored closely for any change in health or appearance (e.g. zooxanthellar expulsion) to ensure they remained healthy.

2.5.3 Anemone Exposure to ^{15}N Sediment

The sediment exposure experiments took place in small 50 ml glass containers with screw top lids (named "feeding chambers" - Figure 8). These were acid washed (10% HCl) and autoclaved to remove or kill any attached microorganisms within the chambers prior to the start of experiment. 30 ml 0.22-µm FSW was added and all the chambers placed in the same incubator as above to maintain constant water temperature.

Fig. 8. *Small glass 'feeding chambers'. Chambers had screw top lids (as shown), which remained off prior to the feeding experiment but fitted once the experiment had started.*

10 similarly sized anemones (pedal disc diameter 10 ± 1 mm) were then placed into a separate 50 ml chamber and given 1h to fully extend their tentacles and form a strong attachment to the bottom. Any individual that did not to do so was replaced with another. The chambers were then placed back into the incubator for 5 days prior to use, during which time the anemones were unfed. Six control chambers with just 30 ml 0.22-µm FSW (i.e. no anemones) were also placed in the incubator for this time.

The experiment was run with two different sediment loads which were intended to closely replicate the conditions seen affecting *A. aureoradiata* on a mudflat (Figure 3): the low sediment load which contained 5 g dry weight in 22 ml 0.22-µm FSW - final concentration 0.227 g ml^{-1} (Figure 9A) and the high sediment load which contained 20 g dry weight in 15 ml 0.22-µm FSW - final concentration 1.333 g ml^{-1} (Figure 9B). At the low sediment load, anemones were buried in approximately 10-15 mm of sediment allowing them to bring their tentacles and oral discs to the surface, with light shaking from the orbital shaker keeping smaller sediment particles in suspension (Figure 9A); at the high sediment load, the amount of sediment (approximately 30 mm depth) plus a very low FSW depth 3-5mm) at the surface meant only a small amount of sediment became suspended, however no anemone could extend itself to the surface during the experiment (Figure 9B). In total there were 16 chambers with 8 chambers per sediment load: 5 chambers each contained the low sediment load and a anemone while 3 control chambers contained just the low sediment load and no anemone; 5 chambers each contained the high sediment load and a anemone while 3 control chambers contained just the high sediment load and no anemone.

The uptake of particulate nitrogen from the sediment experiment was designed to firstly detect and measure any uptake of particulate nitrogen by *A. aureoradiata,* and secondly, to ascertain if there was any difference in this uptake between the low and high sediment loads. The labelled sediment for each chamber was weighed into 50 ml centrifuge tubes, then centrifuged (4500 × g) and rinsed with 0.22-µm FSW twice (to remove any unincorporated ^{15}N label). Afterward, the sediment was soaked in 0.22-µm FSW for approximately an 1h before being rinsed and centrifuged another three more times (again to remove any remaining unincorporated ^{15}N label), before finally being ready for use in the experiment. The low sediment load was carefully layered onto the anemone at a level where the anemone's tentacles remained open. The high sediment

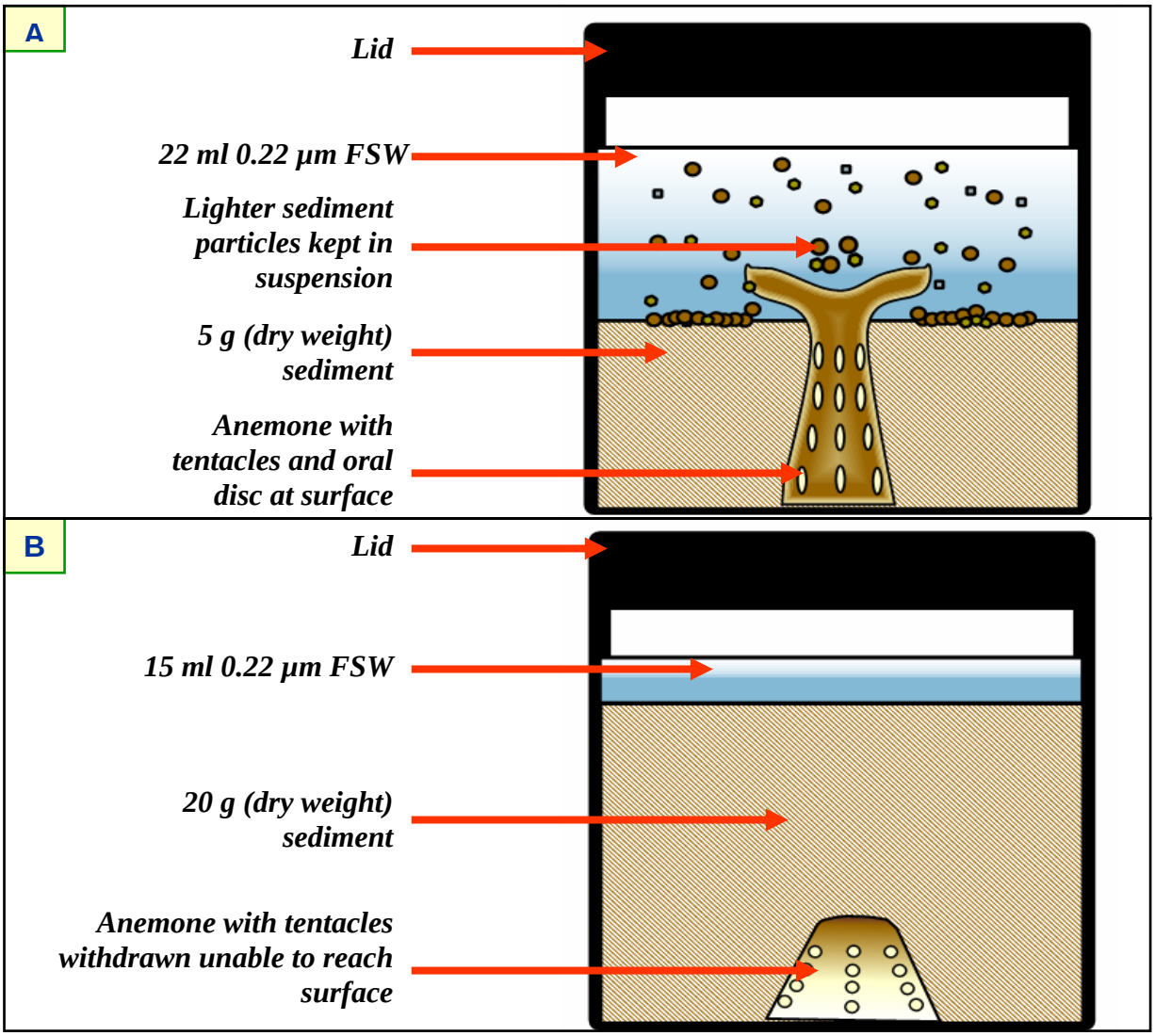

Fig. 9. *Design of sedimentary nitrogen uptake experiments. The high and low sediment loads were intended to closely replicate those that affect* Anthopleura aureoradiata *on the mudflat. 9A. At the low sediment load anemones were buried in approx. 10-15 mm of sediment allowing them to bring their tentacles and oral discs to the surface. Light shaking from the orbital shaker allowed some of the smaller sediment particles to remain suspended. 9B. At the high sediment load, the amount of sediment (approx. 30 mm depth) plus a very low FSW depth (3-5mm) at the surface meant very little sediment became suspended and no anemone could come to the surface during the experiment.*

load was placed on top of the anemone, covering the anemone entirely beneath approximately 30 mm of sediment. Sediment was also added to the anemone-free control chambers at the respective sediment loads. 1 ml (pipetted) sub-samples of the sediment (sediment shaken vigorously to get all sediment in suspension before sample taken) in each chamber were taken immediately to determine the initial atom percent ^{15}N. These samples were dried (55-65°C) and decalcified on pre-combusted 2.5 cm GF/F filters (500°C for 4h) prior to mass spectrometer analysis (S. Bury NIWA, Wellington). All chambers were then placed onto an orbital shaker in a light (80-120

µmol photons m^{-2} s^{-1}), temperature (18°C) and humidity (relative humidity 50-55%) controlled room. The speed of the shaker was gentle enough that most of the sediment in the high sediment load chambers remained settled but agitated enough that in the low load, anemone tentacles remained open and a few of the lighter sediment particles kept in suspension.

The uptake experiment lasted 6h, during which light, temperature and humidity conditions, as well as the anemones and the feeding chambers were closely monitored. At the end of experiment (t = 6h), the chambers were taken off the orbital shaker and the anemones removed. The anemones were rinsed thoroughly in 0.22-µm FSW to clean off any adhered sediment and then placed in "egestion chambers" (Figure 10) filled to the brim with 0.22-µm FSW. Anemones were then given 1h to attach themselves to the bottom of these chambers before the chambers were sealed with screw top lids and placed upside down in light (80-120 µmol photons m^{-2} s^{-1}) for 18h. This allowed time for the digestion and egestion (as shown by Figure 10) of ingested sediment before the anemone tissue was processed.

Meanwhile, the remaining sediment content of each of the experimental chambers was vigorously mixed and 1 ml sub samples were taken and filtered onto pre-

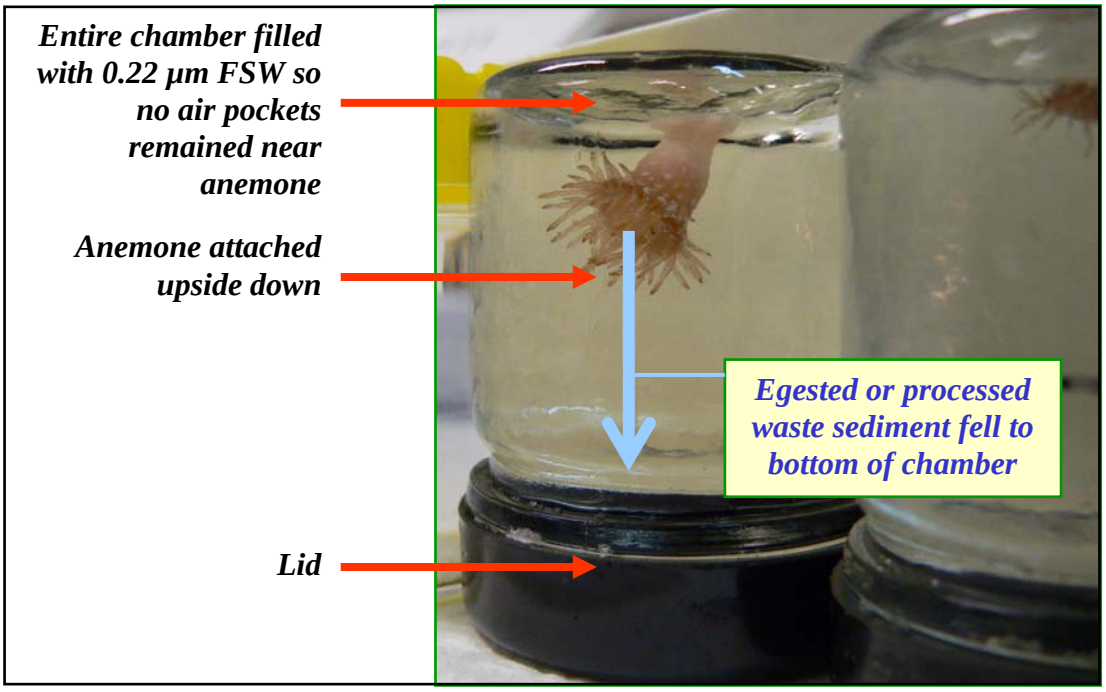

Fig. 10. *Egestion chambers with upside down attached anemones. Any egested sediment fell to the bottom of the chamber as indicated by direction of the Blue arrow.*

combusted 2.5 cm GF/F filters (500°C for 4h) and prepared for ^{15}N analysis as before. At the end of the egestion period, the anemones were thoroughly rinsed in their own egestion chamber's water and each anemone homogenised using a hand-held 15 ml glass tissue grinder in 3 ml 0.22-µm FSW. The resulting homogenate was then made up to 8 ml and centrifuged (2500 × g) for 5 min. The host fraction (supernatant) was then decanted off into a separate tube and refrigerated (6°C) while the symbiont zooxanthellar pellet was resuspended and centrifuged a further three times before being pipetted onto pre-combusted 2.5 cm GF/F filters (500°C for 4h) and dried (55-65°C). Increments (0.5 ml) of the host supernatant were then sequentially spotted onto pre-combusted 2.5 cm GF/F filters (500°C for 4h) with the filters being dried (55-65°C) between each spotting; an approximate total of 0.5-2 ml was spotted onto each filter. Finally, the host and zooxanthellar filters were fumed in a desiccator with concentrated HCl to remove carbonates, dried at 55-65°C to constant weight, and then packed into 6-welled plastic plates as above, ready for ^{15}N analysis.

2.5.4 Natural ^{15}N Abundance of Anemone

Natural ^{15}N abundance values were taken to determine the natural amount of ^{15}N in anemone and zooxanthellar tissues. These values can then be subtracted from the amount of enrichment detected in anemones and zooxanthellar fractions after exposure from the uptake experiment to give the actual amount of ^{15}N uptake from the sediment rather than the ^{15}N amount taken up plus the natural ^{15}N abundance. Anemones were collected on the 5th March 2007 and maintained as per section 2.2. They remained in the incubator for 90 days, being fed 5 times a week with *Artemia* sp. nauplii after which anemones (n = 5) were homogenised using a hand-held 15 ml glass tissue grinder in 3 ml 0.22-µm FSW. The volume of the resulting homogenate was made up to 8 ml and centrifuged (2500 × g) for 5 min. The host fraction (supernatant) was decanted into a separate tube and made up to 10 ml before two 1.5 ml sub-samples were frozen (-20°C). Meanwhile, the zooxanthellar pellet was resuspended in 0.22 µm FSW and centrifuged a further three times, and the resulting pellet frozen at -20°C. At the time of analysis, both host and symbiont sub-samples were hand thawed and pipetted onto pre-combusted 2.5 cm GF/F filters (500°C for 4h) with host samples sequentially spotted and zooxanthellar pellets pipetted on the filter as described above. Host and

zooxanthellar filters were fumed in a desiccator with concentrated HCl to remove carbonate, dried at 55-65°C to constant weight, and prepared for ^{15}N analysis as before.

2.5.5 Isotopic Analysis

All samples were sent to the National Institute of Water and Atmospheric Research (NIWA) stable isotope laboratory in Wellington, New Zealand where stable isotope analyses were all done with a Delta^{Plus} (Thermo-Finnigan, Bremen, Germany) continuous flow, isotope ratio mass spectrometer. Solid samples were prepared in tin boats and combusted in an NA 1500N (Fisons Instruments, Rodano, Italy) elemental analyser combustion furnace (at 1020°C) in a flow of oxygen and He carrier gas. Oxides of nitrogen were converted to N_2 gas in a reduction furnace at 640°C. N_2 and CO_2 gases were separated on a Porapak Q gas chromatograph column before being introduced to the mass spectrometer detector via an open split Conflo II interface (Thermo-Finnigan, Bremen, Germany). CO_2 and N_2 reference gas standards were introduced to the mass spectrometer with every sample analysis. ISODAT (Thermo-Finnigan) software was used to calculate ^{15}N values against atmospheric air. Percent N values were calculated relative to a solid laboratory reference standard of urea (Elemental Microanalysis, U.K.) at the beginning of each run. Internal standards were routinely checked against National Institute of Standards and Technology (NIST) standards. Repeat analysis of NIST standards produces data accurate to within 0.1-0.5‰ for ^{15}N and a precision of better than 0.5‰. For % N content, data are accurate to within 0.4%, with a precision usually better than 0.3% for N. It should be noted that, due to the high costs associated with mass spectrometry, only single (not duplicate or triplicate) sediment, host and zooxanthellar samples were analysed.

All isotopic results were calculated with the following equations (Equations 1 and 2) and presented as:

Atom % ^{15}N:

$$Atom\ \%\ ^{15}N = \frac{^{15}N}{^{14}N + ^{15}N} \times 100 \qquad \textbf{[Equation 1]}$$

Atom % excess (APE):

$$APE = atom\ \%\ ^{15}N_{final} - atom\ \%\ ^{15}N_{initial} \qquad \textbf{[Equation 2]}$$

APE values were calculated to determine actual ^{15}N enrichment of the host or zooxanthellar fractions. Here, *atom % ^{15}N* values of the host or the zooxanthellae were subtracted from the ^{15}N enrichment found naturally in the host and zooxanthellar fractions before anemones were exposed to sediment; where *atom % $^{15}N_{initial}$* = natural ^{15}N abundance values, and *atom % $^{15}N_{final}$* = the final atom % ^{15}N of the host or zooxanthellar fractions.

2.6 Zooxanthellar Nitrogen Status

To assess the level to which nitrogen status (ammonium enhancement of dark carbon fixation) is influenced by exogenous nitrogen supply in *A. aureoradiata*, anemones

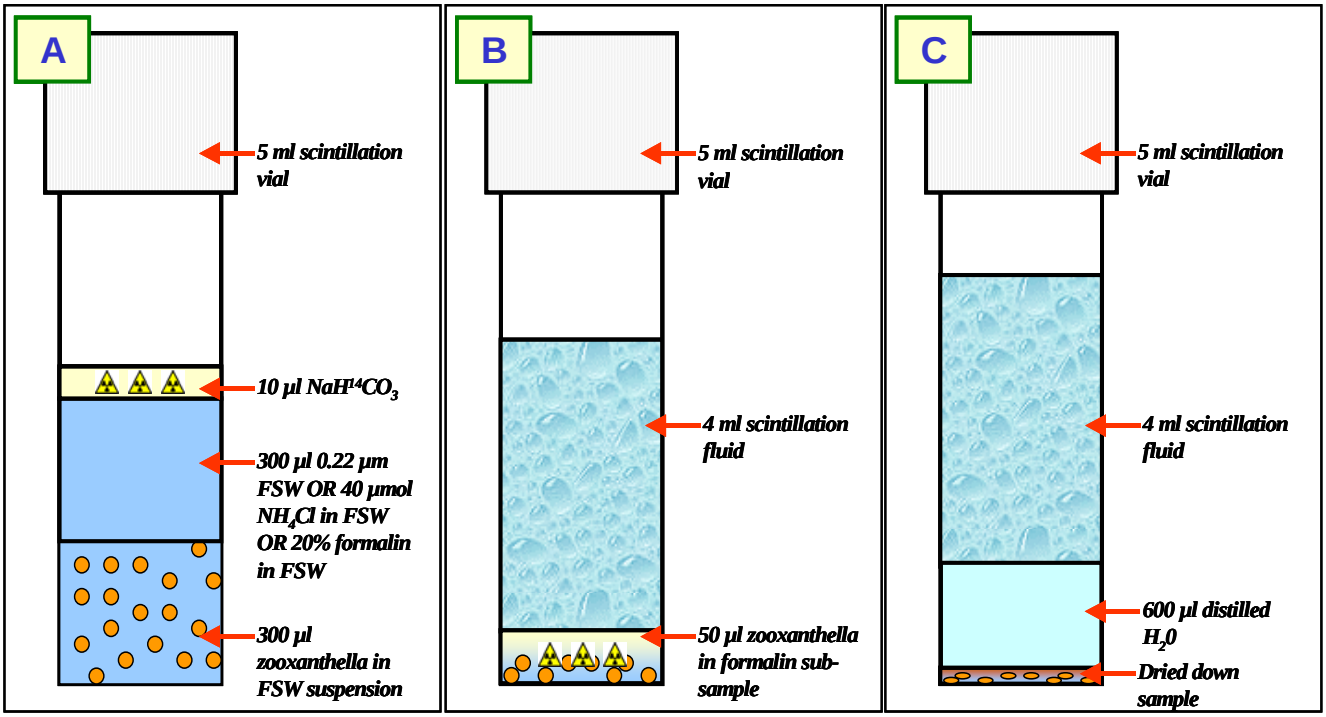

Fig. 11. *^{14}C experiment treatment vials. 11A. Concentrations used during the dark & light carbon fixation. Each vial received 300 µl of zooxanthella solution in 0.22 µm FSW and either 300 µl of 0.22 µm FSW, 300 µl 40 µmol NH_4Cl in 0.22 µm FSW or 300 µl 20% formalin in 0.22 µm FSW solution, before 10 µl $NaH^{14}CO_3$ was added. 11B. Formalin sub-sample vial. 50 µl sub-samples of each of the 3 formalin vials were sampled for added activity immediately after the addition of $NaH^{14}CO_3$ and 4 ml scintillation fluid added. 11C. Sample vials after being dried down following the experiment. 600 µl distilled H_2O + 4 ml scintillation fluid was added before radioactivity was measured. Note, vial contents not to scale.*

were starved before the examination of the nitrogen status of their zooxanthellae. Approximately 250 anemones were collected on the 5th of March 2007 and maintained

as per section 2.2. Anemones were fed twice a week with *Artemia* sp. nauplii for the two weeks following collection after which, anemones were fed 5 times per week for the 3-month period leading up to the start of the following laboratory experiments.

2.6.1 Nutritional History & Zooxanthellar Nitrogen Status

One hundred anemones were divided into 7 groups. One of these groups (n = 5 anemones) was immediately analysed to examine the nitrogen status of zooxanthellae in 'well-fed' anemones, while each of the remaining 6 groups (n = 10-15 anemones per group) was each placed in a glass dish with 150-200 ml nitrogen-free artificial seawater (NFASW). These were then housed in the incubator as per section 2.2. Five of the groups were then starved for 8 weeks while the other one was kept 'well-fed' by feeding *Artemia* sp. nauplii 5 times per week for the same period. Nitrogen status was examined at 2, 4, 6, 8 weeks in the starved groups (n = 5 anemones) with one anemone selected from each of the starved five groups at each two-week period. Nitrogen status was also analysed at 8 weeks in the 'well-fed' group (n = 5 anemones).

Each anemone was homogenised using a hand-held 15 ml glass tissue grinder in 3 ml 0.22-µm FSW and the volume of the resulting homogenate then made up to 8 ml and centrifuged (2500 × g) for 5 min. The supernatant was then discarded, and the remaining zooxanthellae centrifuged and resuspended with clean 0.22-µm FSW twice more. The subsequent algal suspension was finally adjusted with 0.22-µm FSW to give a zooxanthella density of 5-10 × 10^5 cells ml^{-1}. The final density of zooxanthellae was determined from 10 cell counts, conducted with a haemocytometer.

The nitrogen status of zooxanthellae was determined by measuring the ammonium enhancement of dark carbon fixation as described by Cook *et al.* (1992), Cook *et al.* (1994) and Davy *et al.* (2006). Dark carbon fixation was measured in 5-ml white plastic scintillation vials wrapped in black electrical tape and all manipulative work done in dim white light (<1 µmol photons m^{-2} s^{-1}) to reduce the photosynthetic incorporation of ^{14}C. Each treatment vial contained 0.3 ml zooxanthella suspension, to which 0.3 ml 0.22-µm FSW ± 40 µM NH_4Cl (n = 5 for each treatment) was added (Figure 11A). Three further vials contained 0.3 ml + 0.3 ml 20% formalin (Figure 11A) in FSW, and these were used to determine levels of both added ^{14}C and background

activity. All vials were then pre-incubated in darkness for 45 min before receiving 10 µl $NaH^{14}CO_3$ stock solution (containing 14.8 MBq/400 µCi) to give a final concentration of ~148 kBq/4 µCi ml^{-1}. The 3 formalin vials were sampled (50 µl + 4 ml scintillation fluid - Figure 11B) immediately to determine added radioactivity and all the vials subsequently sealed and incubated in darkness at 18°C for 4h. After 4h incubation, the vials were acidified at once with 1 N HCl and dried with a heating block (48h at 45°C), to remove any unincorporated ^{14}C. Once dried down, 0.6 ml distilled water + 4 ml scintillation fluid was added to each vial (Figure 11C) and radioactivity measured by liquid scintillation counting (Wallac 1409 Liquid Scintillation Counter, Turku, Finland). Finally, dark carbon fixation rates, ammonium enhancement ratios (Equation 3) and V_D/V_L values (Equation 4) were calculated following the correction for background activity and conversion of fixation rates to femtograms (1×10^{-15} kg) C $cell^{-1}$ h^{-1} as described by Cook *et al.* (1992).

For carbon fixation in the light, 5-ml white plastic scintillation vials received zooxanthellae, 0.22-µm FSW and ^{14}C as above, but with the following exceptions. Treatment vials (Figure 11A) received 0.3 ml zooxanthella suspension + 0.3 ml 0.22-µm FSW (n = 5) and three vials received 0.3 ml + 0.3 ml 20% formalin in FSW. Vials were then pre-incubated in a clear perspex tube rack on top of a light bank (which illuminated the bottom of the vials with a photosynthesis-saturating irradiance of 160-200 µmol photons m^{-2} s^{-1} - Figure 12) for 20 min, after which tubes were removed and 10 µl $NaH^{14}CO_3$ stock solution added. Formalin vials were again sampled immediately (50 µl + 4 ml scintillation fluid) (Figure 11B) before all the vials were subsequently sealed and re-placed back onto the light bank (18°C) for 30 min. After 30 min incubation, the vials were removed from the light bank, acidified immediately, dried down (48h at 45°C), scintillation fluid added (Figure 11C), radioactivity measured, and measurement of light carbon fixation rates calculated as described for dark carbon fixation.

The ammonium enhancement ratio was calculated as

$$\textit{Ammonium enhancement ratio} = \frac{\textit{dark } NH_4^+ \textit{ C fixation rate}}{\textit{dark FSW C fixation rate}} \qquad \textbf{[Equation 3]}$$

Nitrogen status was also expressed by as V_D/V_L (Cook *et al.* 1992)

$$V_D/V_L = \frac{dark\ NH_4^+\ C\ fixation\ rate - dark\ FSW\ C\ fixation\ rate}{C\ fixation\ in\ the\ light} \qquad \textbf{[Equation 4]}$$

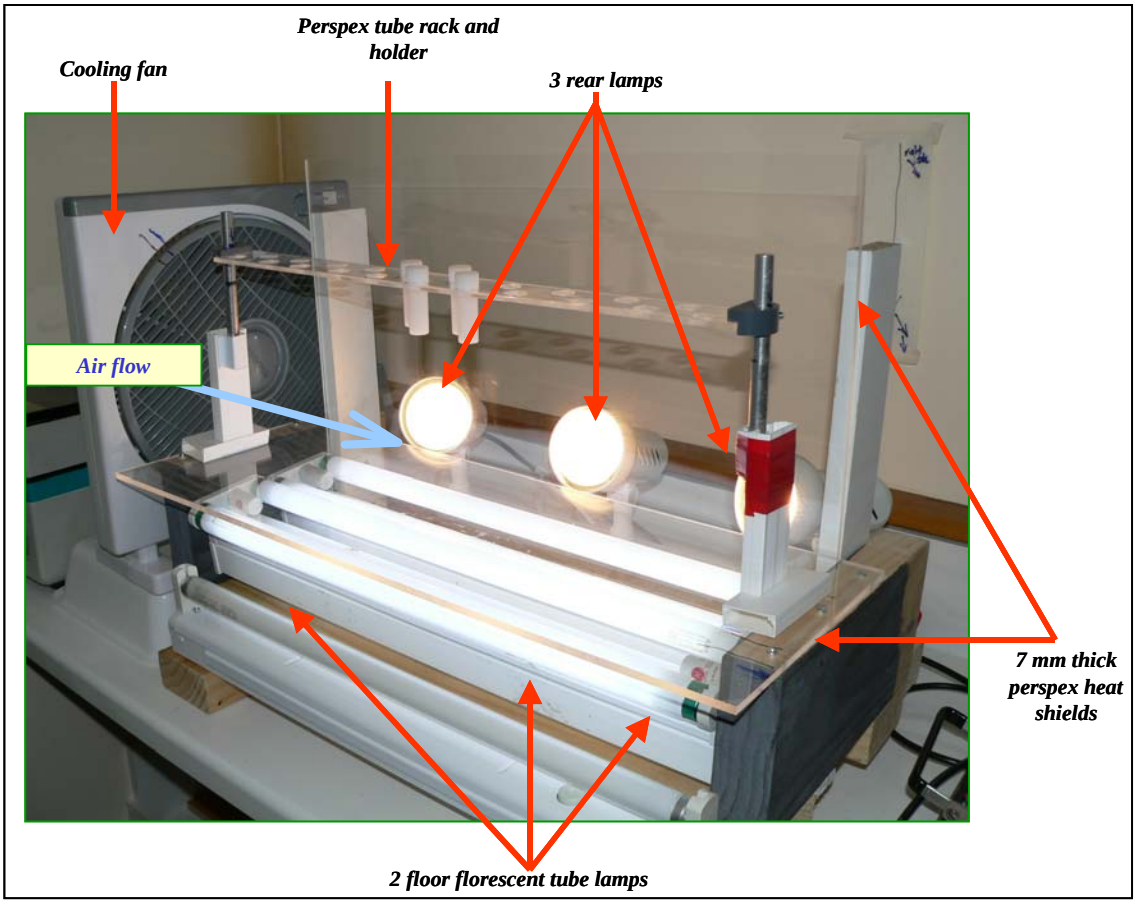

Fig. 12. *The light bank & tube rack used to saturate photosynthesis for the determination of light carbon fixation rates in zooxanthellae. The clear perspex tube rack was held above 2 double-tubed florescent lamps (Phillips 18W Cool-White lamps) and three small incandescent spotlights (Phillips R80 100W reflector bulbs). The 7 mm thick perspex heat shields, in combination with the cooling fan, allowed vials to incubate at a constant temperature of 18°C.*

2.6.2 The Influence of Habitat on Zooxanthellar Nitrogen Status

The nitrogen status of zooxanthellae isolated from *A. aureoradiata* from a mudflat site and a rocky intertidal site were assessed to ascertain if nitrogen sufficiency differed with habitat. Fifteen anemones (5 anemones per day) were collected from the mudflat at Pauatahanui Inlet (Figures 4 & 5C) between the 20th & 22nd June 2007 and the rocky intertidal at Kau Bay (Figures 5D & 6) between the 27th & 29th June 2007. Of those

collected from the rocky intertidal, 8 were from tide pools in the upper-littoral zone (Figure 6B) while the other 7 were from the mid-littoral zone lower down the shore (Figure 6C) (See section 2.1 for more details). Anemones were brought back to the lab and the nitrogen status of their zooxanthellae examined as above within 3h; the anemones were not fed between collection and analysis.

2.6.3 Sediment Uptake & its Influence on Zooxanthellar Nitrogen Status

This experiment was done to determine whether nitrogen taken up and assimilated by *A. aureoradiata* from the sediment had an influence on zooxanthellar nitrogen status. Here, zooxanthellar nitrogen status was examined by starving anemones in mudflat sediment.

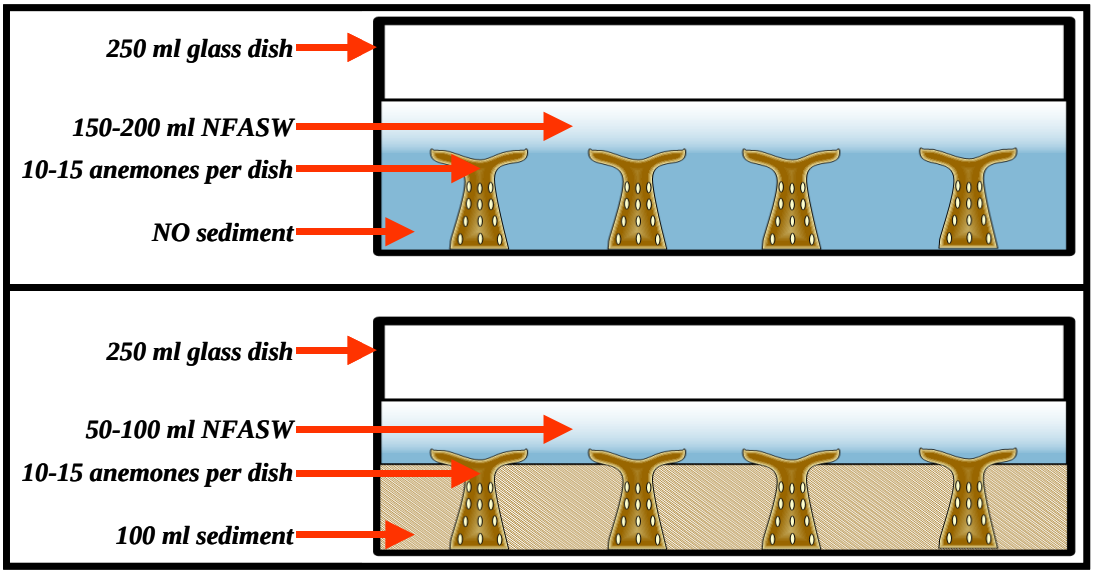

Fig. 13. *Zooxanthella nitrogen status in* Anthopleura aureoradiata *incubated in glass dishes with and without mudflat sediment. 13A. Anemones (n = 10-15) incubated in nitrogen free artificial seawater (NFASW). 13B. Anemones (n = 10-15) incubated in mudflat sediment & NFASW. The depth of the sediment was similar to that seen on the mudflats; 10-15 mm deep, just shallow enough for the anemones to extend their tentacles and oral disc to the surface. Anemones were periodically observed at the surface during the 12h daily light phase but always withdrew back into the sediment during the 12h dark phase*

Anemones (n = 150) were split into 2 groups with one of these (n = 10 anemones) analysed immediately to assess the nitrogen status of 'well-fed' anemones. Half of the leftover group was then placed in 5 glass dishes (n = 10-15 anemones per dish) with 150-200 ml NFASW (Figure 13A) and the other half placed in another 5 glass dishes (n = 10-15 anemones per dish) with 50-100 ml NFASW + approximately 100 ml of

mudflat sediment (Figure 13B - see sediment collection & maintenance below). Both the 'sediment' and 'no sediment' groups were subsequently maintained in an incubator (as per section 2.2) and not fed with *Artemia* sp. nauplii. Nitrogen status was assessed at 2, 4, 6, 8 weeks (1 anemone selected per dish as above in the previous experiment, hence n = 5 for each treatment at each 2 week interval).

Sediment collection & maintenance

Sediment was collected (as per section 2.3) every week between May-August 2007 and prepared as per section 2.3 with the following exceptions. The sediment was thoroughly rinsed 4 times with NFASW and made up to 500 ml with NFASW before half was added immediately to the experimental dishes, and the other half refrigerated at 4°C. The sediment in the experimental dishes was replaced at the beginning of the week with new sediment or with refrigerated sediment 4 days later on in the week. Meanwhile, the NFASW in the no 'sediment dishes' was also replaced twice a week.

2.7 Statistical Analyses

All statistical analyses were carried out with SPSS© 11.0.1 for Windows (2001). *T*-Tests were used to compare the ^{15}N enrichment of the host and symbiont between high and low sediment loads. *T*-Tests were also employed to examine differences between zooxanthellar dark NH_4^+ fixation and the dark FSW rates in all nitrogen status experiments, plus differences in zooxanthellar nitrogen status between Pauatahanui Inlet and Kau Bay, and between low- and high-shore sites at Kau Bay. One-way ANOVA was used to analyse differences between treatments in the sediment ^{15}N-enrichment experiment, and between the ammonium enhancement ratios, photosynthetic rates and V_D/V_L values between each two-week time point in both the nutritional history and sediment/no sediment nitrogen status experiments. Analysis of these differences was further inspected with *Post Hoc* Tukey HSD tests to search for any disparity between treatments or each two-week time point. Two-way (univariate) ANOVA was employed to determine differences in sediment ^{15}N-enrichment between the start and the end of the experiment, at both sediment loads, in the sedimentary particulate nitrogen uptake experiment.

3

RESULTS

3.1 Sediment ^{15}N Enrichment

The sediment enrichment experiment was designed to assess which component of the sediment (biological/chemical/physical) was taking up the ^{15}N label. The natural ^{15}N abundance of the sediment or ^{15}N enrichment of the sediment at 0h was 0.40 ± 0.01 atom % ^{15}N across all treatments. The ^{15}N enrichment of the sediment ($^{15}NH_4^+$ alone) showed a maximum atom % ^{15}N of 0.96 ± 0.02 (mean ± Standard Error [SE]) after 24h (Figure 14), after which enrichment ceased, remaining statistically unchanged between 24 and 48h (*t*-TEST, $T_{[2,4]}$ 0.715, P>0.05). The final atom % ^{15}N of the sediment treated with ampicillin (50 μg ml^{-1}) was 0.85 ± 2 × 10^{-3} after 24h, while the formalin control ($^{15}NH_4^+$ + formalin) was 0.42 ± 2 × 10^{-3} after 24h. A one way ANOVA comparing the atom % ^{15}N of all treatments showed that the $^{15}NH_4^+$ alone treatment was significantly greater than the $^{15}NH_4^+$ ampicillin treatment, and both treatments significantly greater than the $^{15}NH_4^+$ + formalin treatment (Figure 14) (ANOVA, $F_{[2,7]}$ 34.32, P<0.0001 and paired comparisons by Tukey HSD, significant differences p<0.003). Figure 15 illustrates that the uptake of ^{15}N label was primarily biological, with little chemical or physical uptake.

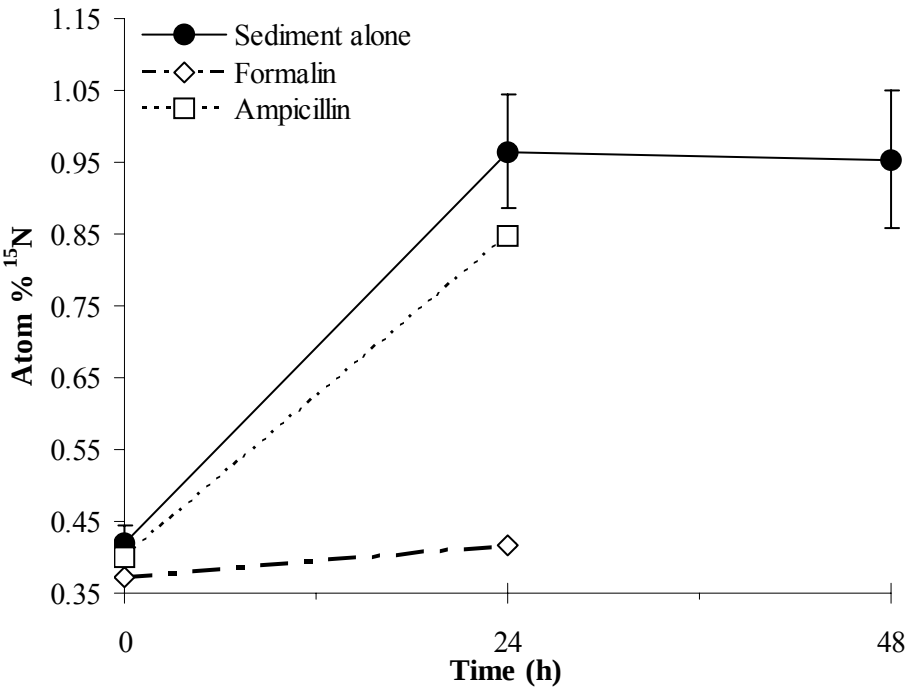

Fig. 14. *^{15}N-NH_4^+ labelling of live, killed (2.5% formalin final concentration) and antibiotic-treated (50 µg mL^{-1} ampicillin) sediment collected from Pauatahanui Inlet. Values are mean ± SE. Error bars too small to be seen on the formalin and ampicillin line plots. Note – Y-axis begins at 0.35 atom % ^{15}N because the natural ^{15}N abundance of the sediment was 0.40 atom % ^{15}N across all treatments. N = 3 replicates for sediment alone, n = 1 replicate for the formalin treatment and n = 1 replicate for the ampicillin treatment.*

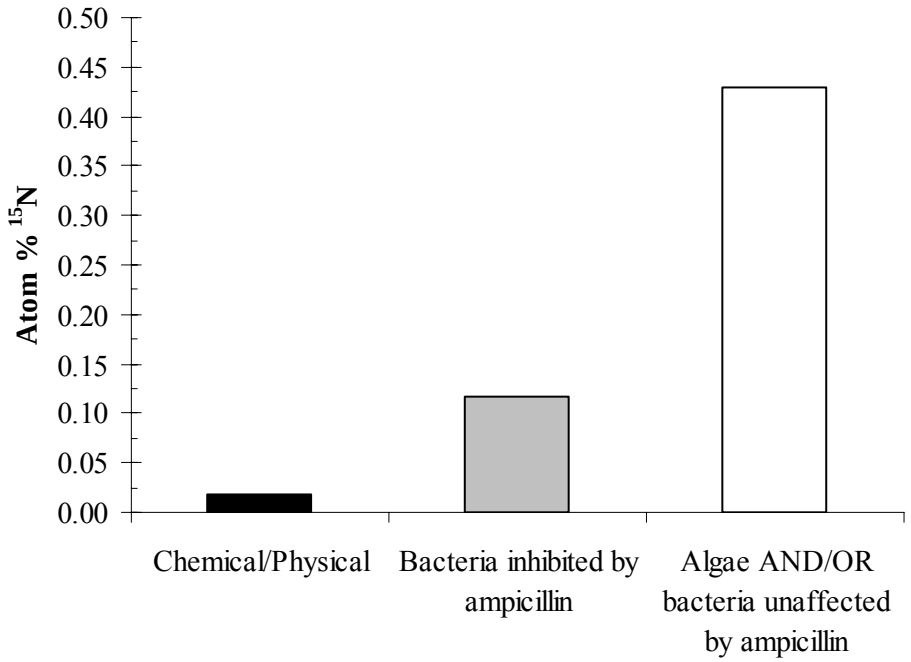

Fig. 15. *Specific uptake of ^{15}N into sediment collected from Pauatahanui Inlet after 24h. Chemical/physical uptake = uptake measured in killed (formalin) treatment; bacteria inhibited by ampicillin = uptake in sediment alone treatment – uptake in sediment ampicillin treatment; Algae and/or bacteria unaffected by ampicillin = uptake in ampicillin treatment – uptake by formalin treatment. Note, these are calculated from mean values so error bars are absent.*

3.2 Particulate ^{15}N Uptake from the Sediment

3.2.1 Isotopic Content of Sediment

This experiment was designed to investigate the potential for nitrogen uptake from the sediment by *Anthopleura aureoradiata* and to ascertain if there was any difference in this uptake between the low and high sediment loads

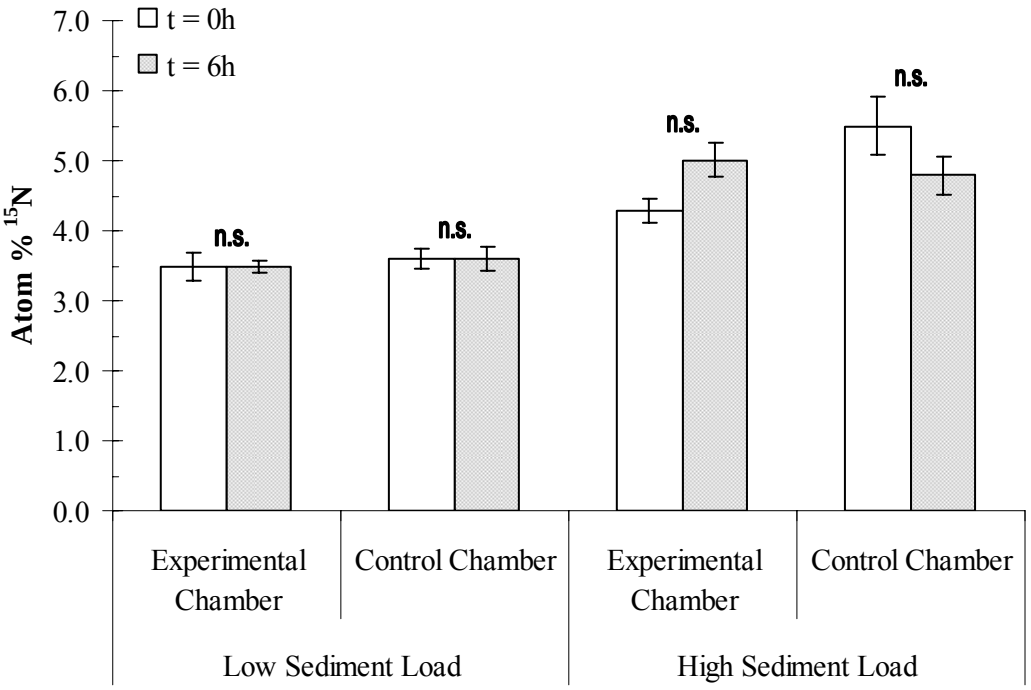

Fig. 16. *Changes in the atom % ^{15}N of the low and high sediment loads during the 6h sedimentary nitrogen uptake experiment. Values are mean ± SE. Sediment samples were taken at the beginning (t = 0h) and the end (t = 6h) of the experiment. n = 5 anemones (1 per experimental chamber) and n = 3 control chambers (no anemones) for each sediment load treatment. n.s. = no significant difference (P> 0.05), where comparisons are between the initial (0h) atom % ^{15}N and the final (6h) atom % ^{15}N.*

The isotopic content of the sediment for each uptake chamber was analysed before and after the uptake experiment to determine if there were any changes during this period. After the 6h experiment, the final sedimentary atom % ^{15}N did not significantly change from the initial sediment atom % ^{15}N (two way ANOVA $F_{[1,8]}$ 0.026, P>0.05) in any of the control and experimental chambers (for both high and low sediment loads), although, sedimentary atom % ^{15}N was significantly higher (two way ANOVA $F_{[3,14]}$ 63.952, P<0.0001) in the high sediment load in contrast to the low sediment load (Figure 16). At the low sediment load, sediment atom % ^{15}N averaged 3.49 ± 0.14 &

3.48 ± 0.17 (initial sediment atom % ^{15}N & final sediment atom % ^{15}N, respectively) in the experimental chambers and 3.60 ± 0.19 & 3.60 ± 0.09 (initial & final atom % ^{15}N, respectively) in the control chambers. Meanwhile, at the high sediment load, sediment atom % ^{15}N was on average 20-25% higher, averaging 4.29 ± 0.41 & 5.01 ± 0.27 (initial & final atom % ^{15}N, respectively) and 5.50 ± 0.17 & 4.79 ± 0.25 (initial & final atom % ^{15}N, respectively) for the experimental and control chambers, respectively.

3.2.2 Anemone Exposure to ^{15}N sediment

Following the uptake experiment, both host and zooxanthellar (symbiont) tissue fractions were analysed for ^{15}N enrichment. The atom percent excess (APE) enrichment values of the host (Figure 17) were not significantly different between the low and high sediment loads (*t*-TEST, $T_{[2,8]}$ 1.186, P>0.05) with average host tissue enrichment of

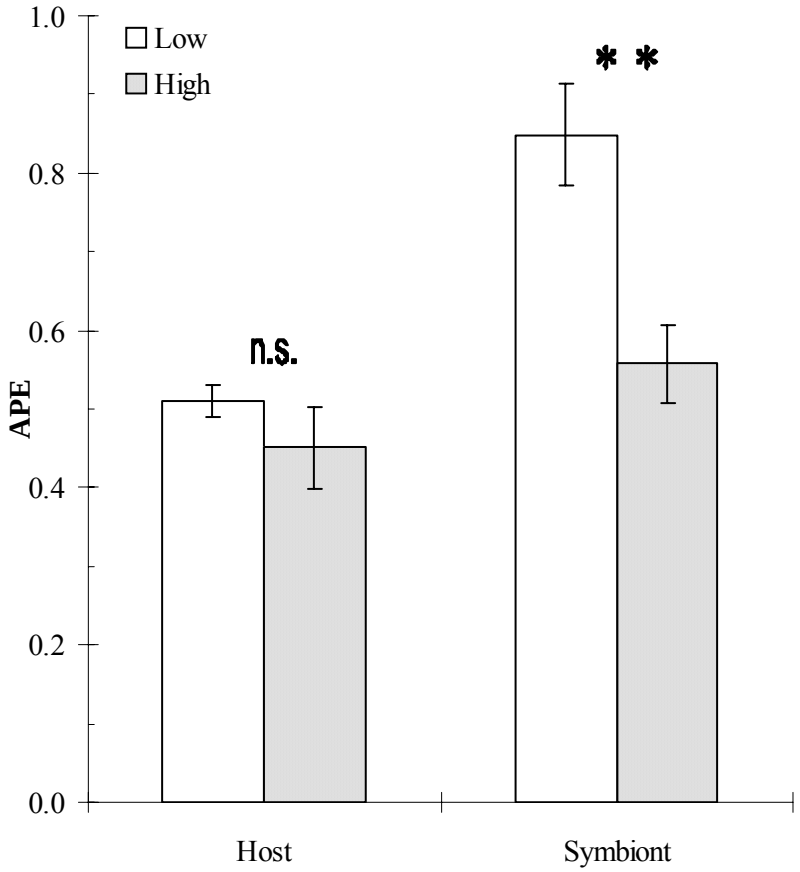

Fig. 17. *Atom % excess (APE) of the host and symbiont fractions at the high and low sediment loads. Values are mean ± SE. n = 5 for each the host/zooxanthellar fractions per sediment treatment. Natural ^{15}N abundance of the host and symbiont were 3.71 (P<0.00001) and 3.69 (P<0.00001) respectively. Significant differences are shown by ** P<0.005 or no significant differences are shown by n.s. where comparisons are between low and high sediment loads (not host and symbiont).*

0.51 ± 0.02 atom % ^{15}N and 0.45 ± 0.05 atom % ^{15}N at the low and high sediment loads, respectively. Conversely, APE values of the zooxanthellae were significantly higher (*t*-TEST, $T_{[2,8]}$ 4.034, P<0.005) in the low sediment load (0.85 ± 0.06 atom % ^{15}N) as opposed to the high sediment load (0.56 ± 0.04 atom % ^{15}N) (Figure 17). Meanwhile, APE of the host was significantly lower (*t*-TEST, $T_{[2,8]}$ 5.637, P<0.001) than the APE of the symbiont at the low sediment load, while at the high sediment load, there was no significant difference (*t*-TEST, $T_{[2,8]}$ 1.660, P>0.05) between host and symbiont.

3.3 Zooxanthellar Nitrogen Status

3.3.1 Nutritional History & Zooxanthellar Nitrogen Status

The level to which nitrogen status (ammonium enhancement of dark carbon fixation) was influenced by exogenous nitrogen supply in starved *A. aureoradiata* was examined (Figure 18). There was a clear relationship between nutritional history and the enhancement of dark carbon fixation by 40 μM ammonium in freshly isolated zooxanthellae of *A. aureoradiata*. No significant enhancement (*t*-TEST, $T_{[2,8]}$ 7.711, P>0.05) of dark carbon fixation was seen between 0-2 weeks of starvation or after 8 weeks of repeated feeding, when the ammonium enhancement ratio (Figure 18A & B) averaged just 0.91 ± 0.08. Significant enhancement (*t*-TEST, $T_{[2,8]}$ 6.029, P<0.0001) was only seen after ≥ 4 weeks of starvation, being highest at 6 weeks (2.90 ± 0.57). There was therefore a clear change in the ammonium enhancement ratio (one way ANOVA, $F_{[5,28]}$ 14.626, P<0.0001) with food supply, with enhancement being significantly more (one way ANOVA, Tukey HSD, significant differences p<0.005 – Table 2) than when anemones were starved for shorter durations or fed regularly.

Photosynthesis was also affected by starvation (Figure 18C). The photosynthetic rate of the zooxanthellae from anemones starved ≥ 2 weeks and anemones well fed after 8 weeks was just 60% of that seen at 0 weeks (1257.42 ± 192.30 fg C fixed $cell^{-1}$ h^{-1}). Thus, the photosynthetic rate at 0 weeks was significantly greater (one way ANOVA, $F_{[6,28]}$ 76.920, P<0.0001) than when anemones were starved for between 2-8 weeks and

well fed after 8 weeks; photosynthesis was similar between all other treatments (one way ANOVA, Tukey HSD, p>0.05 – Table 2).

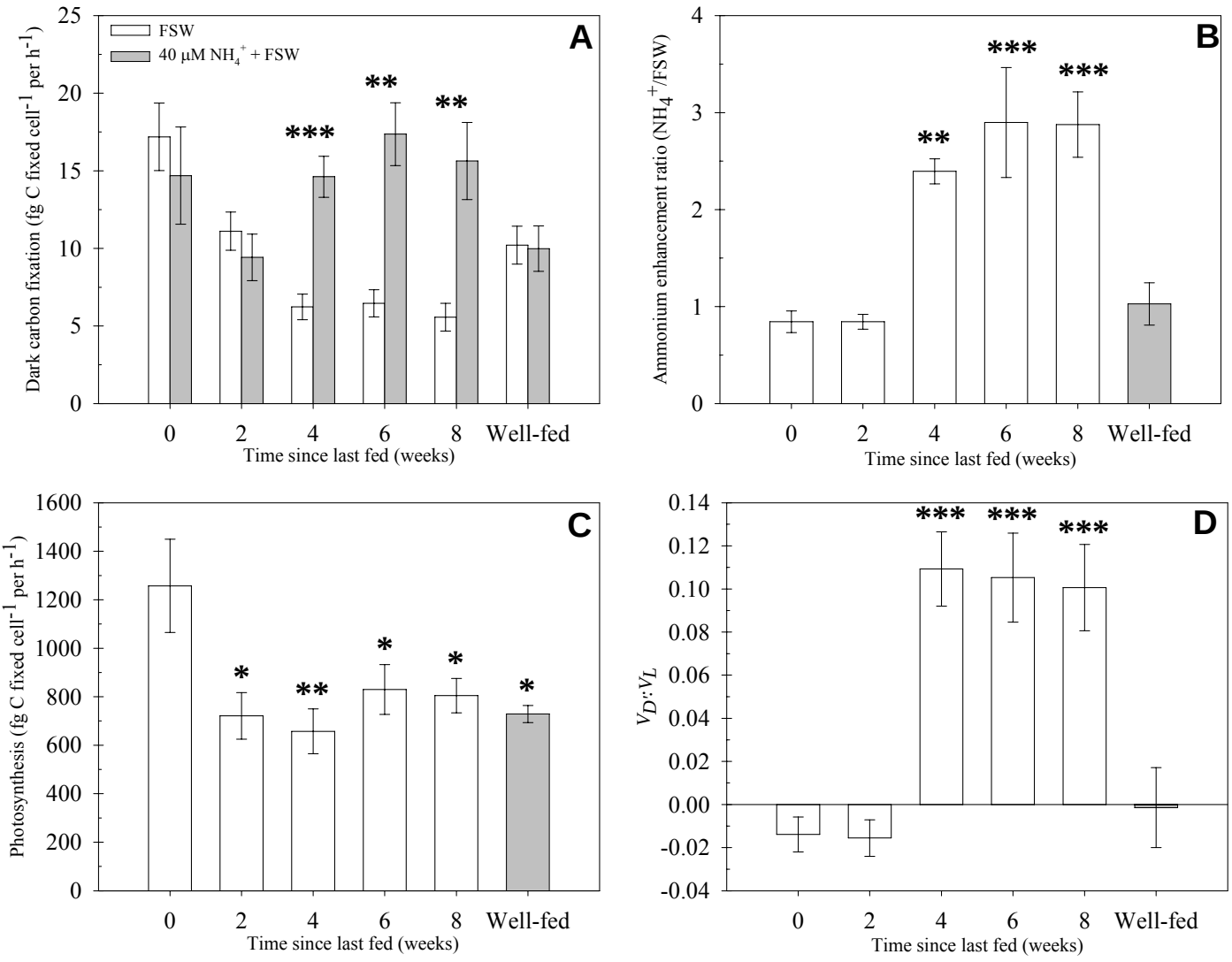

Fig. 18. *The influence of host feeding regime on zooxanthellar nitrogen status and photosynthesis in* Anthopleura aureoradiata*. Anemones were fed 5 times a week for 12-14 weeks prior to starvation for 2-8 weeks; "well fed" anemones were fed 5 times a week throughout the duration of the 8-week experiment. (A) Dark carbon fixation rate (fg) of freshly isolated zooxanthellae (FIZ) incubated with and without 40 µM* NH_4^+*. (B) Ammonium enhancement ratio (dark* NH_4^+ *rate/dark FSW rate) of FIZ. (C) Rate of light saturated photosynthesis (fg) when FIZ were incubated in FSW. (D)* $V_{D'}:V_L$ *of FIZ (another index of nitrogen sufficiency where* $V_{D'}$ *= dark* NH_4^+ *rate – dark FSW rate, and* V_L *= rate of carbon fixation in light). Values are in means ± SE, n = 5 for each treatment. fg = femtograms or* 1×10^{-15} *kg. Figure legends are for each individual plot of the figure only. Significant differences are shown by *P<0.05, ** P<0.005 and * P<0.0005 where comparisons are between rates with and without ammonium (A only) or with the rate/value at time zero (B-D).*

Despite these similarities in photosynthetic rate, there was a significant increase (one way ANOVA, $F_{[5,28]}$ 18.766, P<0.0001) in the ratio of $V_{D'}/V_L$ with starvation (Figure 18D). $V_{D'}/V_L$ values after ≥ 4 weeks of starvation were significantly higher (one

way ANOVA, Tukey HSD, p<0.0001 – Table 2) than $V_{D'}/V_L$ values between 0-2 weeks of starvation or following 8 weeks of feeding. Figure 18D demonstrates that $V_{D'}/V_L$ values after 4-8 weeks of starvation (0.11 ± 0.02) were, on average, approximately 6 times higher than in well fed anemones (0 and 8 weeks) or anemones starved for 2 weeks (-0.10 ± 0.01).

3.3.2 The Influence of Habitat on Zooxanthellar Nitrogen Status

The nitrogen status of zooxanthellae isolated from *A. aureoradiata* were assessed to ascertain if nitrogen sufficiency differed with habitat (Figure 19). There was a significant difference (*t*-TEST, $T_{[1,28]}$ 5.401, P<0.0001) between the nitrogen status of zooxanthellae from the Pauatahanui Inlet mudflat and the Kau Bay rocky intertidal sites, (n = 15 for both field sites). There was no significant enhancement (*t*-TEST, $T_{[0,13]}$ 0.675, P>0.05) of dark carbon fixation in freshly isolated zooxanthellae from mudflat anemones, where the ammonium enhancement ratio (Figure 19A & B) averaged just 1.263 ± 0.160. At the rocky intertidal site, dark carbon fixation, was significantly enhanced by ammonium in zooxanthellae from both the low (mid-littoral zone) (*t*-TEST, $T_{[0,5]}$ 10.178, P<0.0001, n = 7) and high (tide pools in the upper-littoral zone) (*t*-TEST, $T_{[0,6]}$ 5.124, P<0.0001, n = 8) shore heights. However, there was no significant difference (*t*-TEST, $T_{[0,13]}$ 0.220, P>0.05) in ammonium enhancement ratios between the low (2.87 ± 0.27) and high rocky intertidal sites (2.99 ± 0.46). Differences between sites were also analysed with one way ANOVA, Tukey HSD (Table 3).

The average photosynthetic rates of zooxanthellae from mudflat anemones (1534.60 ± 112.14 fg C fixed $cell^{-1}$ h^{-1}) were significantly higher (*t*-TEST, $T_{[1,28]}$ –2.099, P<0.05 [p = 0.045]) than those from rocky shore anemones from both shore heights (Figure 19C). There was no significant difference (*t*-TEST, $T_{[0,13]}$ 1.361, P>0.05) between photosynthetic rates of zooxanthellae from the low (780.59 ± 200.63 fg C fixed $cell^{-1}$ h^{-1}) and high (1295.01 ± 306.09 fg C fixed $cell^{-1}$ h^{-1}) rocky intertidal shore height sites.

Lastly, $V_{D'}/V_L$ values (Figure 19D) were significantly higher (*t*-TEST, $T_{[1,28]}$ 2.396, P<0.05) in zooxanthellae from the rocky intertidal anemones compared with zooxanthellae from the mudflat anemones. Mudflat $V_{D'}/V_L$ values averaged 6×10^{-3} ± 4

$\times 10^{-3}$, while rocky intertidal $V_{D'}/V_L$ values averaged 0.24 ± 0.13 and 0.11 ± 0.05 for the low and high shore heights, respectively. However, this difference between low and high rocky intertidal shore height was again insignificant (*t*-TEST, $T_{[0,13]}$ −1.039, P>0.05).

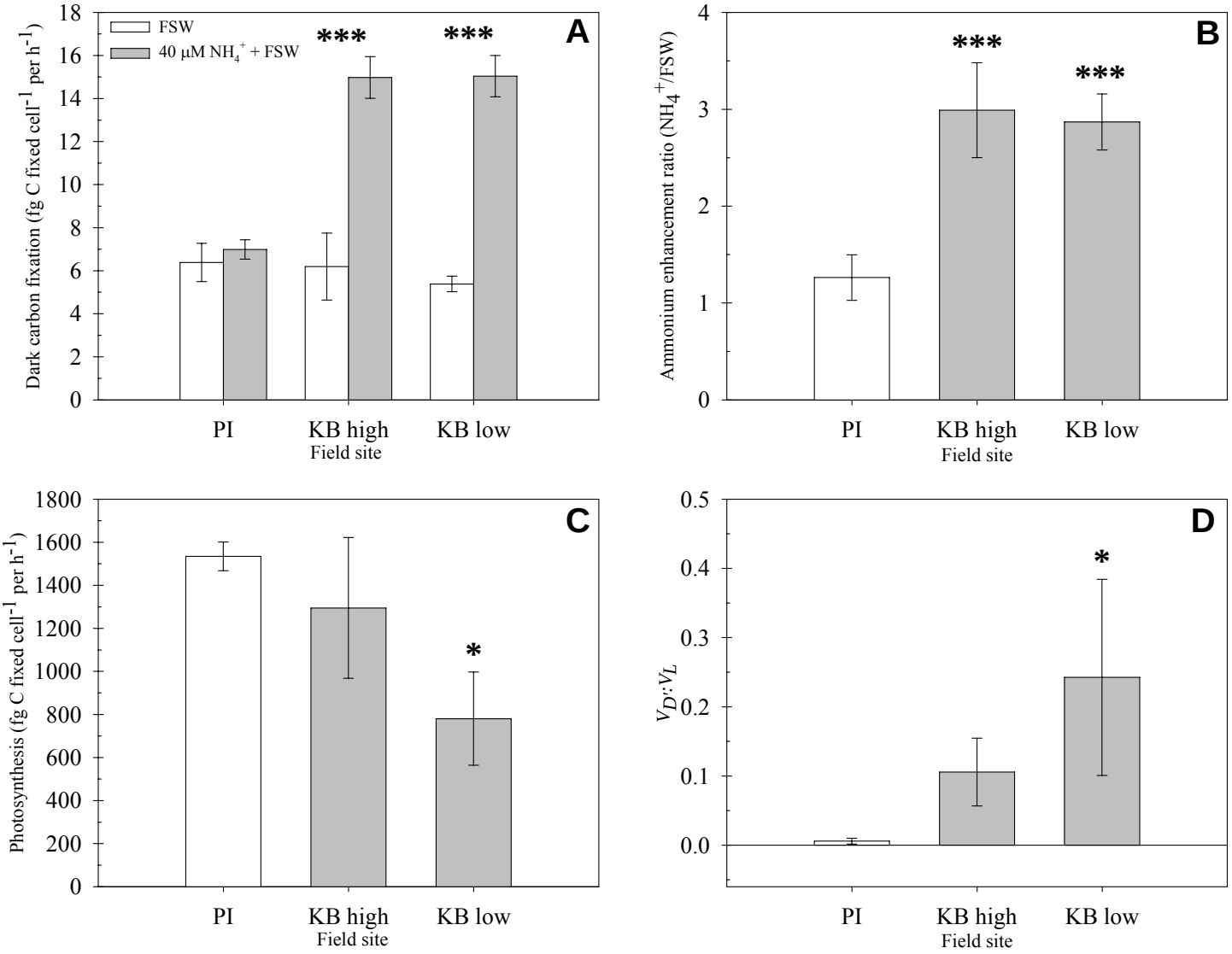

Fig. 19. *Nitrogen status of zooxanthellae in* Anthopleura aureoradiata *at different field sites. 5 Anemones collected daily for 3 days and one week apart for Pauatahanui Inlet and Kau Bay field sites. (A) Dark carbon fixation rate (fg) of freshly isolated zooxanthellae (FIZ) incubated with and without 40μM NH_4^+. (B) Ammonium enhancement ratio (dark NH_4^+ rate/dark FSW rate) of FIZ. (C) Rate of light saturated photosynthesis (fg) when FIZ were incubated in FSW. (D) $V_{D'}$:V_L of FIZ (another index of nitrogen sufficiency where $V_{D'}$ = dark NH_4^+ rate – dark FSW rate, and V_L = rate of carbon fixation in light). PI = Pauatahanui Inlet, KB high = Kau Bay upper-littoral tide pools, KB low = Kau Bay mid-littoral zone. Values are in means ± SE, n = 15 for PI, n = 8 for KB high, n = 7 for KB low. fg = femtograms or 1 × 10^{-15} kg. Figure legends are for each individual plot of the figure only. Significant differences are shown by *P<0.05 and * P<0.0005 where comparisons are between rates with and without ammonium (A only) or with the rate/value at Pauatahanui Inlet (B-D).*

3.3.3 Sediment Uptake & its Influence on Zooxanthellar Nitrogen Status

A. aureoradiata was starved with and without sediment, and the zooxanthellar nitrogen status measured (Figure 20). Once more, there was a significant relationship between nutritional history and the enhancement of dark carbon fixation by ammonium in freshly isolated zooxanthellae of *A. aureoradiata* incubated in both sediment (one way ANOVA, $F_{[3,22]}$ 6.611, P<0.001) and without sediment (one way ANOVA, $F_{[3,22]}$ 7.812, P<0.001) (Figure 20A, B & D). In the 'sediment' treatment, no significant enhancement (*t*-TEST, $T_{[2,8]}$ 2.296, P>0.05 [p = 0.051]) of dark carbon fixation was seen between 0-4 weeks of starvation, where the average ratio was 2.21 ± 0.32. Significant enhancement (*t*-TEST, $T_{[2,8]}$ 9.006, P<0.0001) was seen after ≥ 6 weeks of starvation, where enhancement was its highest after 6 weeks starvation (3.73 ± 0.46). Similarly, no significant enhancement (*t*-TEST, $T_{[2,8]}$, 2.244, P>0.05 [p = 0.055]) of dark carbon fixation was seen after 0-4 weeks of starvation in the no sediment treatment, when the average enhancement ratio was 1.94 ± 0.81. Significant enhancement (*t*-TEST, $T_{[2,8]}$ 3.908, P<0.0001) was again only seen after ≥ 6 weeks of starvation, where enhancement peaked (2.74 ± 0.54) after 6 weeks starvation. However, due to the extreme closeness of the p-values at 4 weeks to the 0.05 significance threshold and the extremely high ammonium enhancement ratios (>1.94 for both treatments) there was reasonable evidence for enhancement between 2 and 4 weeks in both the sediment and no sediment treatments. More importantly, there was no significant difference (One way ANOVA, $F_{[4,48]}$ 2.144, P>0.05, Tukey HSD – Table 2) in the ammonium enhancement ratios of freshly isolated zooxanthellae between anemones starved (at each time point) in sediment or without sediment (Figure 20D).

Photosynthesis at 0 weeks was 1835.30 ± 229.51 and 1519.05 ± 113.26 fg C fixed $cell^{-1}$ h^{-1} for the sediment and no sediment treatments, respectively (Figure 20D). Photosynthesis, declined significantly (one way ANOVA, $F_{[4,48]}$ 15.624, P<0.0001) after ≥ 2 weeks where it averaged just 842.36 ± 106.13 and 722.70 ± 76.01 fg C fixed $cell^{-1}$ h^{-1} (and averaged just 45% and 49% of that seen at 0 weeks), respectively, for both sediment and no sediment treatments. However, there was no significant difference (one way ANOVA, $F_{[3,43]}$ 1.044, P>0.05 Tukey HSD – Table 2) between the photosynthetic rates of zooxanthellae from anemones starved between 2-8 weeks. Despite the decline after ≥ 2 weeks, there was no significant difference (one way ANOVA, $F_{[4,48]}$ 2.063,

P>0.05) in the zooxanthellar photosynthetic rates at each time point between anemones starved with sediment or without sediment.

***Table 2**. One-Way ANOVA Tukey HSD post hoc P-values for the starvation and sediment/no sediment treatments. Values at each week starved are differences from that at 0 weeks starved except for the sediment VS no sediment values, where differences are between sediment and no sediment at each respective week. Significant differences (p<0.05) are shown by a *.*

	Weeks starved					
Experiments	0	2	4	6	8	Well-fed after 8 weeks
Nutrition & nitrogen status						
Ammonium enhancement ratio		1.000	*0.004	*0.000	*0.000	0.996
V_D/V_L		1.000	*0.000	*0.000	*0.000	0.990
Photosynthesis		*0.008	*0.003	*0.050	*0.033	*0.009
Nitrogen status in the sediment						
Ammonium Enhancement ratio		0.997	0.909	*0.003	*0.035	
V_D/V_L		1.000	0.665	*0.019	*0.007	
Photosynthesis		*0.001	*0.003	*0.002	*0.000	
Nitrogen status without sediment						
Ammonium enhancement ratio		0.998	0.147	*0.004	*0.006	
V_D/V_L		0.993	0.298	*0.008	*0.002	
Photosynthesis		*0.014	*0.036	*0.038	*0.004	
Sediment VS no sediment						
Ammonium enhancement ratio	1.000	1.000	1.000	0.776	1.000	
V_D/V_L	1.000	1.000	1.000	1.000	1.000	
Photosynthesis	0.874	1.000	1.000	1.000	1.000	

***Table 3.** One-Way ANOVA Tukey HSD post hoc P-values for the field sites. Values at each field site listed are differences from that of the Pauatahanui Inlet field site. Significant differences (p<0.05) are shown by a *.*

	Ammonium enhancement ratio	V_D/V_L	Photosynthesis
Field site			
Kau Bay high shore	*0.000	0.434	0.640
Kau Bay low shore	*0.001	*0.023	*0.029

V_D/V_L values (Figure 20E) increased significantly with starvation in sediment (One way ANOVA, $F_{[3,22]}$ 6.700, P<0.001) and without it (One way ANOVA, $F_{[3,22]}$ 7.957, P<0.001). In the sediment treatment, V_D/V_L values after ≥ 6 weeks of starvation were significantly higher (one way ANOVA, Tukey HSD, significant differences p<0.02 – Table 2) than V_D/V_L values of those starved between 0-4 weeks. Figure 20E demonstrates, that the V_D/V_L value at 6 weeks starved was 0.17 ± 0.06, more than

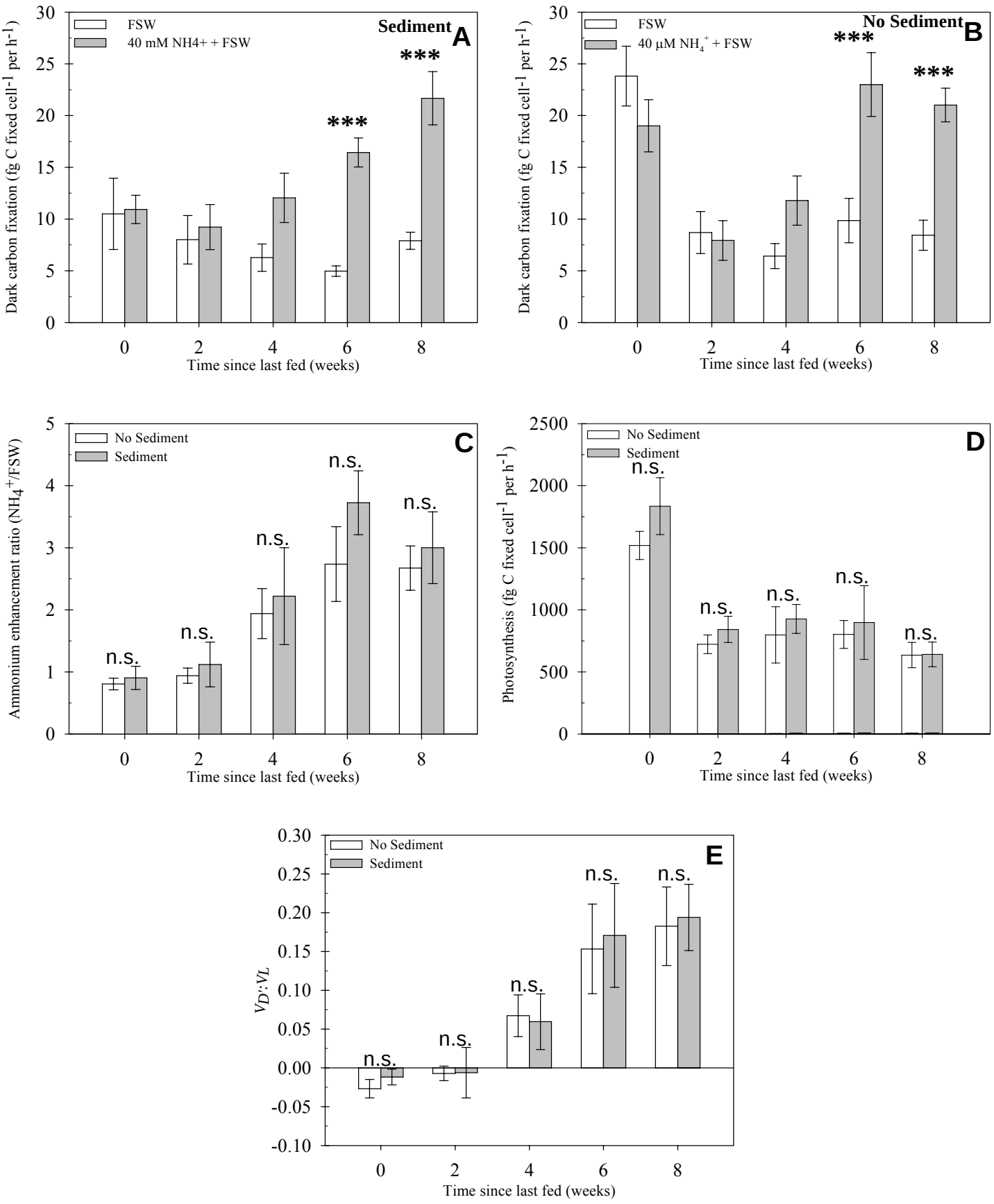

Fig. 20. *The influence of host feeding regime on zooxanthellar nitrogen status and photosynthesis in* Anthopleura aureoradiata *incubated with or without mudflat sediment from Pauatahanui Inlet. Anemones were fed 5 times a week for 12-14 weeks prior to starvation for 2-8 weeks. (A) Dark carbon fixation rate (fg) of freshly isolated zooxanthellae (FIZ) from anemones incubated in mudflat sediment. FIZ were incubated with and without 40µM NH_4^+. (B) Dark carbon fixation rate (fg) of FIZ from anemones incubated without mudflat sediment. FIZ were incubated with and without 40µM NH_4^+. (C) Ammonium enhancement ratio (dark NH_4^+*

Fig. 20 continued. *rate/dark FSW rate) of FIZ from anemones incubated with/without sediment. (D) Rate of light saturated photosynthesis (fg) when FIZ from anemones incubated with/without sediment. (E) $V_{D'}$:V_L of FIZ from anemones incubated with/without sediment (another index of nitrogen sufficiency where $V_{D'}$ = dark NH_4^+ rate – anemones incubated with/without sediment (another index of nitrogen sufficiency where $V_{D'}$ = dark NH_4^+ rate – dark FSW rate, and V_L = rate of carbon fixation in light). Values are in means ± SE, n = 25 for each treatment with n = 5 for each week. fg = femtograms or 1 × 10^{-15} kg. Figure legends are for each individual plot of the figure only. Significant differences are shown by *** P<0.0005 for where comparisons are between rates with and without ammonium (A & B) while no significant differences are shown by n.s. where comparisons are between sediment and no sediment treatments at each two week time point (C-E).*

twice that seen at 4 weeks starved (0.06 ± 0.03). Meanwhile, in the no sediment treatment, $V_{D'}/V_L$ values were again significantly higher (one way ANOVA, Tukey HSD, significant differences $p<0.008$ – Table 2) after ≥ 6 weeks of starvation than between 0-4 weeks of starvation, with the average $V_{D'}/V_L$ value at 6 weeks starved being 0.15 ± 0.05; more than double that seen at 4 weeks starved (0.07 ± 0.02). There was again no significant difference (One way ANOVA, $F_{[4,48]}$ 0.108, $P>0.05$) in $V_{D'}/V_L$ values of freshly isolated zooxanthellae between anemones incubated with or without sediment (Figure 20E).

4

DISCUSSION

In this study, it was shown that the temperate symbiotic anemone *Anthopleura aureoradiata* can take up particulate nitrogen from ^{15}N labelled sediment. Moreover, this study is the first to examine the effects of the host-uptake of sediment on the zooxanthellae, where in spite of ^{15}N labelled nitrogen being detected in symbiont fractions, host exposure to sediment particles did not alleviate nitrogen deficiency when otherwise deprived of food.

4.1 Sediment ^{15}N Enrichment

Sources of Sedimentary Particulate Nitrogen

The patterns of sediment enrichment imply that the uptake of $^{15}NH_4^+$ by the sediment was primarily biologically driven. The formalin-treated sediment showed very little uptake relative to the sediment plus $^{15}NH_4^+$ alone, indicating that chemical or physical processes were of little importance. However, the influence of ampicillin was minor on ^{15}N uptake, suggesting that either the antibiotic did not inhibit all bacteria in the sediment or uptake was primarily by sediment-associated algae. In addition, the concentrations of ampicillin used may have been too low to stop all bacterial uptake (Mills 2000). These patterns were identical with the measurements of Mills (2000) and Mills *et al.* (2004) who conducted similar experiments with particulate matter collected from a tropical reef site in Bermuda. One exception however, was that enrichment in the

ampicillin-treated particulate matter did not differ from the live particulate matter ($^{15}NH_4^+$ alone treatment) (Mills 2000; Mills *et al.* 2004). Therefore, results indicate that the organic or biological portion of the sediment is what is nutritionally available to *A. aureoradiata* and indeed it is this component that deposit feeding mudflat organisms utilise for their nutrition (Lopez & Levinton 1987).

4.2 Particulate Nitrogen Uptake from the Sediment

Here we show that *A. aureoradiata* can acquire particulate nitrogen from ^{15}N labelled sediment. As in this study, both Rosenfeld *et al.* (1999) and Mills *et al.* (2004) followed the flux of labelled particulate matter or sediment into the symbiotic association. Rosenfeld *et al.* (1999) showed the consumption and transfer of fluorescein-isothiocyanate labelled organic matter from the sediment, into the cells of the solitary tropical reef coral *Fungia horrida* while Mills *et al.* (2004) demonstrated that three tropical scleractinian corals *Siderastrea radians*, *Montastrea franksi* and *Diploria strigosa* had their tissues enriched with ^{15}N following exposure to ^{15}N labelled particulate matter. Nitrogen uptake from sediment or particulate matter sources by corals has also been assessed by examining the particulate matter or sediment itself for signs of uptake. For instance, Mills *et al.* (2004) proposed that the corals *S. radians*, *M. franksi* and *D. strigosa* took up particulate matter from the sediment based on dry weight (dw) declines in the particulate matter used during their uptake experiments. Similarly, Mills & Sebens (2004) measured the nitrogen content of sediments layered onto the surfaces of the corals *Siderastrea siderea*, *Agaracia agaricites* and *Porites astreoides* and found that after digestion, all species expelled sediment with a lower percentage of nitrogen than when they were first ingested. From this they calculated that *S. siderea* assimilated approximately 31 % of the nitrogen associated with the sediment, while *A. agaricites* and *P. astreoides* assimilated 55-74%. Additionally, non-ingested sediment sloughed off by *A. agaricites* and *P. astreoides* was lower in percentage nitrogen than the sediment initially ingested into the gut, which Mills & Sebens (2004) proposed was a result of either *A. agaricites* and *P. astreoides* selectively ingesting particles, which were higher in nitrogen, during the uptake process, or stripping the sediment of organic nitrogen during the sloughing process. The mode of sediment uptake in *A. aureoradiata* however, is unknown, with both the ingestion of sediment

particles into the mouth and the active stripping of nitrogen from particles without ingestion likely utilised in this species.

Increased sediment loads had a significant effect on the uptake of sedimentary nitrogen in *A. aureoradiata*. For instance, uptake of ^{15}N by the host was similar at both high and low sediment loads, but the algal symbionts acquired more nitrogen at the lower load (1.13 versus 0.93 atom % ^{15}N in the low and high loads, respectively). Likewise, Anthony (1999a) measured decreasing particulate organic carbon assimilation efficiencies with increasing concentrations of SPM for the corals *Porites cylindrica, Pocillopora damicornis*, *Montipora digitata* and *Acropora millepora*; at low concentrations (1 mg L^{-1}), estimates of assimilation efficiency ranged from 89 to 95% of the ingested SPM, decreasing to 40–50% at the highest concentrations (30 mg SPM L^{-1}). Similarly, Mills & Sebens (1997) found that the ability of the corals *S. siderea* and *A. agaricites* to sort, distinguish and ingest the more nutritious *Artemia* sp. cysts from the less nutritional sediment particles diminished with increasing particle load suggesting that corals started ingesting particles nonselectively. Futhermore, Mills & Sebens (2004) demonstrated that increasing sediment load in the coral species *A. agaricites* and *P. astreoides* resulted in egested sediment fractions that were no different in percent nitrogen than when they were consumed, indicative of a reduction in the corals' capacities to strip sedimentary nitrogen during the sorting process and also ingest particles of higher nutritional quality. In spite of these results, anemones in our study were exposed to far greater sediment loads (than any of the loads corals experienced in the above studies) with concentrations of 0.227 kg L^{-1} and 1.333 kg L^{-1} at the low and high loads, respectively. For example, Anthony's (1999a) highest concentration was 30 mg of SPM L^{-1}, while Mills & Sebens (1997) and Mills & Sebens (2004) layered 300 & 40 mg cm^{-2} over their corals, respectively. Sediment used in this study also differed significantly from that of those studies above, with the exception of Mills & Sebens (2004). The top 1-5 mm of surficial sediment surrounding *A. aureoradiata* was used in the current study, while previous studies used particulate matter, either filtered (< 10 μm) from the seawater (Anthony, 1999a, 2000) or collected in particle traps <105 μm (Mills et al., 2004) and thus may contain higher proportions of organic material (detritus or micro-organisms such as bacteria and microalgae) as opposed to grains of sediment.

Despite these differences, uptake of ^{15}N was still recorded at both sediment loads. This was understandable at the lower sediment load, where *A. aureoradiata* tentacles remained opened, but in the case of the high sediment load, the anemone had its tentacles withdrawn, being completely smothered throughout the feeding experiment. Thereby, it is possible that ^{15}N may have been actively phagocytosed across the anemone's epidermis into the anemones tissues. Alternatively, the role of mucus as a prey or particle trap in cnidarian feeding is well known (Sebens & Johnson 1991), and when buried in the lab, anemones visibly coated their exterior in mucus (S. Morar, pers. observ.). Mills & Sebens (2004) hypothesised that the corals *S. siderea* and *A. agaricites* used mucus as well as their tentacles to aid them in uptake of nitrogen from the sediment. Therefore it was possible that *A. aureoradiata* may have also been able to actively deplete the surrounding sediment of nitrogen using these mucus secretions. Assuming that uptake rates are correlated with sediment concentrations (Anthony 1999a), and that *A. aureoradiata in situ* takes up similar quantities of sediment as those measured here, an individual with its oral disc and tentacles spread out at the surface should be better at utilising the sediment than an individual that is retracted in to the sediment below the sediment surface.

Evidence thus far in tropical corals indicates that zooxanthellae benefit little from the uptake of particulate matter or sediment sources. For instance, in the corals *Porites cylindrica*, *P. damicornis*, *M. digitata* and *A. millepora*, Anthony (1999a) found that relatively little of the carbon assimilated from ingested SPM was respired (13-34%) by the coral, with the majority used for tissue growth. Meanwhile, Mills *et al.* (2004) discovered no ^{15}N labelled nitrogen in zooxanthellae of the tropical corals *S. radians*, *M. franksi*, and *D. strigosa* following host uptake of ^{15}N labelled particulate matter. This lack of any ^{15}N labelled nitrogen prompted Mills *et al.* (2004) to suggest that nitrogen recycling in the corals *S. radians*, *M. franksi*, and *D. strigosa* did not occur or was minor, and that nitrogen acquired by the host through ingestion was primarily conserved for coral growth (in agreement with the nitrogen conservation hypothesis). Despite these trends, our study not only detected ^{15}N in the zooxanthellae of *A. aureoradiata,* but also showed the zooxanthellae were far more enriched than the animal; all of which took place within 18h of first being exposed to ^{15}N labelled sediment. Precisely speaking, zooxanthellae in this study obtained 55% and 62% of the ^{15}N taken up from the low and high sediment loads, respectively. This partitioning differs somewhat from

previous labelled-prey studies that used other isotopic tracers and a wide variety of symbiotic partners, experimental conditions, and processing techniques. Zoochlorellae obtained 22-26% of prey carbon ingested by hydra in the light, and 25-34% in darkness (Cook 1972). In the coral *Astrangia danae*, 10-20% of the ingested carbon was found in zooxanthellae (Szmant-Froelich 1981). Zooxanthellae from the anemone *Aiptasia pallida* and the temperate scleractinian coral *Oculina arbuscula* both acquired 10-20% of the ingested prey nitrogen after 4h of feeding on ^{15}N labelled *Artemia* sp. (Piniak *et al.* 2003). However, while no subsequent ^{15}N enrichment with time took place in *A. pallida* tissues, zooxanthellar amino acids became increasingly enriched (15-20% greater) over 30h (Piniak *et al.* 2003). Thus in this study, assuming that ^{15}N enrichment of zooxanthellar fractions increased following the 6h feeding experiment, up until host and zooxanthellar tissue samples were analysed 18h later, the 55% and 62% values seem reasonable.

The presence of ^{15}N within zooxanthellae indicates that nitrogen is passing from the host to the zooxanthellae. Therefore, we assume that ^{15}N-labelled sediment is phagocytosed and broken down by the host; the digested products are then incorporated into host tissue and/or immediately catabolised, with waste nitrogen excreted or taken up by the zooxanthellae (Piniak *et al.* 2003). Although, these results show the passing of ^{15}N from host to zooxanthellae, the return of nitrogen from zooxanthellae to host for complete nitrogen recycling to occur has yet to be determined in *A. aureoradiata*. Perhaps, the addition of ^{15}N enriched zooxanthellae cultures to aposymbiotic (zooxanthellae eliminated) *A. aureoradiata* individuals would enable the tracking of ^{15}N from zooxanthellae to host, or may be the use of ^{14}C-labelled amino acids to demonstrate the synthesis and release of essential amino acids by the zooxanthellae to the host (Trench 1971; Swanson & Hoegh-Guldberg 1998; Wang & Douglas 1999) may resolve the issue of nitrogen recycling versus conservation in this species.

The acquisition of both ammonium and nitrate in symbiotic cnidarians is a light-dependent process (Grover *et al.* 2002; Furla *et al.* 2005). The light-stimulated uptake of ammonium, for instance, indicates the involvement of the enzyme glutamate synthase, which, in many other photosynthetic systems, is driven by photochemically derived reduced ferredoxin (Lea & Mifflin 1979; Furla *et al.* 2005). Consequently, zooxanthellae are thought to be the main site of assimilation of this inorganic nitrogen

(Grover *et al.* 2002; Furla *et al.* 2005). Following exposure to ^{15}N sediment, anemones in this study were placed in the light for 18h (24h after first being exposed to ^{15}N sediment) and as a result, higher uptake by *A. aureoradiata* at the lower sediment loads brought about greater enrichment of the zooxanthellae. Alternatively, it is well documented that symbiotic zooxanthellae take up dissolved inorganic nitrogen (DIN) from the water column at much faster rates than does host tissue and indeed, this is certainly the case in the temperate anemone *Anemonia viridis* (Roberts *et al.* 1999b) as well as the tropical anemones *Aiptasia pulchella,* (Swanson & Hoegh-Guldberg 1998) *A. pallida* and *Bartholomea annulata* (Lipschultz & Cook 2002). It is therefore possible that zooxanthellae could also have taken up dissolved ^{15}N from the overlying or interstitial water during the anemones exposure to ^{15}N labelled sediment. Dissolved ^{15}N may have come from any unincorporated ^{15}N label or ^{15}N catabolised by flora (bacteria and microalgae) within the sediment during the 6h uptake experiment (C. B. Cook, pers. comm.). This however, seems unlikely as the freeze drying process would have likely killed sediment associated microbes while repeated washing of the sediment would have removed any remains of unincorporated dissolved ^{15}N label. Moreover, no change in sediment ^{15}N enrichment for all treatments during the feeding experiment and very little sediment ^{15}N enrichment in the formalin treatment of the sediment ^{15}N enrichment experiments (the former indicative of very little label being lost into the surrounding chamber water from sediment and the latter indicative of little abiotic ^{15}N uptake), suggests the amount of label potentially gained by this means, is likely small compared to the amount actively taken up from the sediment by the anemone.

4.2 Zooxanthellar Nitrogen Status

4.2.1 Nutritional History & Zooxanthellar Nitrogen Status

The nitrogen sufficiency of symbiotic dinoflagellates, as indicated by the ammonium enhancement of dark carbon fixation and V_D/V_L was clearly dependent on host feeding in *A. aureoradiata*. Food ingested by the host represents a significant source of inorganic nutrients for the algae and thus it was an ideal proxy for measuring the effects of sediment ingestion on nitrogen status in this species.

There was little evidence of nitrogen deficiency in zooxanthellae of *A. aureoradiata* after 2 weeks of starvation, with the ammonium enhancement ratio (NH_4^+ /FSW) still being just below 0.95 and V_D/V_L being negative (-1.03). The situation changed strikingly between 2-4 weeks with NH_4^+/FSW and V_D/V_L rising to 2.35 & 0.11, respectively. These measurements were consistent with zooxanthellae from another temperate cnidarian, the scleractinian coral *Plesiastrea versipora,* where NH_4^+/FSW increased from 1.03 to 1.78 and V_D/V_L values increased from $<10 \times 10^{-4}$ to 53×10^{-4} from 2 to 4 weeks (Davy *et al.* 2006). This response time, however, was slow compared to the times reported in tropical cnidarians. For instance, in zooxanthellae from the tropical sea anemone *A. pallida*, NH_4^+/FSW increased from 1.33 to 2.35 after just one week of host starvation (Cook *et al.* 1992). Similarly, NH_4^+/FSW in the reef coral *Madracis mirabilis,* increased from 1.2-1.4 to 1.7-1.9, while values of V_D/V_L increased from 2×10^{-3} to $5\text{-}6 \times 10^{-3}$ after 15 days of host starvation (Cook *et al.* 1994). Ammonium enhancement ratios, meanwhile, peaked at 6 weeks of starvation in both the temperate cnidarians *A. aureoradiata* and *P. versipora*, in stark contrast to the tropical cnidarians where ratios peaked at 1 week and 15 days for *A. pallida* and *M. mirabilis*, respectively (Cook *et al.* 1992; Cook *et al.* 1994; Davy *et al.* 2006).

Photosynthetic rate (fg C $cell^{-1}$ h^{-1}), the other indicator of nutrient stress measured here decreased rapidly in *A. aureoradiata* soon after starvation began, before remaining relatively constant for the next 8 weeks. However, well-fed anemones after 8 weeks also had reduced photosynthetic rates remarkably similar to those seen between 2 to 8 weeks of host starvation. In contrast, the photosynthetic rate of zooxanthellae from *P. versipora* decreased by 19% between 4-6 weeks and a further 21% after 8 weeks since last being fed (Davy *et al.* 2006). Reduced levels of photosynthetic pigments (chlorophyll *a*) as well as densities (cells mg^{-1} protein) of the zooxanthellae are most likely the cause of the declines in photosynthetic rate (Davy *et al.* 2006), though both were not assessed here. Indeed, declines in chlorophyll *a* levels and zooxanthellar densities were evident within just one week of starving *A. pallida*, where these factors decreased by about 90% and 80%, respectively, after 3 months of starvation (Cook *et al.* 1988). Similarly, photosynthetic rates in the hydroid *Myrionema amboinense* decreased by 40 % after just 5 days of starvation (McAuley & Cook 1994). Correlated with this rapid change was a decrease in mitotic index (the percentage of zooxanthellae

undergoing mitosis) from 10.08% to 3.90% (McAuley & Cook 1994) and even more rapidly from 10-15% to below 1% after 2-4 days of starvation in a subsequent study (Fitt & Cook 2001). Associated with this was a decline in the ratio of glutamine/glutamate, which is another sensitive indicator of nitrogen status (Flynn 1990) Additionally, after 14 days of starvation, zooxanthellar densities and the mitotic index in the anemone *A. pulchella* were just 60% and 35% of those seen in well-fed individuals, respectively (Smith & Muscatine 1999). Meanwhile, the decline in the photosynthetic rate in well-fed anemones after 8 weeks, maybe linked with the nitrogen free artificial seawater (NFASW) in which anemones were incubated throughout the duration of the experiment; although, no explanations to why NFASW would reduce photosynthetic rate but not increase the ammonium enhancement ratio or V_D/V_L (i.e. nitrogen deficiency) in well-fed individuals can be offered.

The time taken for starvation to affect zooxanthellar nitrogen status appears to vary between tropical and temperate host species, with zooxanthellae in *A. aureoradiata* and *P. versipora* being less susceptible to changes in host feeding regime than are zooxanthellae in the tropical hosts studied to date (Davy *et al.* 2006). Certainly, zooxanthellar growth in tropical cnidarians fluctuates rapidly with changes in food supply (Muscatine *et al*. 1989; Fitt & Cook 2001; Grover *et al.* 2002), as well as changes in (DIN) (Hoegh-Guldberg & Smith 1989; Muscatine *et al.* 1989; Stambler *et al.* 1991; Snidvongs & Kinzie 1994; Stambler *et al.* 1994). Conversely, Roberts *et al.* (2001) found that when the temperate anemone *A. viridis* was starved and exposed to low levels of DIN for 47 days, the glutamine/glutamate ratio of the zooxanthellae was comparable to that seen after starvation under DIN-enriched conditions and suggested continuing nitrogen sufficiency. There are no known explanations for the tolerance of zooxanthellae from temperate hosts such as *A. aureoradiata*, *P. versipora* and *A. viridis* to starvation, however it could be due to a relatively low reliance on host feeding as a source of nitrogen (Cook *et al.* 1994). Alternatively, such hosts may have greater internal stores of nitrogen than hosts that are more prone to changes in food supply (McAuley & Cook 1994) or their zooxanthellae may be better at utilising their own or their host's nitrogen reserves during periods of host starvation (Davy *et al.* 2006). McAuley & Cook (1994) also proposed that hosts with high concentrations of zooxanthellae maybe more reliant on external sources of nitrogen than those with lower concentrations. However, a density of 3×10^6 zooxanthellae cm^{-2} in *P. versipora* (Kevin

& Hudson 1979) is comparable to or even higher than a number of tropical reef corals (Muscatine *et al.* 1989; Cook *et al.* 1994), including *M. annularis* in which densities of 1.46×10^6 zooxanthellae cm^{-2} were reported and whose zooxanthellae showed rapid signs of nitrogen deficiency when starved (Cook *et al.* 1994; Davy *et al.* 2006).

4.2.2 The Influence of Habitat on Zooxanthellar Nitrogen Status

4.2.2.1 Mudflat

The results of this study indicate that zooxanthellae from mudflat anemones were nitrogen sufficient (or approached nitrogen sufficiency) with NH_4^+/FSW and V_D/V_L being just 1.26 and -6.0×10^{-3}, respectively. The data from mudflat *A. aureoradiata* are consistent with those of field populations of *P. versipora* in the temperate waters of south eastern Australia (Davy *et al.* 2006). Although our field measurements of nitrogen status were only conducted during winter of 2007, Davy *et al.* (2006) examined nitrogen status seasonally, reporting spring, summer, autumn and winter, enhancement ratios of 1.25, 1.32, 1.00 and 1.08, respectively. While nitrogen sufficiency was greater during autumn and winter compared to spring and summer, zooxanthellae in *P. versipora* remained nitrogen sufficient all year round (Davy *et al.* 2006). These results, combined with our mudflat data, suggest that zooxanthellae from temperate hosts are far more nitrogen sufficient than are zooxanthellae from tropical corals and anemones. For example, on the tropical reefs around Bermuda, reef corals *M. mirabilis* and *M. annularis* had NH_4^+/FSW values of 1.2 to >3 and 1.4 to 2.0, respectively (Cook *et al.* 1994). Additionally, nitrogen deficiency was reported in the subtropical anemone *A. pallida*, even in individuals collected from an enclosed, nutrient-rich mangrove site; NH_4^+/FSW and V_D/V_L values ranged from 1.30 to 2.88 and 1.09 to 7.85×10^{-3}, respectively, being higher in the summer and lower in the winter (Cook *et al.* 1992). Exceptions to this in tropical waters are corals which reside on reefs heavily impacted by terrestrial watersheds and anthropogenic inputs (Davy *et al.* 2006). For instance, zooxanthellae in the coral *Montastraea faveolata* in the Florida Keys are nitrogen sufficient, with NH_4^+/FSW values of just 1.07 and 1.17 measured at offshore and inshore sites, respectively (Cook *et al.* 2002, cited in Davy *et al.* 2006).

Given that zooxanthellar nitrogen status in *A. aureoradiata* changed markedly when anemones were fed or starved in the laboratory, with anemones fed 5 times a week for ≥ 12 weeks having nitrogen status indices (expressed as either NH_4^+/FSW or V_D/V_L) comparable to anemones collected on the mudflat in winter, it is likely that the nitrogen sufficiency seen in this temperate symbiosis on the mudflats is related to a ready supply of plankton and/or assimilation of dissolved nitrogen direct from the overlying or interstitial water. The relative importance of particulate versus dissolved nitrogenous sources cannot be determined at present, due to lack or relevant field data for *A. aureoradiata*; however, it is likely that it fluctuates over the course of the year in response to seasonal changes in nutrient levels and plankton productivity (Muller-Parker & Davy 2001).

Nitrogen sufficiency at Pauatahanui Inlet can be explained by high levels of (DIN) in interstitial water of the sediment (J. Davy & M. Palka unpubl. - 5.1 Appendix A – Dissolved Inorganic Nitrogen Concentrations). Ammonium in particular was nearly 20 times higher in the interstitial water than it was in the overlying seawater (Figure 21 - Appendix A). Such elevated levels of ammonium are likely to come from several different sources. Firstly, the decomposition of organic material in the anoxic layer of the sediment by anaerobic bacteria results in high levels of reduced compounds such as methane, hydrogen sulphide, iron and ammonium. These compounds subsequently diffuse upwards into the aerobic layer (Kemp *et al.* 1990; Nybakken & Bertness 2005) where *A. aureoradiata* resides on a mudflat. Although, measurements of DIN were taken in the late spring of 2007, it is believed the DIN levels from this source would be expected to remain elevated all year round. Secondly, the intertidal region at Pauatahanui Inlet is also rich in benthic infauna such as bivalves and polychaetes (Healy 1980), which are capable of turning over large volumes of sediment (Pickrill 1979). This process, known as bioturbation is well recognised for re-releasing nutrients such as nitrate, ammonium and phosphorus into the interstitial water (Aller 1982; Clavero *et al.* 1994). Finally, Mourtisen & Poulin (2003) and Mourtisen & Poulin (2005) established that on mudflats around New Zealand, *A. aureoradiata* forms a facultative (see section 1.1) symbiotic relationship with the New Zealand cockle *Austrovenus stuchburyi* in which the former is provided with a suitable substrate for attachment and the latter obtains protection against parasitic infections; this intimate relationship may provide an

opportunity for nutrient exchange. Mokady *et al.* (1998) discovered that a seemingly parasitic boring bivalve *Lithophaga simplex*, found inhabiting the calcium carbonate skeletons of the scleractinian coral *Astreopora myriophthalma*, produced considerable amounts of ammonium (as a nitrogenous waste product), which provided the coral and its zooxanthellae with significant portions of their total ammonium needs. Therefore, in addition to providing an attachment substrate, perhaps *A. stuchburyi* is releasing waste ammonium. The high ammonium concentrations produced in the field by the processes above would help explain why field populations of *A. aureoradiata* are nitrogen sufficient, but also why sediment with interstitial water removed had no effect on zooxanthellar nitrogen status.

4.2.2.2 Rocky Intertidal

The nitrogen deficiency rates demonstrated in zooxanthellae from the rocky intertidal field populations of *A. aureoradiata* are the first data to report nutrient deficient zooxanthellae in temperate waters. Enhancement ratios of 2.87 and 2.99 for the low and high rocky intertidal sites, respectively, even exceeded those exhibited in two tropical Bermudan reef corals, *M. mirabilis* and *M. annularis,* and a subtropical anemone *A. pallida* (Cook *et al.* 1992; Cook *et al.* 1994). In fact, they were more comparable to those seen in zooxanthellae from anemones starved >6 weeks, when enhancement peaked in all of the starvation experiments. DIN (ammonium and nitrate + nitrite) levels at Kau Bay (J. Davy & M. Palka unpubl. - 5.1 Appendix A – Dissolved Inorganic Nitrogen Concentrations) from the water column in late spring of 2007 were comparable to observed DIN values of the water column at Pauatahanui Inlet (Figure 21 - Appendix A). However, ammonium and nitrate + nitrite levels at Kau Bay were 25 and 2.5 times lower, respectively, than concentrations of ammonium and nitrate + nitrite detected in the interstitial water of the sediment on the mudflat. While no direct measurements of nutrient levels were taken in winter of 2007 when nitrogen status in zooxanthellae of *A. aureoradiata* was measured, it is proposed that DIN levels would have been somewhat higher than those observed in late spring (Heath 1977). Although low DIN levels at Kau Bay, compared to the sediment at Pauatahanui Inlet explain why zooxanthellae from rocky intertidal anemones maybe nitrogen deficient, the degree to which these temperate zooxanthellae were nitrogen deficient is unexpected. Several authors have suggested that Wellinton Harbour is a sheltered, well-mixed, nutrient-rich, semi-enclosed body of water that acts as a basin for the Hutt River to the north

(Maxwell 1956; Brodie 1958; Booth 1975; Heath 1977; Gardner 2000). Also, storm events and terrestrial runoff from several local freshwater streams, as well as high levels of seston (particulate organic material suspended in the water column) around Kau Bay (Helson *et al.* 2007) all year round should promote nitrogen sufficiency. It may be possible that *A. aureoradiata* living in the rocky intertidal zone has very high densities of zooxanthellae in its tissues, and indeed anemones are visibly browner (indicative of higher concentrations of zooxanthellae or photosynthetic pigment) than their mudflat counterparts (S. Morar, pers. obs.). Moreover, the comparatively higher light levels experienced by rocky shore anemones over their conspecifics on the mudflat would encourage more photosynthesis and therefore higher densities of zooxanthellae. This, according to McAuley & Cook (1994), would put higher demands on external sources of nitrogen to maintain nitrogen status than it would for anemones with lower zooxanthellae densities, leading to greater degrees of nitrogen deficiency.

The difference in nitrogen status in zooxanthellae from *A. aureoradiata* between at the two field sites is unusual and was not entirely expected considering previous studies of temperate nitrogen status. On the other hand, it could be argued that two very different environments are highly likely to differ in zooxanthellar nitrogen status. Nevertheless, such "snapshot" measurements (Davy *et al.* 2006) may well be misleading and only more frequent measurements especially of nitrogen status in addition to DIN levels, will confirm whether the results found in this study are indeed accurate. Moreover, assessment of zooxanthellar characteristics such as chlorophyll *a* levels and zooxanthellae density per animal will further corroborate measurements of nitrogen status.

4.2.2 Sediment Uptake & its Influence on Zooxanthellar Nitrogen Status

Despite the uptake of ^{15}N from the sediment by *A. aureoradiata*, this nitrogen did not assist the nitrogen status of the zooxanthellae; nitrogen deficiency was evident after 2 weeks of starvation even in the presence of sediment. As in the earlier starvation experiment, the ammonium enhancement ratios of the sediment/no sediment treatments peaked at 6 weeks of starvation while photosynthesis, again declined after >0 weeks of starvation. More importantly however, was no difference in photosynthetic rate between the sediment and no sediment treatments. These results clearly indicate that host uptake

of sediment did not provide zooxanthellae with the essential nutrients required to delay the onset of nitrogen deficiency in *A. aureoradiata*. This combined with the result that field populations of *A. aureoradiata* are nitrogen sufficient on the mudflat suggests that sediment was not the major source of nitrogen to zooxanthellae in this anemone species, with the major source of nitrogen perhaps eliminated before being supplied to the anemone in these experiments. Certainly, DIN levels in interstitial water were higher than those in the water column and *in situ* water was eliminated from collected sediment before it was provided to *A. aureoradiata*.

4.3 Ecological Implications & Conclusions

Under turbid conditions, enhanced particle feeding may be necessary to counteract the reduction in phototrophy and to sustain a positive energy budget (Anthony 2000). Research thus far measuring the uptake of sediment or particulate matter has only been carried out in corals which inhabit turbid tropical waters with very low levels of planktonic food and dissolved inorganic nutrients (nitrates and ammonium). Such conditions promote the utilisation of particulate matter or sediment as a source of nutrition (Abelson *et al.* 1993; Stafford-Smith 1993; Anthony 1999a; Anthony & Fabricius 2000; Fabricius & Dommisse 2000). On the other hand, temperate associations inhabit waters which contain high levels of dissolved inorganic nutrients and plentiful supplies of planktonic food (Muller-Parker & Davy 2001). One might therefore assume that there is no need for the intake of sediment particles, as most associations are nutritionally suffice. Alternatively, intake of some of the sedimentary particles may be necessary to supplement nutrition, particularly in cases where symbiotic species reside in severely light limited, turbid habitats.

While the importance of sediment as a food source to *A. aureoradiata* is questionable, there is no doubt that this species possesses the ability to utilise it as a source of nitrogen. The oxidised sediment surrounding *A. aureoradiata* contains diatoms, as well as large numbers of both autotrophic bacteria, and bacteria which break down the copious amounts of organic matter present on a mudflat (Nybakken & Bertness 2005). Regardless of the low quality of this sedimentary food source, deposit feeding organisms consume and prosper from these microbes in this environment

(Lopez & Levinton 1987). Also, one such deposit feeder, the mud snail *Amphibola crenata*, which is abundant at Pauatahanui Inlet (Kennedy 1986), produces continuous strings of mucus-bound faeces (Juniper 1986), which have been shown to stimulate colonisation and metabolic activity of adhering bacteria and fungi (Juniper 1981). According to Juniper (1987), such faecal material contains more active bacterial flora than unprocessed sediment and these clumps may constitute a significant portion of the particulate food available not only to deposit feeding organisms but also to *A. aureoradiata*.

However, while *A. aureoradiata* was able to take up nitrogen from the sediment, the sediment's negligible effects on the zooxanthellae suggest it was not an important source of nitrogen to this particular symbiosis. This combined with high levels of interstitial DIN imply that there is no need for sediment consumption to maintain nitrogen status, or that sediment may just be important in times of low food and dissolved nutrient availability rather than on a regular basis. However, if the latter was the case, sediment should have eased nitrogen deficiency of the zooxanthellae, which it did not.

In the tropics, however, it has been suggested that carbon acquisition from the sediment, rather than nitrogen acquisition may be especially important, compensating for reduced photosynthesis caused by turbidity. (Anthony 1999a; Anthony 2000). The potential importance of sedimentary carbon to symbiotic cnidarians in temperate light-limited environments such as a mudflat must therefore be considered. While nitrogen maybe obtained from DIN in the interstitial waters, the primary source of carbon is photosynthetically derived from the zooxanthellae. With the large amounts of time *A. aureoradiata* remains buried when no overlying water is present, and only a third of the population at the sediment surface at any one time when overlying water is present (5.2 Appendix B – Preliminary Field Study) (Figure 22 - Appendix B), zooxanthellae are spending the majority of time in low light unable to harvest light for photosynthesis. Therefore carbon may be more limiting than nitrogen in this species, and as a result the ability of *A. aureoradiata* to take up particulate carbon from the sediment versus the carbon loss associated with reduced phototrophy needs to be further examined. Finally, investigations of particulate nitrogen and carbon uptake from the sediment and nitrogen status in other temperate symbiotic anemones (*Cereus pedunculatus*, *Anthopleura ballii*,

Anthopleura artemisia) with similar burrowing habits (Davy *et al.* 1997; Anderson 2000) to *A. aureoradiata* may establish the generality of these findings. If sedimentary nitrogen and carbon uptake are indeed general phenomena in these temperate symbioses, then they may help explain the marked robustness and stability of such symbioses under conditions that would be otherwise expected to be intolerable.

In summary, although particulate nitrogen can be taken up from the sediment by *A. aureoradiata*, the importance of sediment as a food source is less evident. Perhaps, dissolved nutrients such as ammonium and nitrate are more valuable on a mudflat, or carbon rather than nitrogen is the most important factor. The results certainly suggest that further research on the nutritional role of sediment in this species as well as other temperate cnidarian-zooxanthellate associations is still warranted. Moreover, this will provide a further branch of evidence for differences between tropical and temperate dinoflagellate-invertebrate associations.

5

APPENDICES

5.1 Appendix A: Dissolved Inorganic Nitrogen Concentrations

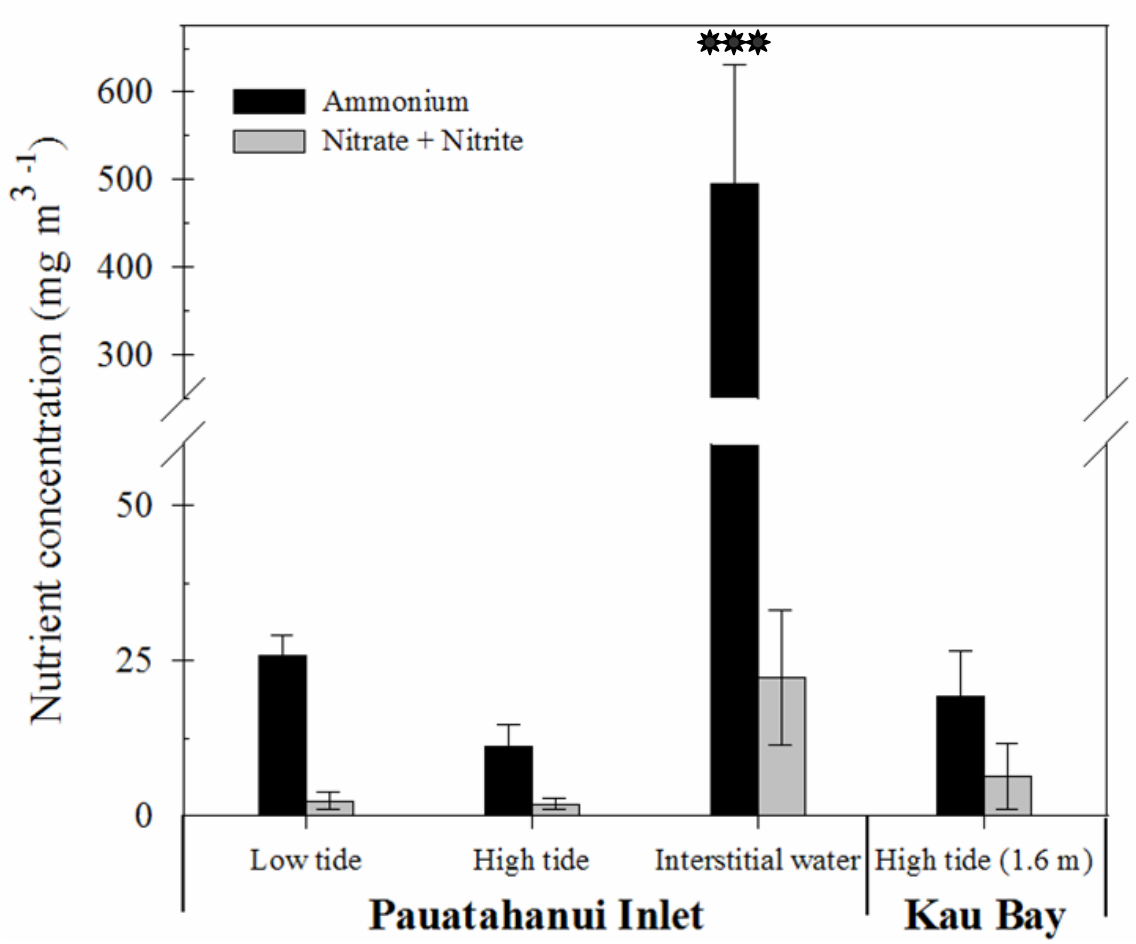

Fig. 21. *Dissolved ammonium and nitrate + nitrite nutrient concentrations at the different field sites. Concentrations are in mg m$^{3\ -1}$. Values are mean ± SE, n = 3 for each site. Note there is a break and a change in scale in the y-axis. Nutrient concentrations of low tide and high tide at both field sites were of the water column while interstitial water concentrations were of the water within the sediment. Significant differences are shown by *** P<0.0001 where comparisons are between concentrations of the respective nutrient between collection sites. Samples were collected on November 21 2007 and analysed by NIWA, Hamilton. Data courtesy of J. Davy & M. Palka, unpublished.*

5.2 Appendix B: Preliminary Field Study

5.2.1 Methods

The vertical distribution of *Anthopleura aureoradiata* in the sediment (either at the sediment surface or buried – see Figure 3) was examined in response to tide. The objective of this experiment was to determine how many anemones were at the sediment surface at high tide compared to how many were buried at low tide in the same location.

On the mornings of 29th & 30th of December 2005 and 14th January 2006 during high tide, 5 50 m transects were set up lengthways (in a direction from the shore into the water) 25 m apart in an area where *A. aureoradiata* was known to be abundant. Transects were marked with thin rope and placed in different areas of the shore (along the shore not up and down the shore) on each day to survey a much larger portion of the shore. While snorkelling at high tide (water level >0.8 m), 5 50 × 50 cm quadrats were randomly selected along each transect and the amount of anemones present at the surface counted. Once each quadrat was completed, large 60 cm long pegs with a small pink flags at the top were placed at each of the 4 corners of the quadrat. These pegs allowed us to identify the exact position of the quadrat when it came to digging up the quadrat at low tide. At low tide (no overlying water present) during mid-afternoon on the same day, the top 5-8 cm of each quadrat was carefully dug up with a shovel and the sediment sieved through a 2 mm mesh size 50 × 50 cm sieve. The remaining contents of each sieve were examined carefully for *A. aureoradiata* individuals attached to the cockle shells (*Austrovenus stuchburyi*) or cockle-shell debris. The number of *A. aureoradiata* specimens in each quadrat was then noted.

The number of anemones at the surface of the sediment was compared to the number buried at low tide to give us the proportion of anemones at the surface of the sediment when samples were taken at high tide.

5.2.2 Results & Discussion

On average, 18.4%, 41.4% and 41.8% of the anemones in the quadrat were at the surface of the sediment when samples taken at high tide for days 1, 2 and 3, respectively (Figure 22). Thus, 81.6%, 58.6% and 58.2% of the anemones were buried at the time the samples were taken at high tide suggesting that approximately two thirds of the anemones were below the surface at high tide. The low percentage at the surface during day 1 (Figure 22) was perhaps due to the overcast conditions at the time high tide samples were taken as opposed to the clear sunny conditions on days 2 and 3. Certainly in this case, the number of anemones at the surface of the sediment may depend on the overhead weather conditions and anemones indeed withdraw back into the sediment during periods where the cloud covers the sun on a mostly clear, somewhat cloudy day (S. Morar, pers. obs.). Similarly, the presence of *A. aureoradiata* at the surface will also depend on tide especially when low tide sometimes results in no overlying water. Additionally, water temperature, seasonal factors, as well as the behaviour of the cockle may also have an influence. In conclusion, it is possible that mudflat *A. aureoradiata* spends the majority of its life buried beneath the surface.

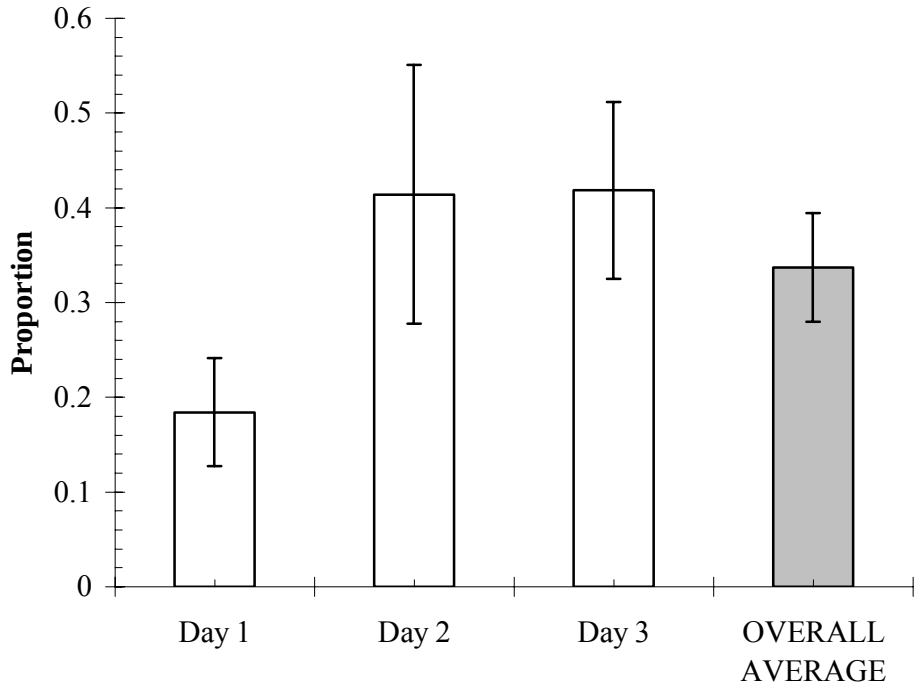

Fig. 22*. Mean proportion of* Anthopleura aureoradiata *at the sediment surface at high tide ± SE on each day and the overall mean proportion for the 3 days. N = 25 on each day and N = 75 overall. Note, no statistical analyses were conducted.*

6

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