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Stable isotopes of nitrogen as potential indicators of nitrogen contamination in Port Curtis - a pilot study

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Introduction

Stable isotopes are widely used in environmental studies (Lajtha and Michener 1994). The ratio of nitrogen stable isotopes, $^{15}N : ^{14}N$, in vegetation relative to that ratio in air (delta (δ)^{15}N) is used to track particular nitrogen species through the environment to understand natural processes such as nitrogen cycling in plant communities (eg Nadelhoffer and Fry 1994, Rao *et al.* 1994, Bartkow and Udy 2004), nutrient and food web relationships in ecosystems (eg Lee 2000) and to pursue pollution issues (Macko and Ostrom 1994).

Dennison, Costanza and others (Anon. 1998) used this approach to define the extent of sewage pollution in Moreton Bay. They used the measured δ^{15}N in deployed sentinel plants (macro algal species *Catenella nipae*) suspended in the water column to map the extent of "zones of impact" throughout Moreton Bay. The same approach was used to map the extent of the influence of industrially sourced nitrogen from a licensed marine discharge in Halifax Bay near Townsville (Dennison *et al.* 1999). At the same location they used δ^{15}N in mangrove foliage (*Avicennia marina*) to define contamination in estuarine waters. The method does not easily distinguish between nitrogen sourced from industry, sewage, septic or aquaculture sources (Wormington *et al.* 2004) but does identify regions of elevated nitrogen from human sources. Further, Costanzo *et al.* (2003) found that elevated δ^{15}N with elevated %N reflected sewage inputs while depressed δ^{15}N with elevated %N was indicative of agricultural inputs in a sub-tropical estuary indicating some understanding of regional nitrogen pathways is necessary to interpret some results. Despite these limitations the use of mangroves does provide a tool for monitoring medium to long-term nitrogen inputs especially those that are influencing sediment nutrients – through contaminated groundwater for example.

Port Curtis has been the receiving environment for sewage and diffuse nitrogen sources from a small number of settlements fringing the port as well as nitrogen discharges to Port Curtis from industrial sources. The significance of these potential sources in the nitrogen economy of port sediments and waters is poorly understood.

The objectives of this pilot study were to examine the applicability of δ^{15}N assessment as a monitoring tool in Port Curtis, to identify likely candidate plant species that could be used as sentinels and consider the approach to further work. It was decided to utilise the foliage of *in situ* aquatic macrophytes rather than deploy macro algae as mangroves fringe much of the coastline and seagrass and macro algal beds are common and because there is no local macro algae similar to *Catenella nipae* in south-east Queensland.

Methods

Samples were collected within Port Curtis, around Curtis Island, through the Narrows and south of Port Curtis around Wild Cattle Island and Rodds Bay. The sites south of Port Curtis were included as "industry free" reference environments while the upper Fitzroy Estuary was included as a "developed estuary" reference with a significant sewage discharge.

At each site, five newest fully expanded leaves from mangrove trees and shrubs and foliage from seagrass and fragments of macro algae were collected, washed in deionised water and placed on ice for transport back to the laboratory. Samples were placed in paper bags and dried at <50°C until constant weight. Dry foliage was ground and then sub samples were oxidised in a Roboprep CN Biological Sample Converter (Europa Tracermass, Crewe, U.K.). The resultant N_2 was analysed by a continuous flow isotope ratio mass spectrometer (Europa Tracermass, Crewe, U.K.). Total %N of each sample was determined, and the $^{15}N : ^{14}N$ expressed as the relative difference between the sample and a standard (N_2 in air) using the following equation (Peterson and Fry, 1987):

$$\delta^{15}N = (^{15}N/^{14}N \text{ (sample)} / ^{15}N/^{14}N \text{ (standard)} - 1) \times 1000 \text{ (‰)}$$

Results

Seven mangrove species, one seagrass species and two macro algae were collected from 28 locations around Port Curtis, upper Fitzroy Estuary and in Rod's Bay. Sampling locations and data are listed in Appendix 1.

The $\delta^{15}N$ values identified among the 10 macrophytes across the 28 locations ranged from –1.167 in *Ceriops tagal* at Yellow Patch and –1.410 in *Aegiceras corniculatum* to 13.08, 11.396 and 11.595 in *Aegiceras corniculatum* at the top of the Fitzroy Estuary. All other records ranged from 0.079 to 6.371.

Of the macrophytes sampled, only three mangrove species provided a sufficiently wide coverage of the region of interest for effective monitoring in this pilot study. The three mangroves are *Rhizophora stylosa*, *Avicennia marina* and *Aegiceras corniculatum*. The data for these three species are summarised in Table 1 ($\delta^{15}N$) and Table 2 (% foliar N) and the $\delta^{15}N$ data are displayed spatially in Figures 1, 2 and 3. Here the size of the red data point reflects the magnitude of data at that point. The summary $\delta^{15}N$ data are also displayed in Figure 4.

The $\delta^{15}N$ data from *Aegiceras corniculatum* foliage in the upper Fitzroy Estuary are different from the equivalent data from the other regions. There were no differences among the regions in the data from *Rhizophora stylosa* or *Avicennia marina.*

The %N in foliage tended to be higher in *Avicennia marina* than in *Rhizophora stylosa* and *Aegiceras corniculatum* but there were no differences among the regions.

Discussion

<u>Using mangroves for regional assessment</u>

A quick scan of the data in the summary tables and figures suggests that there is spatial variation in $\delta^{15}N$ within each species. $\delta^{15}N$ in *Rhizophora stylosa* appears to be lower around Rodds Bay than around Port Curtis and Curtis Island (with two exceptions). $\delta^{15}N$ in *Avicennia marina* appears to be quite variable across the region with the highest determinations near to a small settlement on the north eastern end of Curtis Island. $\delta^{15}N$ in *Aegiceras corniculatum* is greatest in the Barrage Reach of the Fitzroy estuary.

With the exception of the Fitzroy Estuary data, these results are consistent with generally unpolluted mangroves analysed in north Queensland where $\delta^{15}N$ ranged from 1.06 ^{o}/oo in reference environments to 40.2 ^{o}/oo in estuaries contaminated with industrial nitrogen (Dennison *et al.* 1999). In Moreton Bay $\delta^{15}N$ around 10 ^{o}/oo was considered indicative of waters influenced by sewage effluent while parts of the bay well flushed with oceanic water had $\delta^{15}N$ less than 3 ^{o}/oo (Anon. 1998).

We conclude that, no evidence of general nitrogenous contamination was detected in Port Curtis or Rodds Bay using $\delta^{15}N$ in mangrove foliage. There is some slight indication of elevated $\delta^{15}N$ near a small settlement on the northwest tip of Curtis Island. There was no indication of any residual influence of the recently decommissioned sewage discharge into the Calliope River.

In contrast, however, $\delta^{15}N$ in the upper Fitzroy Estuary suggests chronic nitrogenous contamination is present. The $\delta^{15}N$ in *Avicennia marina* from the same reach of the estuary was not elevated. This suggests that the two mangrove species that utilise slightly different environmental zones are subject to different nitrogen regimes. Further monitoring in the Estuary is required to ascertain the nature and extent of the nitrogenous contamination here. Measuring the concentration of stable isotopes of nitrogen in mangrove foliage in Port Curtis and the adjacent regions identifies regional differences around the coastline and the Fitzroy Estuary and Rodds Bay hold promise as reference environments for Curtis Coast waters.

<u>Alternative approaches</u>
Using plants *in situ* has limitations as their nitrogen utilisation may not be correlated to aquatic nitrogen concentrations (Costanzo *et al.* 2003). We have used terrestrial and mangrove trees *in situ* to monitor contaminated groundwater and chronic exposure in impounded estuarine waters (Wormington et al. 2004) but used deployed macro algae to monitor open water nitrogen at the same site (Dennison et al. 1999). Macro algae derive nutrients directly from the water column. In this pilot study we sampled *Sargassum* sp. and other macro algae within the region but their distribution is too disjointed to provide an *in situ* monitoring tool. There is potential to apply these algae as deployed monitors in the manner of *Catenella nipae* in southeast Queensland waters after some research in on appropriate methodologies.

<u>Negative $\delta^{15}N$ values</u>
The negative $\delta^{15}N$ detected in this investigation are not unusual and have been detected near Townsville (Wormington *et al*. 2003) in unpolluted natural environments and In areas of native vegetation in the Northern Territory(Cook 2001).

<u>Nitrogen concentrations in mangrove foliage</u>
The foliar nitrogen concentrations were within the range reported elsewhere (Rao *et al*. 1994; Fry *et al*. 2000, Alongi *et al*. 2003; Costanzo *et al*. 2003, Wormington *et al*.

2004) and for *Avicennia marina*, were lower than the elevated concentrations from a heavily contaminated tropical estuary (Wormington *et al*. 2004).

Conclusions

We make six conclusions from this pilot study:

1. Certain mangroves can be used as sentinels of some forms of nitrogenous contamination in Port Curtis because of the pattern of distribution those species.
2. The use of mangroves is probably limited to situations of chronic aquatic contamination and contaminated groundwater or sediments.
3. Development of techniques to deploy local macro algae would provide a tool for monitoring aquatic contamination – especially acute impacts.
4. There are indications of anthropogenic nitrogen contamination in the upper Fitzroy Estuary and further investigation is warranted.
5. There were no indications of widespread chronic contamination in Port Curtis or Rodds Bay.
6. The Fitzroy Estuary and the Rodds Bay region provide contrasting reference environments for monitoring Port Curtis.

A more thorough exploration of δ^{15}N along the Fitzroy Estuary has now been completed and will be reported separately in the near future (early 2005).

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Table 1 Summary of $\delta^{15}N$ in foliage of widely distributed mangrove species in Port Curtis and adjacent regions.

Region/ Species	**Rodds Bay region**	**Keppel Bay & open waters**	**Fitzroy Estuary**	**Port Curtis**
Aegiceras corniculatum	x=2.771, n=2	x=0.508, n=2	x=12.024, n=3, sd=0.920	x=2.502, n=2
Avicennia marina	x=3.125, n=5, sd=0.933	x=4.850, n=4, sd=1.435	x=4.986, n=1	x=4.112, n=7, sd=1.041
Rhizophora stylosa	x=2.645, n=5, sd=0.708	x=4.447, n=4, sd=0.882	n/a	x=3.325, n=10, sd=1.161

Table 2 Summary of %N in foliage of widely distributed mangrove species in Port Curtis and adjacent regions.

Region/ Species	**Rodds Bay region**	**Keppel Bay & open waters**	**Fitzroy estuary**	**Port Curtis**
Aegiceras corniculatum	x=1.163, n=2	x=1.101, n=2	x=1.056, n=3, sd=0.033	x=1.3, n=2
Avicennia marina	x=2.01, n=5, sd=0.416	x=1.953, n=4, sd=0.170	x=1.94, n=1	x=1.899, n=7, sd=0.248
Rhizophora stylosa	x=1.026, n=5, sd=0.113	x=0.912, n=4, sd=0142	n/a	x=0.901, n=10, sd=0.126

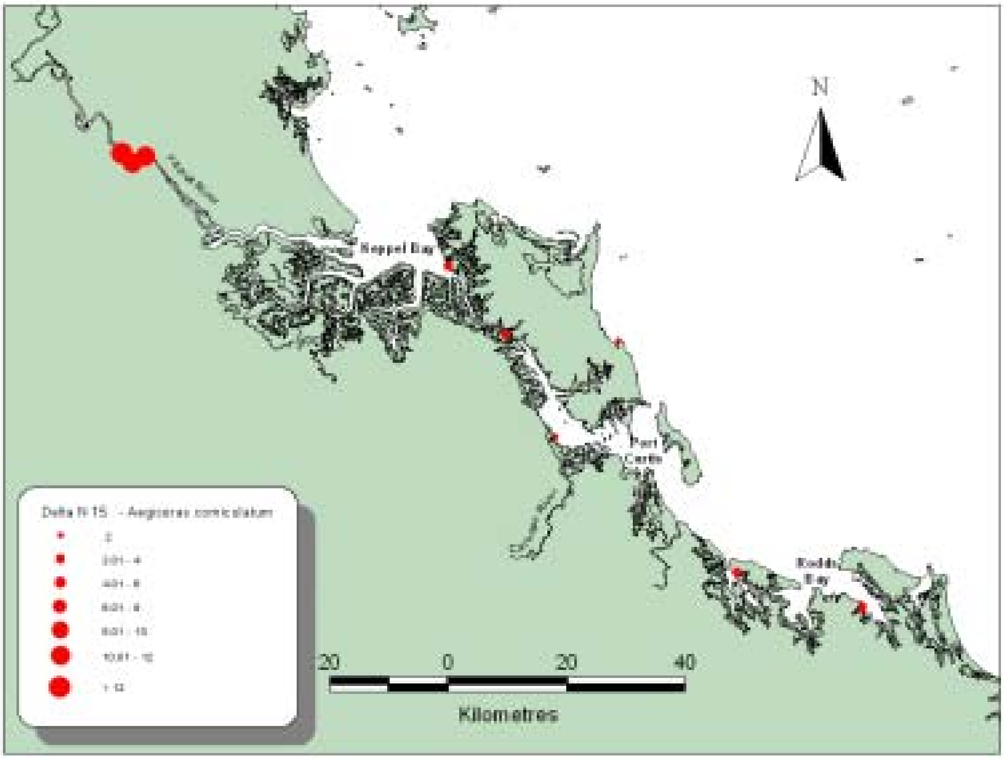

Figure 1 δ^{15}N in *Aegiceras corniculatum* foliage along the Curtis Coast and in the Fitzroy Estuary. The size of the red points reflects the magnitude of the δ^{15}N value.

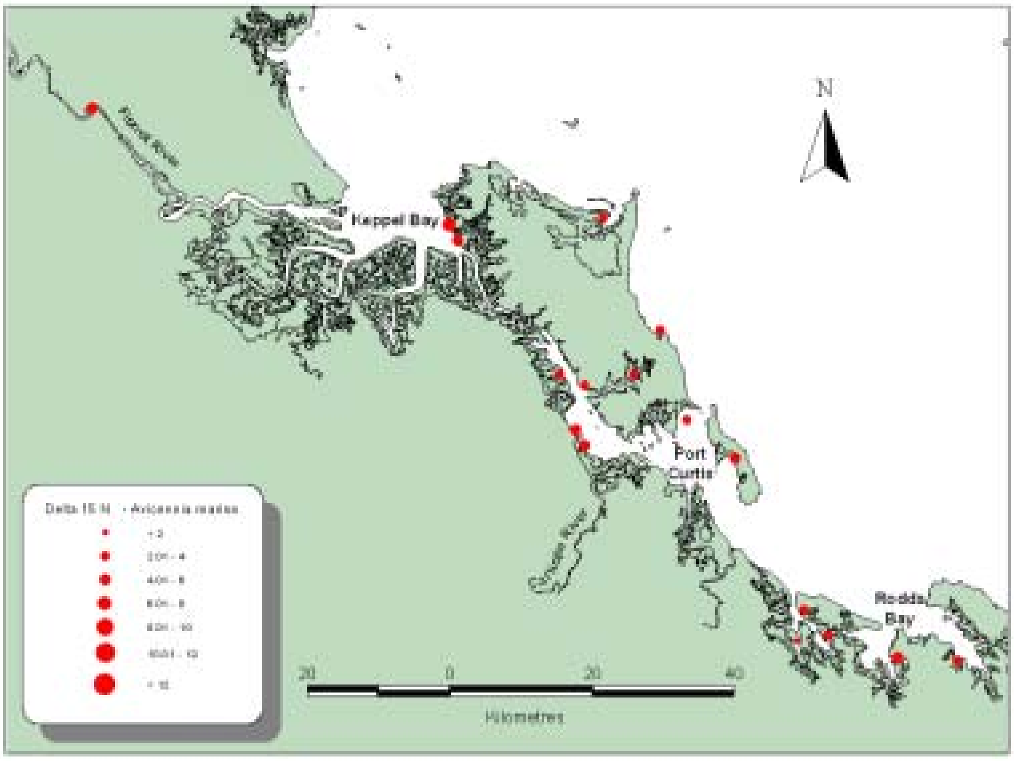

Figure 2 δ^{15}N in *Avicennia marina* foliage along the Curtis Coast and in the Fitzroy Estuary. The size of the red points reflects the magnitude of the δ^{15}N value.

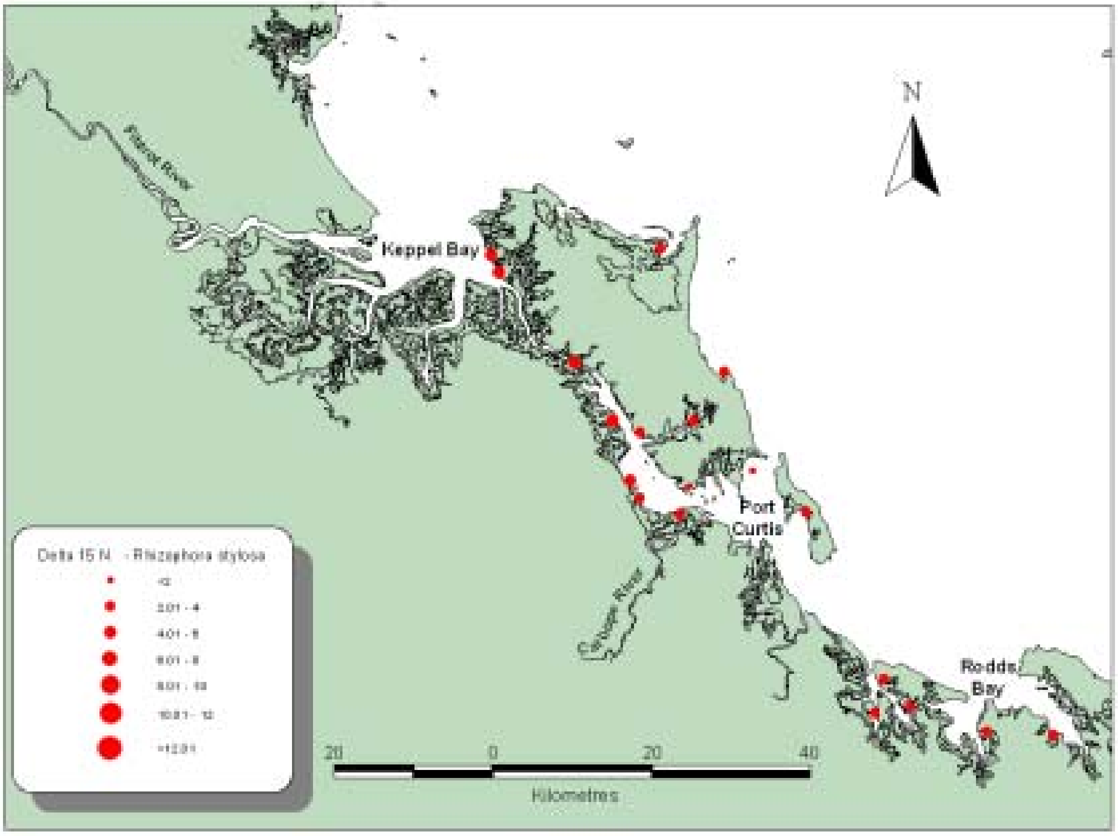

Figure 3 δ^{15}N in *Rhizophora stylosa* foliage along the Curtis Coast and in the Fitzroy Estuary. The size of the red points reflects the magnitude of the δ^{15}N value.

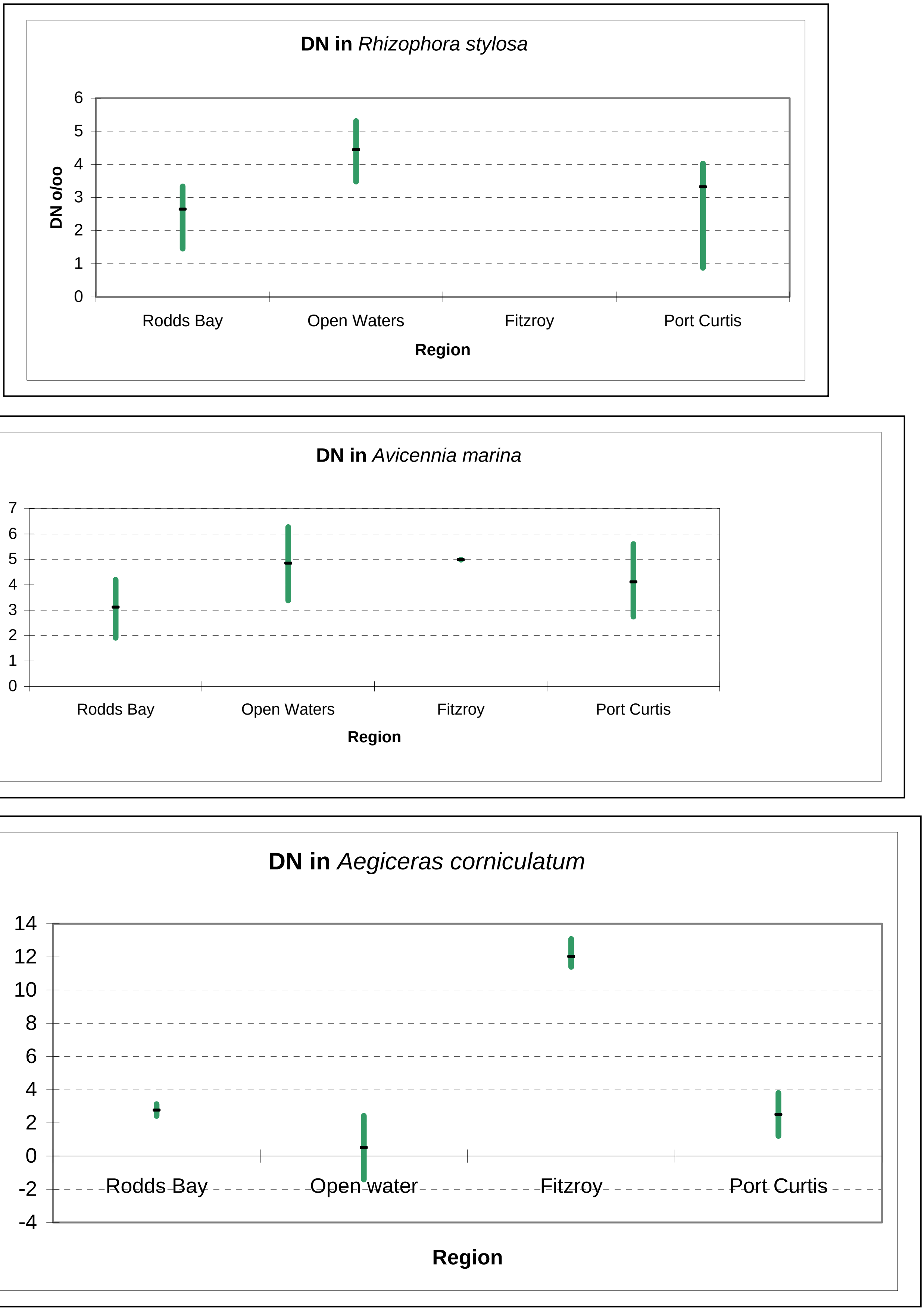

Figure 4 A comparison of the mean (dash) and range (bar) of δ^{15}N among regions. The values for Port Curtis encompass the range within the reference environments – apart from falling well below the δ^{15}N range in *Aegiceras* from the upper Fitzroy estuary where further investigation is indicated.

Appendix 1

Location of sampling sites, $\delta^{15}N$ ($^o/_{oo}$) and %N in macrophytes sampled around Port Curtis

$\delta^{15}N$

Region	Site Name	*Rhizophora stylosa*	*Avicennia marina*	*Aegiceras corniculatum*
Port Curtis	Stuart bund wall	4.774	4.200	
Port Curtis	Graham Creek Mouth	3.218	3.424	
Port Curtis	Boat Creek	4.023		3.797
Port Curtis	Curtis Island C1-C2	1.856		
Port Curtis	Inside Facing Island	3.447	5.602	
Port Curtis	Mouth of Boat Creek	3.502	5.358	1.206
Port Curtis	Mouth of Calliope River	3.875		
Port Curtis	Pelican Banks	0.877	3.518	
Port Curtis	Upper Graham Creek	3.344	2.747	
Port Curtis	Worthington Island	4.336	3.932	
Fitzroy Estuary	F2-E			13.080
Fitzroy Estuary	F3-E			11.396
Fitzroy Estuary	F4		4.986	11.595
Keppel Bay & Open Waters	Northern End of Narrows	5.070	6.272	
Keppel Bay & Open Waters	Black Head	3.480	3.388	-1.410
Keppel Bay & Open Waters	Little Creek	5.308	5.894	2.426
Keppel Bay & Open Waters	Yellow Patch	3.928	3.867	
Rodds Bay	Hummock Hill	1.456	3.930	2.414
Rodds Bay	Colosseum Inlet	2.910	1.917	
Rodds Bay	Boyne Creek North Mouth	2.672	2.839	
Rodds Bay	Seven Mile Creek	3.334	4.197	
Rodds Bay	Turkey Beach	2.854	2.742	
Rodds Bay	Turkey Beach			3.128

$\delta^{15}N$

Region	Site Name	*Ceriops tagal*	*Lumnitzera racemosa*	*Xylocarpus* sp.
Port Curtis	Boat Creek			0.079
Keppel Bay & Open Waters	Black Head	4.065	5.837	
Keppel Bay & Open Waters	Little Creek			0.330
Keppel Bay & Open Waters	Northern End of Narrows	0.891		
Keppel Bay & Open Waters	Yellow Patch	-1.167		

δ^{15}N

Region	Site Name	*Zostera capricorni*	*Sargassum* sp.	Algae	*Osbornia octodonta*
Port Curtis	Worthington Island	3.820			
Port Curtis	Black Swan	3.433			
Port Curtis	Mouth of Boat Creek				1.923
Port Curtis	Mouth of Calliope River	5.127		6.371	
Port Curtis	Pelican Banks	3.230			
Port Curtis	Richard Point		3.778		
Port Curtis	Pelican Banks	3.230			
Port Curtis	South End		3.918		
Keppel Bay & Open Waters	North of Black Head		2.844		
Keppel Bay & Open Waters	Northern End of Narrows			6.622	
Keppel Bay & Open Waters	Black Head		2.832		0.654
Keppel Bay & Open Waters	Seal Rocks - GPS158		2.733		

% N

Region	Site Name	*Rhizophora stylosa*	*Avicennia marina*	*Aegiceras corniculatum*
Port Curtis	Stuart bund wall	0.901	1.791	
Port Curtis	Pelican Banks	0.786	2.200	
Port Curtis	Worthington Island	1.078	2.212	
Port Curtis	Boat Creek	0.737		1.295
Port Curtis	Graham Creek Mouth	0.802	1.623	
Port Curtis	Curtis Island C1-C2	1.068		
Port Curtis	Inside Facing Island	0.851	1.624	
Port Curtis	Mouth of Boat Creek	0.797	1.837	1.305
Port Curtis	Mouth of Calliope River	0.955		
Port Curtis	Upper Graham Creek	1.033	2.007	
Fitzroy Estuary	F2-E			1.085
Fitzroy Estuary	F3-E			1.063
Fitzroy Estuary	F4		1.940	1.021
Keppel Bay & Open Waters	Yellow Patch	1.026	1.805	
Keppel Bay & Open Waters	Little Creek	0.711	1.892	1.204
Keppel Bay & Open Waters	Black Head	0.911	2.678	0.872
Keppel Bay & Open Waters	Northern End of Narrows	0.998	1.918	
Rodds Bay	Boyne Creek North Mouth	0.987	1.840	
Rodds Bay	Colosseum Inlet	0.968	2.198	
Rodds Bay	Hummock Hill	0.946	1.652	1.112
Rodds Bay	Seven Mile Creek	1.004	1.741	
Rodds Bay	Turkey Beach	1.225	2.139	
Rodds Bay	Turkey Beach			1.214

% N

Region	Site Name	*Ceriops tagal*	*Lumnitzera racemosa*	*Xylocarpus* sp.
Port Curtis	Boat Creek			1.843
Keppel Bay & Open Waters	Black Head	0.738	0.833	
Keppel Bay & Open Waters	Little Creek			1.775
Keppel Bay & Open Waters	Northern End of Narrows	0.842		
Keppel Bay & Open Waters	Yellow Patch	0.876		

% N

Region	Site Name	*Zostera capricorni*	*Sargassum* sp.	Algae	*Osbornia octodonta*
Port Curtis	Pelican Banks	1.829			
Port Curtis	Black Swan	0.746			
Port Curtis	Boat Creek Mouth				1.865
Port Curtis	Calliope River Mouth	2.151		0.761	
Port Curtis	Richard Point		0.938		
Port Curtis	South End		0.795		
Keppel Bay & Open Waters	Northern End of Narrows			0.573	
Keppel Bay & Open Waters	Black Head		0.904		1.376
Keppel Bay & Open Waters	North of Black Head		0.737		
Keppel Bay & Open Waters	Seal Rocks - GPS158		0.784		