Temporal and spatial morphological variability of the seagrasses *Halophila ovalis* and *Halodule uninervis* throughout the Great Barrier Reef region

Preliminary analysis

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Executive Summary

The objective of this study was to explore seagrass meadow dynamics in relation to proximity of catchments, nutrient quantity and quality in order to enhance the current understanding of anthropogenic impacts on the Great Barrier Reef World Heritage Area.

Previous studies suggest that seagrass plant morphometrics can indicate the state of a seagrass meadow and the surrounding environment. Seagrass morphometric data (rhizome internode length and leaf width) was collected for a range of species from study sites of the Reef Rescue Marine Monitoring Program seasonally from the late dry season (late-Dry) of 2006 to late-Dry 2008. The most comprehensive datasets were from the Wet and Dry Tropics, and although highly variable, some relationships at spatial (> 1 km) and temporal scales (> 1 year) were apparent. *Halophila ovalis* internode length was found to increase during the Late-Monsoon each year at reef habitats in the Dry Tropics. However, internode lengths for both *H. ovalis* and *Halodule uninervis* increased during the late-Dry at both coastal and reef habitats in the Wet Tropics. *H. uninervis* leaf width was found to have a strong association with habitat type, with narrow leaves found at coastal locations and the wide leaves at offshore locations. A significant amount of variation was also found for both internode length and leaf width for *H. ovalis* among all locations.

Preliminary findings indicate that two of the most abundant seagrass species throughout the Great Barrier Reef undertake morphological acclimation to different environmental conditions, which loosely follows trends that have been observed in other regions of the globe. Variation was observed both over short term seasonal periods, and annually over a period of up to two years. It is likely that many of these temporal changes are short-term responses to variables such as light and nutrient availability. Further investigation of this data set as a multivariate analysis relative to a full suite of water quality indicators is required to fully understand the patterns of morphological change in seagrasses of the Great Barrier Reef.

Introduction

The biological and economic importance of seagrasses as structural components of coastal ecosystems is well recognised (Watson *et al.*, 1993; Duarte and Cebrian, 1996; Costanza *et al.*, 1997). Unfortunately seagrass meadows are globally under anthropogenic stress within the coastal zone, often as a result of increased nutrient and sediment loads (Udy *et al.*, 1999; Orth *et al.*, 2006). These anthropogenic impacts also threaten other significant marine ecosystems, such as coastal and offshore coral communities, and their impacts can often be identified hundreds of kilometers away from the source (Risk and Erdmann, 2000). In the case of Queensland, excess nutrients, sediments and herbicides from catchment run-off are detrimentally impacting upon the Great Barrier Reef (Humphrey *et al.*, 2008; Wooldridge, 2009).

A key component of the Australian Government's Reef Rescue programme, which aims to improve water quality in the catchments of the Great Barrier Reef, implemented by the Great Barrier Reef Marine Park Authority, is the implementation of a long-term water quality and ecosystem monitoring program. Successful water quality improvements are often difficult to determine without the development of novel water quality indicators that change over short time scales and act as early warning signals of environmental change.

Two of the critical environmental parameters for seagrass, nutrient and light levels, are highly influenced in coastal areas by terrestrial human activity, and are often the cause of significant loss of seagrass (Udy *et al.*, 1999; Cabaço *et al*., 2008).

Due to the high plasticity of growth and physiology of seagrass communities, they can accommodate a wide range of environmental conditions (Duarte, 1991; Hemminga and Duarte, 2000). Their fast growth can allow them to recover quickly from periods of short-term stress, or acclimate to changed conditions. These attributes are in contrast to those of coral communities that acclimate slowly to environmental change and have very long recovery times, making them highly susceptible to stress. Seagrasses have therefore been proposed as a good barometer for the health of estuarine and coastal environments (Dennison *et al.*, 1993).

The ability of seagrasses to modify their form in response to environmental conditions suggests that seagrass plant morphometrics could indicate the state of the seagrass meadow and the surrounding environment and act as a sentinel prior to change occurring on highly sensitive reef environments. For example, in a Portuguese seagrass meadow, the length of the internode of a species of *Zostera* was found to be a useful indicator of seagrass response to a human influenced environmental gradient (Cabaco *et al.*, 2009). Such responses can also be observed over relatively short time scales (Japar Sidik *et al.*, 1999; Leoni *et al*., 2008), making morphological characteristics potentially powerful indicators of the water quality of a coastal area.

The aim of this investigation was to explore the possibilities of utilising such morphometric measurements of seagrass as indicators of marine environmental change in the Great Barrier Reef. This was conducted by providing a preliminary assessment of the major temporal and spatial trends in the morphometrics of *Halophila ovalis* and *Halodule uninervis* throughout the Great Barrier Reef region.

Methodology

Seagrass morphometric data was collected as part of the Great Barrier Reef Water Quality Protection Plan (Reef Rescue) Marine Monitoring Program. Surveys were focused upon *Halophila ovalis* and *Halodule uninervis* due to their extensive distribution throughout the Great Barrier Reef region.

Sampling was undertaken twice annually between 2006 and 2008 (commencing October 2006), in the late dry season (September and October), and in the late monsoon season (April and May) at fifteen locations (thirty sites) throughout the Great Barrier Reef coastline. Sites spanned the eastern Queensland coast from Cooktown to Hervey Bay. Further details of the seagrass Reef Rescue Marine Monitoring Program sites, sampling procedures and protocols can be found in the latest summary report (McKenzie *et al.*, 2008).

Fifteen random sediment core samples were collected in an area adjacent to each of the Seagrass-Watch monitoring sites. Cores were taken with a 100mm diameter PVC pipe section, pushed 100mm deep into the sediment. Each of the cores were sieved on site through a 2mm mesh sieve for the removal of silt and sediment, and then placed into individual sealed bags for laboratory processing. Morphometric data could be only collected when *Halophila ovalis* was present in the sample.

The two key morphometric parameters that were collected were internode length and leaf width. Measurements were taken using a ruler to measure distances between nodes on the rhizome to the nearest millimetre. Up to ten random measurements were taken per species per sample. Similarly for leaf width, measurements were taken using a ruler (to the nearest millimetre) and up to ten measurements were taken per species per sample. Samples were retained according to Reef Rescue QA/QC protocols (McKenzie *et al.*, 2008).

The most comprehensive datasets available from the three years of sampling were from the Wet and Dry Tropics, therefore analysis within the present report focused mostly upon these two areas.

Preliminary Results

Inter- and intra-annual variability of leaf width and internode length (Dry Tropics)

Seasonal and inter-annual variability of the internode length of *Halophila ovalis* was high within the Burdekin Dry Tropics region (Figure 1). Within the offshore reef environment of Magnetic Island, *H. ovalis* internode length was generally longer within the late monsoon season relative to the late dry season, but had shown very little change between years. Within the coastal sites, in contrast to the offshore sites, no overall seasonal trend was observed, however inter-annual variability was high, with internode length in October 2006 being much smaller than in 2007 and 2008. Leaf width of *H. ovalis* showed no clear seasonal patterns within either of the environments in the Burdekin Dry Tropics region, but inter-annual variability was high, with leaf width in the late dry season of 2006 and the late monsoon 2008 being much smaller than at other times.

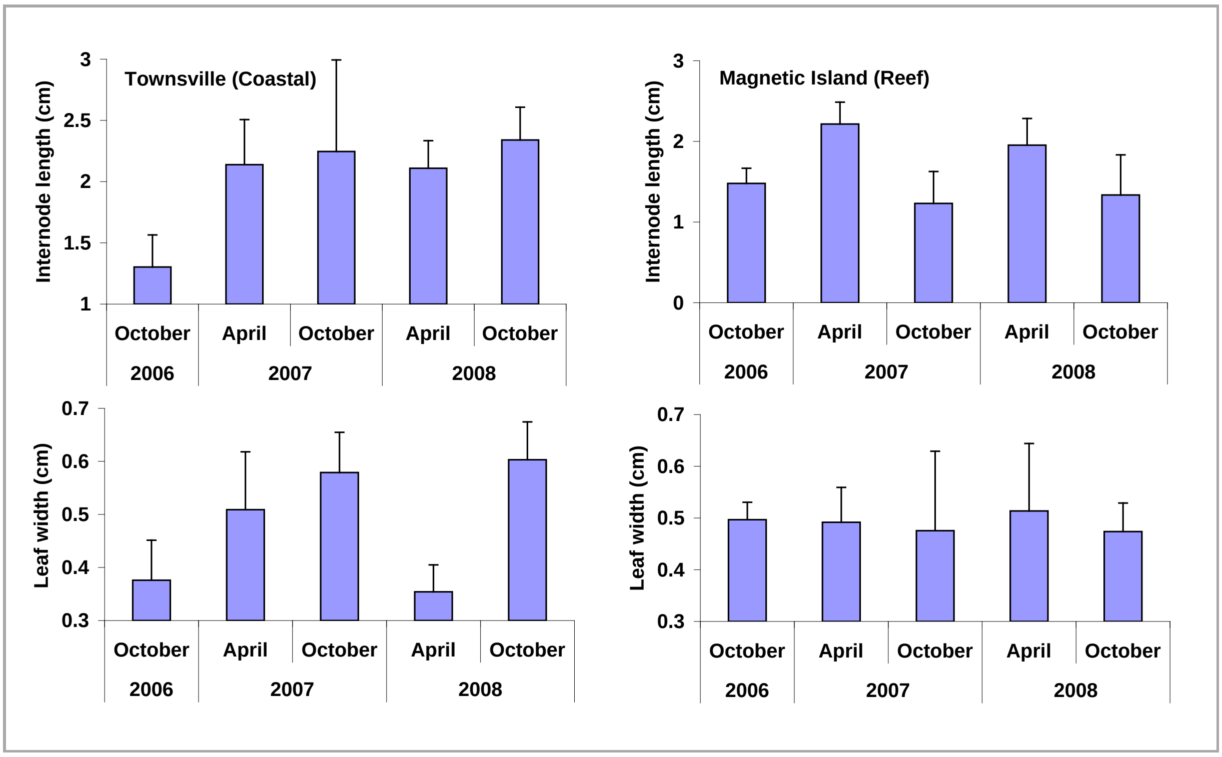

Figure 1: Mean (±95%CI) seasonal *H.ovalis* internode length and leaf width for Townsville reef sites (Magnetic Island) and Townsville coastal sites (Bushland Beach and Shelley Beach).

Seasonal and environmental variability (Wet and Dry Tropics regions)

Examination of all data across years indicates that within the Wet Tropics the internode lengths of both *Halodule uninervis* and *Halophila ovalis* were much larger during the late dry season relative to the late monsoon season (Figure 2).

Within the Wet Tropics there was little variability between the internode lengths of *H. ovalis* between coastal and offshore environments; however the internode lengths of *H. uninervis* were larger but more variable in offshore environments relative to coastal environments.

In the Burdekin Dry Tropics region, an opposite seasonal trend was encountered to the Wet Tropics, as overall across-years analysis of internode lengths revealed that both *H. ovalis* and *H. uninervis* had longer internode lengths during the late wet season relative to the late dry season. As with the Dry Tropics the internode lengths of *H. uninervis* changed with environment, but this pattern was opposite to the Wet Tropics with *H. uninervis* internode lengths less within offshore environments.

The leaves of *H. uninervis* were much wider within offshore environments relative to coastal environments within both the Wet and Dry Tropics regions (Figure 3).

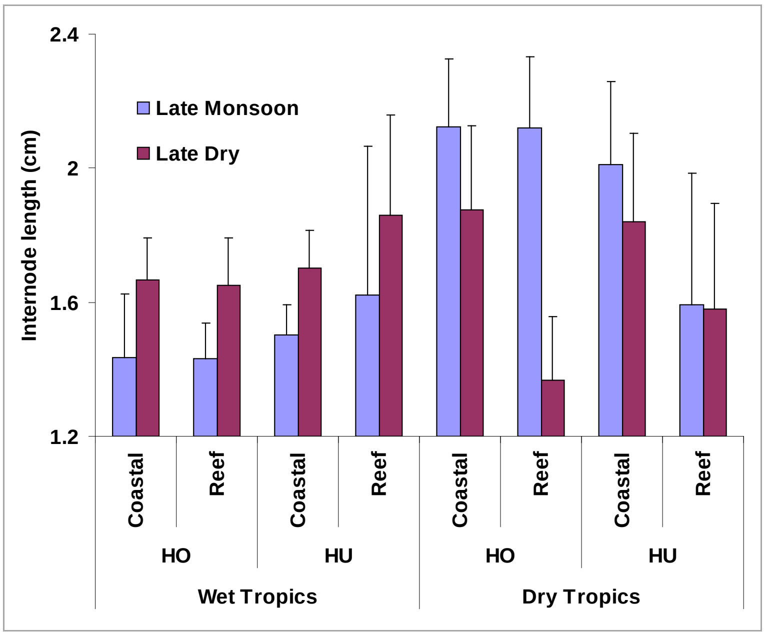

Figure 2: Mean (±95%CI) seasonal internode length of *Halophila ovalis* (HO) and *Halodule univervis* (HU) in the Wet and Dry Tropics, at both coastal and offshore sites.

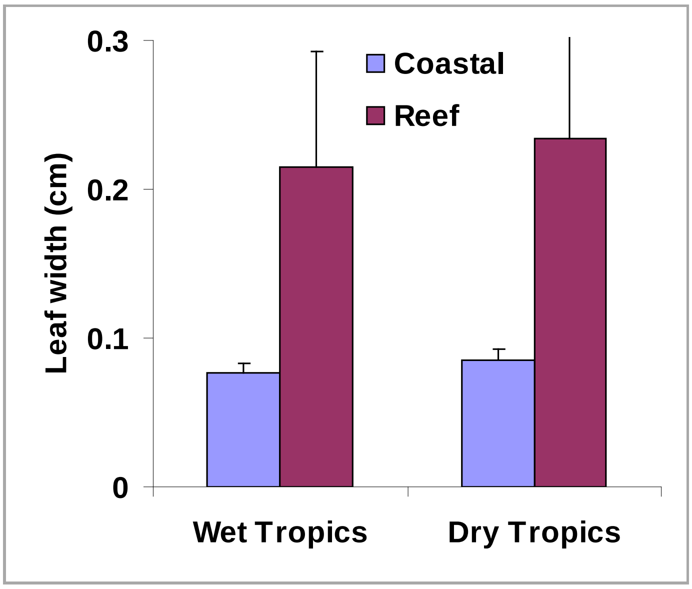

Figure 3: Mean (±95%CI) leaf width of *H.uninervis* at coastal and offshore reef sites in the Wet and Dry Tropics.

Spatial variability (Great Barrier Reef coastal region)

There was high variability of both the leaf widths and the internode lengths of *Halophila ovalis* throughout the Great Barrier Reef region (Figure 4). The largest leaf width was present in Rodds Bay and Urangan; the smallest leaf width in Shoalwater and Pioneer Bays. The internode length of *H. ovalis* was highest in Urangan, Townsville, and Rodds Bay, and as with leaf width, internode length was smallest in Shoalwater and Pioneer Bay. No clear patterns exist between tissue nutrient status and the morphologies of *H. ovalis*, however the locations where large leaf widths have been observed are generally N-limited rather than replete or P-limited (McKenzie *et al.*, 2008).

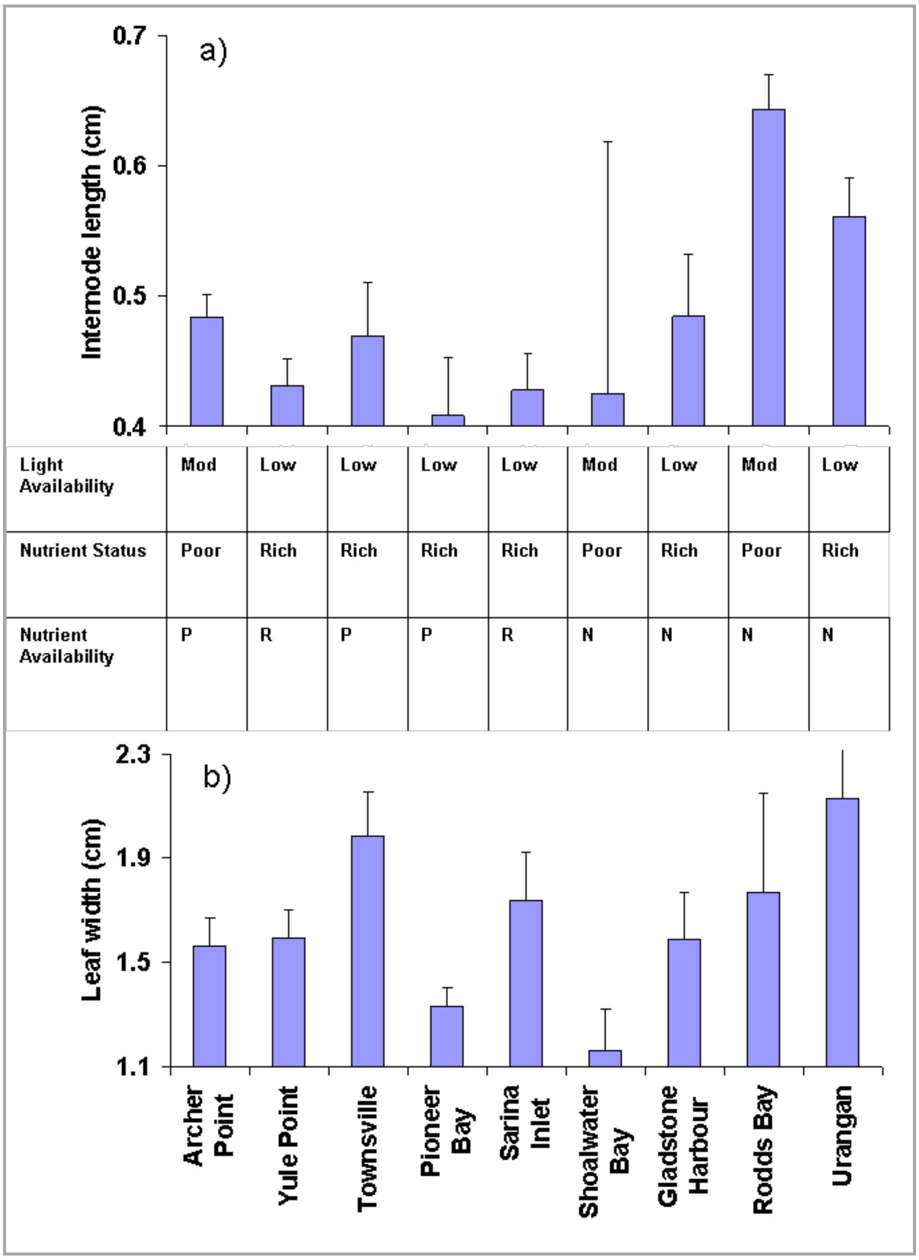

Figure 4: Leaf width and internode length of *Halophila ovalis* at nine coastal locations throughout the Great Barrier Reef, and their tissue nutrient status superimposed. Tissue nutrient ratios of C:N:P are used as indicators of light availability (high, moderate and low), nutrient status (poor or rich), and nutrient availability (N-limited, P-limited or replete). Nutrient data taken from McKenzie *et al.*, (2008).

Discussion

The present study documented the variability of the leaf width and internode length of *Halophila ovalis* and *Halodule uninervis* at sites throughout the Great Barrier Reef, with analysis focusing mostly upon the Wet and Dry Tropics regions within the northern Queensland region. Both species were observed to incur spatial and temporal changes in both of these two morphological parameters. These changes presumably reflect differences in the local environment through space and time (Martinez-Crego *et al.*, 2008).

The internode length of *H. ovalis* and *H. uninervis* in North Queensland changed with season, but the direction of this change was dependent upon location, such that internodes were generally longer during the late Monsoon season than in the late dry season within the Dry Tropics, and longer in the late dry season within the Wet Tropics. Internode length of both of these species was also generally longer in the Dry Tropics than in the Wet Tropics.

Internode length has been found to vary as a direct result of a number of factors including: increased disturbance (Peralta *et al.*, 2005), exposure to reduced salinity (Benjamin *et al.*, 1999), and intertidal exposure (Cabaco *et al.*, 2009). Some studies have suggested that a longer internode length may reflect the presence of a more mature meadow, where environmental conditions are more stable and water quality is higher (Miller *et al.*, 2005, Leoni *et al.*, 2008, Cabaco *et al.*, 2009). Sites in the Dry Tropics were either stable or in decline, while sites in the Wet Tropics were either stable or increasing (area and percent cover). Therefore the shorter internode lengths in the Wet Tropics may reflect meadows that are expanding. The presence of shorter internode lengths could be due to the requirement of a growing plant to rapidly produce more energy. Shorter internode length would mean more shoots and hence greater photosynthetic capabilities for the metabolic needs of horizontal growth.

Although variable internode lengths may reflect the differences in the growth dynamics of different locations, it is also likely that any one of numerous environmental factors could be driving morphological change. There exists considerable variability between the environmental characteristics of the seagrass sites in Townsville and surrounding Magnetic Island (Dry Tropics) relative to those in Yule Point and Green Island (Wet Tropics). Therefore different variables are likely to drive the dynamics of these meadows differently. For example, subtle differences in intertidal height result in large inter-site differences in the amount of exposure that plants and may in turn impact upon their internode lengths (Cabaco *et al.*, 2009).

The seagrass meadows that are monitored within the Dry Tropics generally have a lower light environment than their comparative Wet Tropics sites (pers. observ.). These Townsville sites are probably more highly influenced by freshwater inputs due to the presence of three major river mouths close to Townsville (including the Burdekin River), and the close location of Magnetic Island to the coast relative to Green Island in the Wet Tropics.

In the present study, the mean leaf width of *Halodule uninervis* was found to vary highly with its environmental position (e.g. coastal or offshore reef), with larger widths observed in offshore reef rather than coastal locations of both the Wet and Dry Tropics. Numerous investigations have considered the morphology of *H. uninervis* (den Hartog, 1970; Johnstone, 1978; McMillan, 1983; Japar Sidik *et al.*, 1999) (see Table 1), with researchers observing the presence of two major forms (a wide and a narrow form), and classifications commonly based on this for monitoring. For example, these forms are utilised for seagrass assessments throughout Queensland (Coles *et al.*, 2007). Our research loosely follows previous investigations of leaf morphology (see Table 1), as the offshore sites were sandy higher light reef-top environments (McKenzie *et al.*, 2008), where the large leaf width form was more prominent. Investigations of the photosynthetic capacity of *Zostera noltii* have found inverse relationships between leaf width and photosynthetic capacity (Peralta *et al.*, 2005). Such a growth strategy may be beneficial to a coastal plant under low light stress where energy maybe scarce, as less biomass is required for the same photosynthetic output of a narrow leaf relative to a wide leaf (Voronin *et al.*, 2003; Peralta *et al.*, 2005).

Table 1: Summary of observations on the leaf morphology of the *Halodule uninervis* complex.

Author/ Year	Narrow Type	Wide Type
Den Hartog, 1970 (Indo-Pacific)	<1.0mm Prefers exposed sandy shores exhibiting unsuitable salinity.	1.0-3.5mm Prefers sheltered muddy habitats. Suggests this is a response to nutrient/salinity/light flux.
Johnstone, 1978 (Papua New Guinea)	High intertidal zone, often pure stands, well above upper limit for other seagrasses (ability to resist desiccation and salinity flux), prefers compact sand.	0.5m below mean low water to 5m subtidal, prefers coarse coral substrate, found in community with other species.
McMillan, 1983 (Shark Bay, WA) *Suggests depth profile for complex*	Prefers firm silty substrates, exposed during spring low tide. Size range <1.8mm. Found with small leaved *H. ovalis*.	Prefers muddy areas, mangrove fringes. Lower intertidal zone, not exposed during spring low tides. Size range >1.8mm.
Japar Sidik, 1999 (Malaysia)	Size range 0.5-1.5mm, lower intertidal to subtidal, sandy/muddy and sandy/coral rubble substrate.	Size range 1.5-4.0mm, subtidal zone, sandy/muddy to muddy areas and mangrove fringes.

At a broad spatial scale within only coastal sites, there was also high variability. The leaf width of *Halophila ovalis* varied across nine locations (e.g. where sufficient data existed) with the mean leaf being approximately one-third wider in Rodds Bay relative to Pioneer Bay, and the widest leaves recorded at Rodds Bay and Urangan. Similar variation in internode length at broad spatial scales was also recorded for *Halophila ovalis*. This variation is again likely to reflect the variable environmental conditions that exist throughout the coast of the Great Barrier Reef; however comparison to existing data sets of environmental indicators (tissue nutrient ratios) does not produce any clear trends.

Although in the present study inter-site variability at one moment in time was reasonably low, sampling was spatially limited. Numerous studies have found that the variability of leaf width and internode length for a number of species has been high over only very small spatial scales (Nakaoka and Aioi, 1999, Miller *et al.* 2005, Cabaco *et al.*, 2009). To determine greater long-term patterns of change it might be appropriate to quantify local small scale variability in seagrass morphology as well as conducting larger scale temporal and spatial assessments.

In conclusion we find that two of the most abundant seagrass species throughout the Great Barrier Reef undertake morphological acclimation to different environmental conditions, this variability loosely follows trends that have been observed in other regions of the globe. Variation was observed both over short term seasonal periods, and annually over a period of up to two years. It is likely that many of these temporal changes are short-term responses to variables such as light and nutrient availability. Further investigation of this data set as a multivariate analysis relative to a full suite of water quality indicators is required to fully understand the patterns of morphological change in seagrasses of the Great Barrier Reef.

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