Tropical Water Quality Hub

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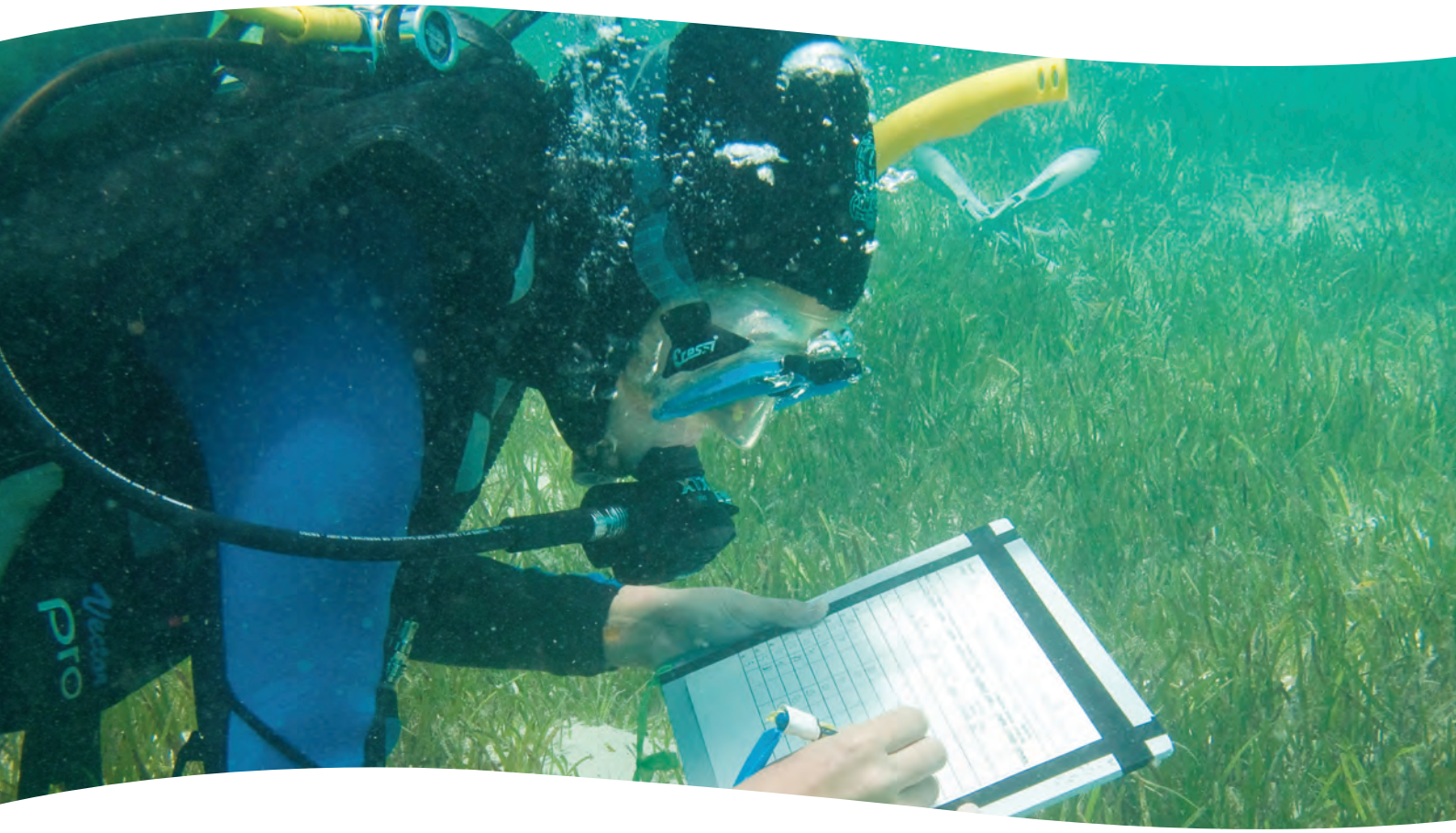
Final Report

Developing and refining biological indicators for condition assessments in an integrated monitoring program

Catherine Collier, Lucas Langlois, Rahel Zemoi, Katherine Martin and Len McKenzie



JAMES COOK UNIVERSITY AUSTRALIA

Developing and refining biological indicators for condition assessments in an integrated monitoring program

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Cover photographs: Catherine Collier

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ACRONYMS

AIC..... Akaike Information Criterion
DOE..... Department of the Environment
GAM ..... Generalised Additive Model
GBR..... Great Barrier Reef
GLM..... General Linear Model
MMP ..... Marine Monitoring Program
NESP..... National Environmental Science Programme
NIR ..... Near Infrared Spectroscopy
NRM ..... Natural Resource Management
QA/QC ..... Quality Assurance Quality Control
RIMREP..... Reef Integrated Monitoring and Reporting Program
RRRC ..... Reef and Rainforest Research Centre Limited
SAFS ..... School of Agriculture and Food Science
StdDev..... Standard deviation
TWQ ..... Tropical Water Quality
TNSC..... Total non-structrural carbohydrates

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EXECUTIVE SUMMARY

Indicators representative of ecosystem condition are required for the long-term monitoring of the Great Barrier Reef (GBR) in a Reef Integrated Monitoring and Reporting Program (RIMREP), which tracks progress towards Reef 2050 Plan targets and objectives. Seagrass meadows are highly sensitive to climatic conditions and environmental pressures such as water quality, as seen through recent (past 10 years) changes in abundance in the GBR (McKenzie, et al., 2016). Due to these impacts, GBR seagrass meadows underwent a period of decline from 2009 to 2011. Widespread loss of seagrass occurred, but in 2015 many meadows had started recovering.

The storage reserves within seagrass rhizomes were tested for suitability as a complimentary indicator in the MMP/RIMREP because previous studies had suggested that they are good indicators. We set out to test the relationships between total non-structural carbohydrates (TNSC) and seagrass condition (i.e. *trend* in abundance, either declining pre 2011 or recovering post 2011), seagrass abundance, water temperature and daily light in a *temporal analysis* using linear models. Samples were collected quarterly from 2008 to 2015 from four locations (8 sites) for three species (917 samples in total) in the Wet Tropics and Burdekin regions. TNSC was significantly ($p<0.001$) lower pre 2011 during the period of decline (181 and 192 mg gDW^{-1} for intertidal sites pooled and subtidal sites pooled, respectively) than post 2011 during recovery (277 and 289 mg gDW^{-1}) for *H. uninervis*. A similar trend was observed for *T. hemprichii,* which occurred at intertidal sites only (168 mg gDW^{-1} in decline and 208 mg gDW^{-1} in recovery), but not for *C. serrulata* which had the fewest available data points. The differences were even greater when investigating individual sites. TNSC were also correlated ($p<0.001$) to seagrass abundance during both the decline and recovery phases. TNSC was positively correlated to water temperature, though the period being assessed was relatively mild in terms of temperature extremes. Therefore, light was the main pressure assessed in this project. A direct effect of light limitation (daily light, average of 30 days prior to TNSC collection) on TNSC was not observed, in fact there was a slight negative effect of light in some analyses. This was contrary to our hypothesis, as low light, at least in part, drove declines in seagrass abundance from 2009 – 2011. In an additional *spatial analysis*, differences in TNSC among regions and habitat types were assessed from 39 sites collected in late 2014 across the GBR. This *spatial analysis* was carried out to explore representativeness of the sites used in the *temporal analysis*. There was little difference in TNSC among habitats; however, TNSC varied among NRMs and were lowest in the Mackay Whitsunday and Fitzroy NRMs.

This exploration of storage reserves, undertaken at a time of dynamic meadow changes, has yielded exciting results on their variation with meadow condition and abundance. However, we did not provide conclusive evidence to support the inclusion of TNSC as an indicator in monitoring programs such as the MMP at this stage, because the link to the main environmental pressure tested – light – was not demonstrated by this analysis. Irrespective of this, TNSC was an indicator of cumulative stress (being correlated to abundance and condition), but the specific pressure(s) could not be identified. This provides justification for further inquiry into the effect of other pressures (e.g. nutrients and flood plume exposure), other biological processes (e.g. reproduction and meadow expansion) and to obtain further data on other species.

We also tested the relationship between %cover and biomass, with the aim of developing biomass calibration formulae. Above-ground biomass and %cover was measured in seven mono-specific meadows for four species and four habitat types. Above ground biomass was highly correlated ($p<0.001$) to % cover, and the correlation was further improved (lower AIC) by factoring canopy height into the calibration. Even after canopy height was included in the calibration, canopy height strongly affected the calibration values and highlighted the importance of habitat/morphology-specific calibration formulae. Further work is required to capture all species and habitat/morphology combinations that are routinely monitored. With further work, these calibration values will enable integration among seagrass monitoring programs including Queensland Ports Seagrass Monitoring Program and GBR historical baseline data.

1. INTRODUCTION

The Reef Integrated Monitoring and Reporting Program (RIMREP) is strategically linked to the Reef 2050 Plan (Great Barrier Reef Marine Park Authority and Queensland Government, 2015), which is the overarching framework for protecting and managing the Great Barrier Reef (GBR) from 2015 to 2050. One of the objectives of the RIMREP is to "enable the early detection of trends and changes in the Reef's environment, inform the assessment of key threats and future risks and drive adaptive management". Thus, indicators are required for the identification of cumulative pressures on the GBR, and for determining current condition and future desired condition (Great Barrier Reef Marine Park Authority, 2014). Monitoring protocols already in use in the GBR inshore Marine Monitoring Pogram (MMP) (McKenzie, et al., 2016) will form the basis of the ongoing development of the RIMREP.

For implementation into the RIMREP, indicators should fit within the DPSIR (driver, pressure, state, impact, and response) framework (Australia. Dept. of Sustainability, 2011) and therefore show quantitative links to environmental pressures. Seagrass meadows are highly sensitive to environmental change, and are referred to as coastal sentinels because of their sensitivity to multiple pressures including inshore water quality (Orth, et al., 2006). Recent historical changes in seagrass abundance within the GBR (2005 – 2015) have demonstrated their vulnerability to environmental conditions. Widespread seagrass loss was driven by multi-annual climatic conditions, which brought widespread rainfall, extremely high run-off and reductions in water quality from 2009 – 2011. Then at a time of depressed seagrass resilience (sensu. Unsworth, et al., 2015), TC Yasi brought physically destructive wind and tidal surge to areas in the Wet Tropics and Burdekin, followed by extremely high terrestrial runoff in February 2011. The cumulative impact of this sequence of events led to unprecedented and widespread decline in seagrass abundance and extent in 2011 (Figure 1). While there were many pressures acting on seagrass meadows, declining water quality was a major cause of loss, with quantitative links between abundance and water quality and/or light conditions made in some locations (Collier, et al., 2012; Petus, et al., 2014). Following on from this was a loss of ecosystem function, as turtle and dugong mortality also reached unprecedented levels (Meager and Limpus, 2012).

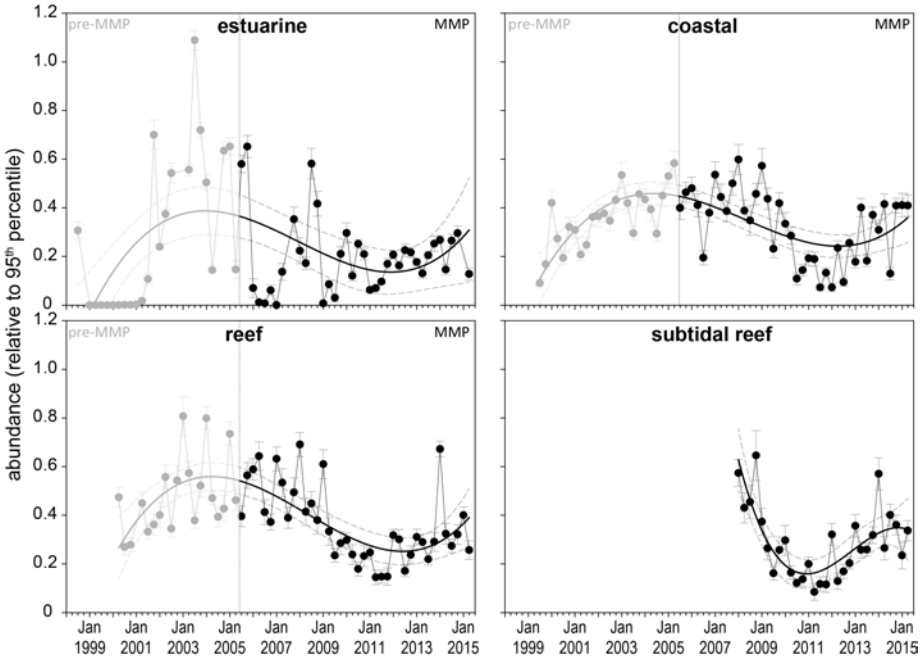

Figure 1: GBR-wide trends in seagrass abundance from 1999 to 2015 (From McKenzie et al, 2016)

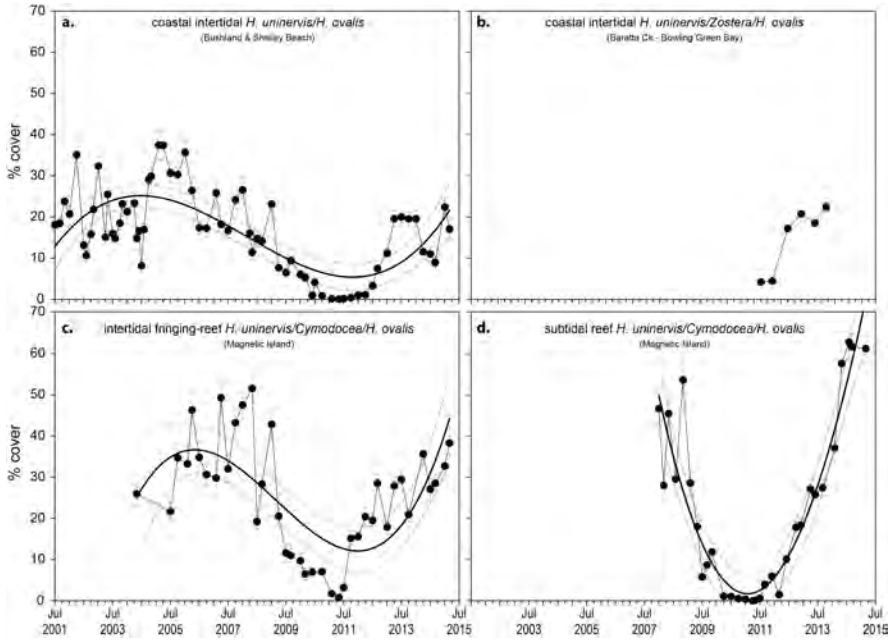

Figure 2: Seagrass abundance at Burdekin NRM region sites from 2001 to 2015, highlighting trends in abundance at Magnetic Island intertidal (c), and subtidal (d), which has undergone loss and recovery since carbohydrate collection, began in 2008 (From McKenzie et al, 2016)

These events highlighted: 1. that healthy seagrass is vital to the ecosystem services of the GBR; 2. seagrasses are highly sensitive to environmental conditions, and; 3. the need to continue to improve monitoring indicators to detect changes in condition (because of 1 and 2). Indicators are aspects of the seagrass that have units of measure and describe seagrass condition, and there are many morphological and physiological traits that can be measured as indicators of seagrass condition (McMahon, et al., 2013). A number of indicators are used in the condition assessment of inshore seagrass meadows in the MMP. Among these, three

indicators are used for the annual Reef Report Card (Australian and Queensland Governments, 2015), because of the empirical links made between these indicators and seagrass condition and resilience (Unsworth, et al., 2015). These are seagrass abundance (measured as percent cover at fixed sites), seagrass reproductive health, and leaf tissue nutrients. However, there is an ongoing need to assess new indicators and continue to refine existing indicators based on evolving monitoring objectives. Thus, refinement of biological indicators for condition assessment is an immediate priority as further highlighted in a recent review of the GBR Marine Monitoring Program (Kuhnert, et al., 2014).

Seagrasses store carbohydrates (sugars and starch) predominantly within their rhizomes and the total reserves stored varies with environmental conditions, including seasonal changes in light and temperature (Alcoverro, et al., 2001; Burke, et al., 1996). Carbohydrates are also used as storage reserves for when photosynthetic rate is insufficient to meet demands from growth and respiration (Touchette and Burkholder, 2000; Unsworth, et al., 2015). For example, when light is reduced to levels below light requirements, storage reserves can be used to subsidise carbon requirements and therefore, they decline under unfavourable conditions (Collier, et al., 2009; Longstaff, et al., 1999). Therefore, storage reserves are rated as robust indicators of environmental stress (McMahon, et al., 2013; Roca, et al., 2016), but they do not respond to environmental stress in all studies (e.g. Soissons, et al., 2016). We explored carbohydrate content as a complimentary indicator of seagrass condition and resilience.

Seagrass abundance is collected in the MMP as percent cover at fixed sites following the rigid QA/QC protocols outlined in McKenzie et al (2014a). Data are reported as percent cover, but in other data sets, including north Queensland Ports Seagrass Monitoring Program, data are presented as above ground biomass estimates (transformed from visual estimates of biomass). Furthermore, historical baseline seagrass abundance prior to the 1990's was reported as % cover, while most mapping surveys since were reported as visually estimated biomass (e.g. Coles, et al., 2009; Coles, et al., 2002; Lee Long, et al., 1996). To enable integration among data sets, percent cover needs to be converted to biomass. This will provide a means to quantitatively assess temporal and spatial trends in seagrass abundance using multiple data sets.

The aims of this National Environmental Science Programme (NESP) Tropical Water Quality Project 3.4 were to:

1. test a new indicator (storage reserves) for potential use in the MMP/RIMREP; specifically, to test the relationship between storage reserves and abundance, and storage reserves and light/temperature as key environmental pressures, and
2. refine existing indicators; specifically develop calibration formulae to convert between percent cover and biomass to facilitate integration of datasets between monitoring programs.

This 8-month project was undertaken from July 2015 to February 2016 and was dependant on a large in-kind contribution from historical collections of samples for carbohydrate analysis and new sample collection carried out during routine monitoring.

2. STORAGE RESERVES

2.1. Storage reserves: Methods

2.1.1. Collection and analysis

This study focussed on *temporal analysis* (quarterly from 2008 to 2014) of storage reserves from 8 sites (4 locations) in order to test the effects of: changing condition and abundance, water temperature, seasonality and light. An additional unplanned *spatial analysis* of storage reserves from 39 sites in late 2014 was undertaken to explore spatial variability and representativeness of sites used in the temporal analysis.

Temporal analysis

Seagrass rhizomes were collected for storage reserves (total non-structural carbohydrates, TNSC of the rhizome) each quarter from 2008 to 2015 for the temporal analysis. Samples were available for intertidal and shallow subtidal sites at Low Isles, Green Island and Dunk Island in the Wet Tropics NRM, and Magnetic Island in the Burdekin NRM. Sampling was focussed on the dominant species *Halodule uninervis* (present at all sites), *Cymodocea serrulata* (subtidal only) and *Thalassia hemprichii* (intertidal only). It did not include *Halophila ovalis* despite it's occurrence at all sites, because it is colonising and is thought to respond too quickly to environmental change to be suited to this frequency of sampling (Longstaff, et al., 1999). These species vary in their physiological tolerance to disturbance (due in part to investment into below-ground biomass and storage reserves), with *T. hemprichii* being the most tolerant and called a persistent species, while *H. uninervis* can be considered a colonising or opportunistic species.

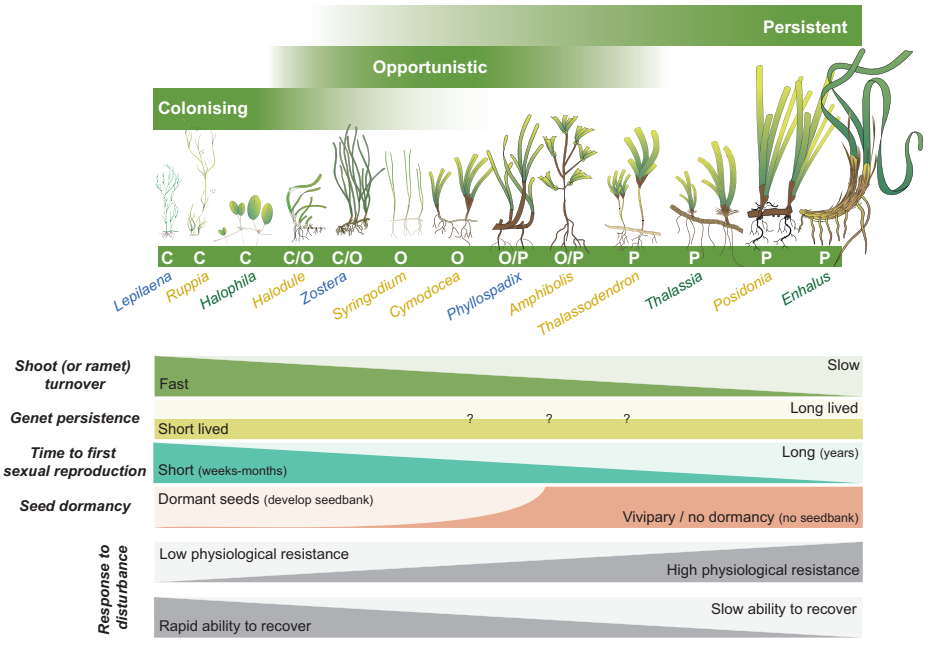

Figure 3: Diagram showing dominant traits of colonising, opportunistic and persistent species. Adapted from Kilminster *et al* (2015)

Sampling was undertaken by hand or using a hand trowel to remove rhizomes from the sediment. A single sample was collected from a small area (approximately 1m diameter, but

depended on seagrass density), with one to six replicates of each species collected more than a few metres apart. Total sample number for each site depended on availability of seagrass (i.e. abundance and area) and time since site was established (e.g. the Magnetic Island site was the first established, so has the most samples, (Table 1). These samples were collected opportunistically when undergoing routine monitoring for the inshore seagrass Marine Monitoring Program (MMP). Gaps in data occur due to a species not being present at the site, or because widespread seagrass loss in 2011 resulted in no seagrass available for collections. Collections were restarted when the meadow was perceived to be able to support the collections without causing an impact. Rhizomes were bagged and placed in a cooler bag with ice before being stored in a freezer (4°C). They were stored frozen prior to cleaning, and removal of leaves. Rhizome samples were then ground to a fine powder using a ball mill and stored in 5 ml tubes within airtight containers prior to analysis.

TNSC were measured at the School of Agriculture and Food Science at the University of Queensland. Soluble sugars were extracted in 80% ethanol at 80 °C for 3 min (repeated 3 times and centrifuged at 2500 rpm for 5 min between each extraction). The supernatant was retained for soluble CHO determination**.** TNSC remaining in the pellet was then solubilized by mixing in deionized water and heating at 95 °C for 1 h. The TNSC was then digested with amylase enzyme and incubated at 55 °C for 2 h. The sample was then centrifuged and the supernatant filtered. The samples were then analysed colorimetrically using a ferricyanide reagent. The values for soluble and TNSC components were summed for total carbohydrates. This was then expressed in terms of the above ground biomass supported by the carbohydrates by multiplying the carbohydrate concentration in the rhizome by the below ground biomass and comparing weight of carbohydrates against weight of leaf material.

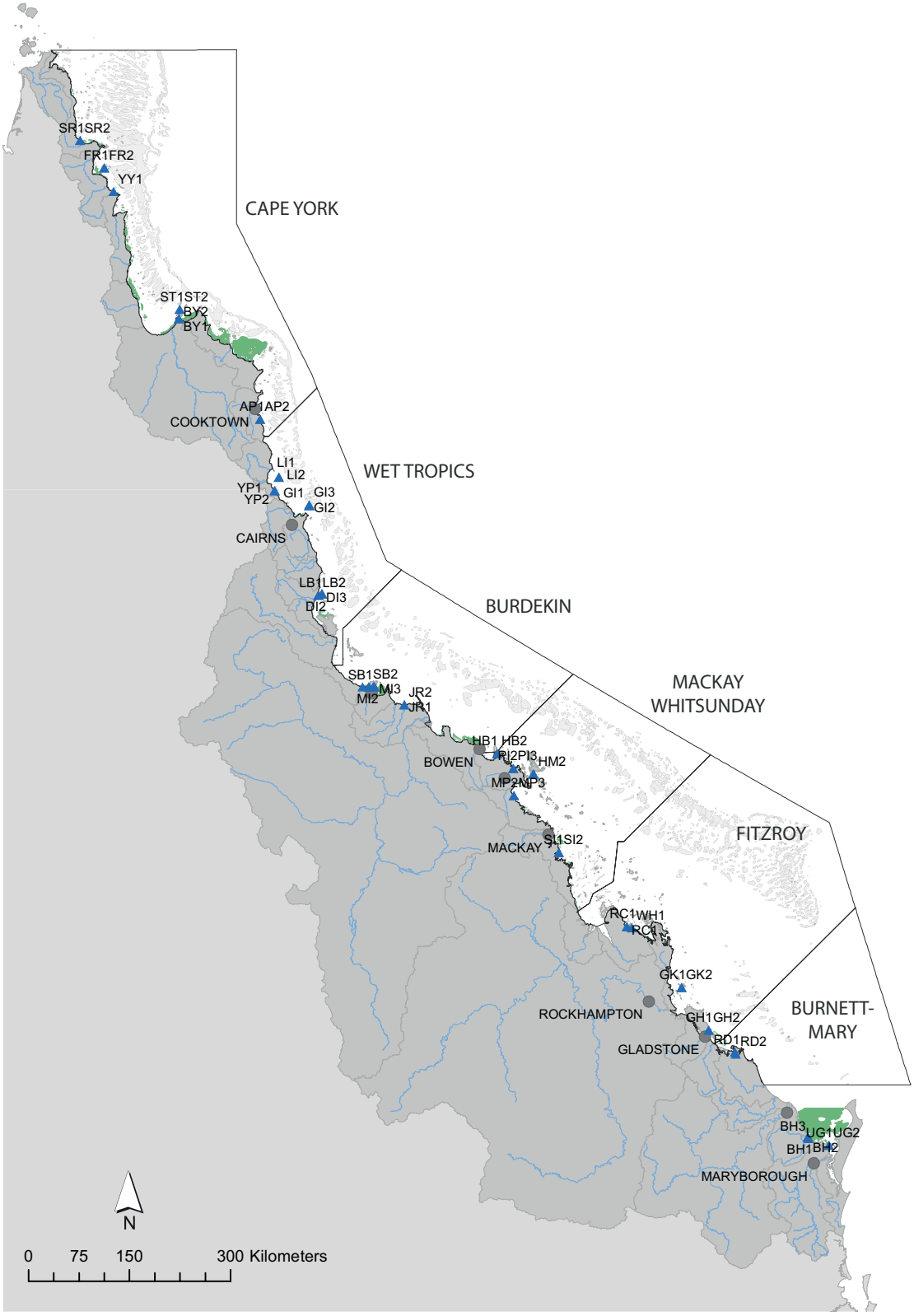

Figure 4: Map of the Great Barrier Reef showing Marine Monitoring Program sampling sites (blue triangles), which were also used for collection of TNSC samples in this study. *Temporal analysis* was focussed on Low Isles (LI), Green Island (GI) and Dunk Island (DI) in the Wet Tropics NRM region and at Magnetic Island (MI) in the Burdekin NRM region, while the *spatial analysis* included all sites. The mapped composite area of seagrass (i.e. seagrass has been present during surveys conducted from 1984 to 2010) is shown in green (McKenzie, et al., 2014b). Also shown is the GBR Marine Park boundary and the National Resource Management (NRM) boundaries.

Table 1: Number of samples collected for each species used in the *temporal analysis* (2008-2015) at Low Isles (LI), Green Island (GI) and Dunk Island (DI) in the Wet Tropics NRM region and at Magnetic Island (MI) in the Burdekin NRM region.

Location	Site	*H. uninervis*	*T. hemprichii*	*C. serrulata*
Low Isles	Intertidal	42	45	-
	Subtidal	33	-	-
Green Island	Intertidal	83	84	-
	Subtidal	90	-	46
Dunk Island	Intertidal	63	48	-
	Subtidal	54	-	-
Magnetic Island	Intertidal	74	30	-
	Subtidal	103	-	98
TOTAL		**542**	**207**	**144**

Spatial analysis

In 2014, an additional GBR-wide sample collection was undertaken in order to assess spatial patterns in carbohydrate content according to habitat characteristics. Samples were collected from 39 sites extending throughout the entire GBR from the northern Cape York region to the Burnett Mary region. Samples for the *spatial analysis* were collected in the late dry season (September to October) of 2014 when plant growth is at a maximum (Figure 1), because environmental conditions are optimal for plant growth. Sample number ranged from one to six (median = 3 for all species), depending on seagrass abundance. Again, analysis was focussed on *H. uninervis*, *C. serrulata*, *T. hemprichii* and also included *Z. muelleri*, which is dominant at coastal and estuarine sites in southern regions but does not occur at sites used in *temporal analysis* (Table 2).

Table 2: Number of samples collected per site for each species used in the *spatial analysis* of trends

NRM	site	*H. uninervis*	*C. serrulata*	*T. hemprichii*	*Z. muelleri*
Cape York	AP1	3			
	AP2	3			
	BY1	3		3	3
	BY2	3		2	
	FR1			3	
	FR2			3	
	SR1	3		3	
	SR2	3		3	
	ST1	1		3	
	ST2	1		2	
Wet Tropics	DI1	3	3		
	DI2	3	3	2	
	DI3	3	3		
	GI1	3	1	3	
	GI2	3		3	
	GI3	6	4		
	LB2	3			
	YP1				1
	YP2	5			
Burdekin	BB1	3			
	JR1				3
	JR2				3
	MI1	6	6		
	MI2	3			
	MI3	6	5		
	SB1	3			
Mackay	HM1	3			
Whitsunday	HM2				3
	MP3				3
	PI2				3
	PI3	3			
	SI1				3
	SI2				3
Fitzroy	GH1				3
	GH2				3
	GK1	3			
	GK2	1			1
	RC1				3
	WH1				3
Burnett-Mary	UG1				3
	UG2				3
	TOTAL	**80**	**26**	**30**	**44**

2.1.2. Supporting data: environment and seagrass abundance

Biological and environmental data are also available for the *temporal analysis* from 2008-2015. Seagrass abundance was measured quarterly, concurrent with collections for carbohydrate content. Abundance was measured as percent cover in fixed transects (33 quadrats) using methods detailed in McKenzie et al (2014a; 2016).

Environmental conditions (light and temperature) were measured continuously from 2008 to 2015. Light was measured at the seagrass canopy height using 2 Pi Odyssey light loggers (calibrated pre-deployment) with a wiper unit to keep the sensor clean, which were typically exchanged quarterly (detailed in McKenzie et al 2016). Loggers continuously recorded instantaneous light (µmol photons m^{-2} s^{-1}) every 15 or 30 minutes and these data were

summed to give a value for total daily light (mol photons m^{-2} d^{-1}) as this is the standard unit used for routine monitoring and reporting, and for compliance and thresholds (Bryant, et al., 2014; Chartrand, et al., Subm; Collier, et al., In Press; McKenzie, et al., 2016). Water temperature was measured every 30, 60 or 90 minutes (depending on deployment time) using ibTag loggers. Water temperature was summarised as mean daily temperature.

2.1.3. Statistical analysis

Generalised additive models (GAM) were used to explore seasonal trends in storage reserves. Two different models were produced: model 1 (M1), later plotted as black line and grey 95% CI, is a basic model with storage reserves vs month (e.g. Figure 6) and model 2 (M2), plotted as coloured lines and dotted lines for 95% CI, is M1 with the addition of a site as an intercept effect. Furthermore to enable the seasonal trend to be plotted as loop (with the last and first months influencing the trend for each other), the analyses were run with month 0 and 13, being a copy of the data from month 12 and 1. The plots were then truncated to show the trend from month 1 to 12 only.

Temporal analysis of total carbohydrate content (TNSC, mg gDW^{-1}) was analysed using linear regression with light, temperature, and abundance as variables and condition (*loss* or *recovery* corresponding to pre- and post-2011) as a fixed factor (GLM; R Core Team, 2013). Light and temperature was calculated as an average of the daily light and temperature measured at the collection site over 30 days prior of collection. Abundance (percent cover) was z-score transformed to enable comparison among sites that have inherently different mean abundances. The z-score transformation standardises each measure based on the mean.

The transformed abundance ($Abund_Z$) was calculated as:

$$Abund_Z = (\%cover - mean\%cover)/ StdDev\%cover \quad \text{Eq 1}$$

A model selection process was applied, testing combinations of variables and factor (condition: loss and recovery) including interactive effects. To determine the best fitting model, the second-order Akaike Information Criterion (AIC_c) was calculated using log likelihood ratios derived from all regression analyses (Burnham and Anderson, 2002). The best model was:

Total Carbohydrates ~ Daily light + Temperature + $Abund_Z$ + Condition

The addition of site as a random effect did not significantly improve the models (i.e. AIC was increased or similar) and therefore was not incorporated into the final model. A model validation process was applied to check for normality (histogram of residuals) and heterogeneity of variance by plotting the residuals against fitted values.

Analysis was performed on all locations pooled (four locations) and on Magnetic Island samples only (one location). Magnetic Island had all species (though very few of *T. hemprichii*, (Table 1) and has been through a particularly extreme cycle of decline and recovery (Figure 1) making it a good test case for the exploration of TNSC in relation to changing abundance and environmental conditions. Species responses were tested

separately, because there are species-specific differences in overall storage capacity, as well as dominant storage compounds (sugar or starch). Analysis was focussed more heavily on *H. uninervis* because the number of samples was greatest for this species as it occurred at all locations and sites (subtidal and intertidal).

2.2. Storage reserves: results and discussion

2.2.1. General trends

Mean total non-structural carbohydrate concentration (TNSC) of the rhizomes was variable among sites and species (Table 3). TNSC was highest in *Z. muelleri* followed by *H. uninervis*, *T. hemprichii*, and *C. serrulata*. *H. uninervis* and *Z. muelleri* stored predominantly starch in rhizomes (70.7% and 73.2%, respectively), while *C. serrulata* stored predominantly sugars (84.5%) and *T. hemprichii* had approximately equal proportions of both (46.1% sugars, 53.9% starch). Dunk Island consistently had the lowest carbohydrate concentration among all sites, while Green Island, which has shown the least decline in seagrass abundance and consistently has highest light also had the highest TNSC (Table 3).

Table 3: Mean TNSC (mg gDW^{-1} ± SE) for all locations pooled and for each of the sites used in temporal analysis. Also shown the mean % sugars and %starch composition for each species

Species	%sugars	%starch	TNSC	Low Isles		Green Is.		Dunk Is.		Magnetic Is.	
				Int	Sub	Int	Sub	Int	Sub	Int	Sub
C.serrulata	84.5	15.5	174.9 (0.5)			291.4 (24.4)	196.1 (11)	154.2 (10.1)	148.9 (10.3)	151.6 (28.1)	161.6 (5.9)
H. uninervis	29.3	70.7	221.9 (0.4)	192 (17.4)	203.7 (21.6)	238.4 (8.7)	227.5 (6.6)	150.1 (8.3)	189.7 (9.7)	209.7 (13.3)	228.8 (8.1)
T. hemprichii	46.1	53.9	197.2 (0.6)	164.2 (11.5)		220.8 (10.2)		155.3 (14.3)		158.0 (17.7)	
Z. muelleri	26.8	73.2	230.0 (1.5)	-	-	-	-	-	-	-	-

TNSC content was plotted over time from 2008 to 2015 (Figure 5) and from this it is very difficult to discern patterns associated with season or environmental conditions. Therefore the effects of season, changing seagrass abundance and environmental conditions (daily light and temperature) are further explored below.

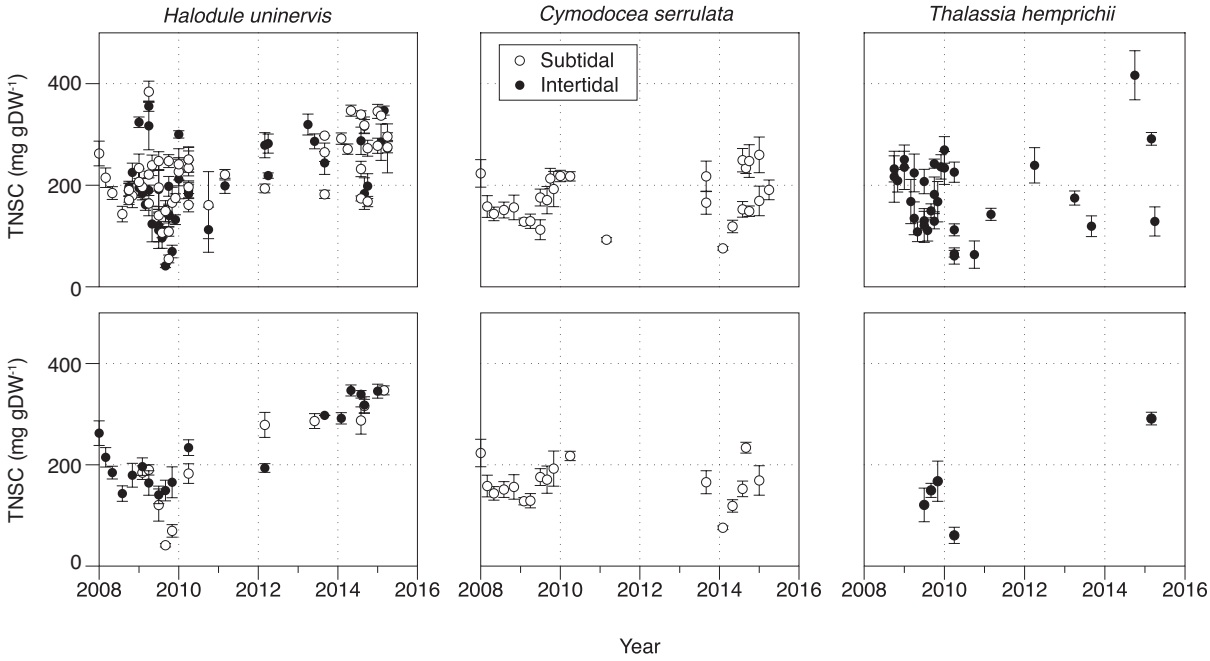

Figure 5: Total non-structural carbohydrates (TNSC, bottom) at all sites (top) and at Magnetic Island only (bottom) for *Halodule uninervis*, *Cymodocea serrulata*, and *Thalassia hemprichii* at intertidal (black dots) and subtidal sites (white dots).

TNSC content for all years were pooled as a preliminary exploration of seasonal changes in allocation to storage reserves. The TNSC of *H. uninervis* tended to be higher in the wet season months from January through to April having accumulated during the growing season from October to December (Figure 6). However, these seasonal patterns were not very strong, in particular for subtidal meadows. The TNSC of *T. hemprichii* and *C. serrulata* (which had fewer data available) also accumulated from October to December, however, the storage reserves of these species were reduced over the wet season, reaching lowest values in May and June at the onset of winter (Figure 7). *T. hemprichii* is a persistent species (Kilminster, et al., 2015), which means that it is able to tolerate disturbances much longer than many other species due in part to investment into below ground biomass and storage reserves. Other persistent species, such as Posidonia spp in the Mediterranean and temperature Australia (Alcoverro, et al., 2001; Collier, et al., 2008), accumulate TNSC over summer (peaking in late summer) and these are then consumed for over-wintering (declining throughout winter).

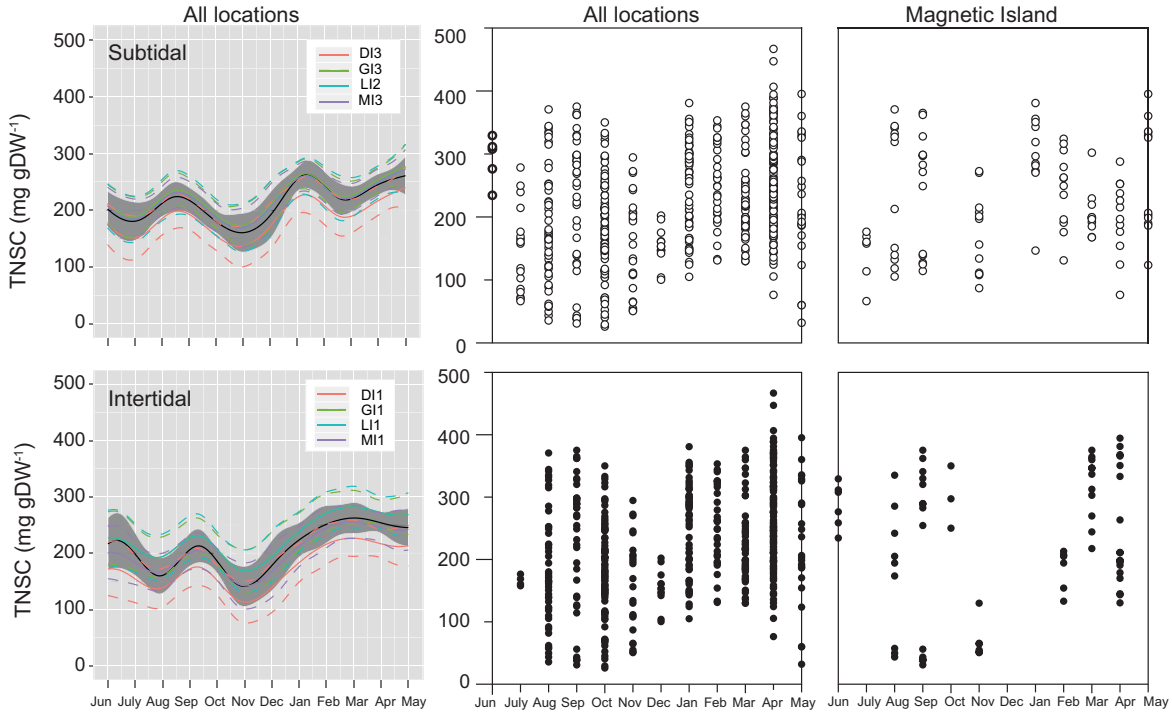

Figure 6: Seasonal patterns of TNSC of *H. uninervis* from all years pooled at all locations as a GAM plot (left), and the raw data (centre) also raw data at Magnetic Island only (right). Data are presented separately for subtidal (top), and intertidal (bottom) sites. Months are ordered according to the MMP reporting year (June-May), which places the late dry season or growing season towards the centre.

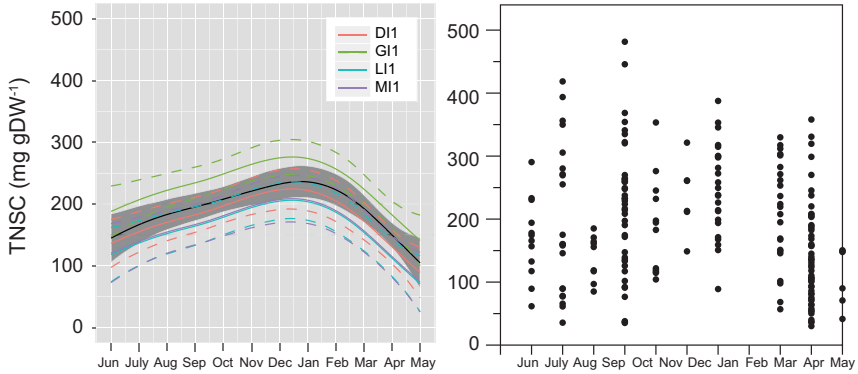

Figure 7: Seasonal patterns of TNSC of *T. hemprichii* from all years pooled at all locations (intertidal only) presented as a GAM plot (left), and as raw data (right). Months are ordered according to the MMP reporting year (June-May), which places the late dry season or growing season towards the centre.

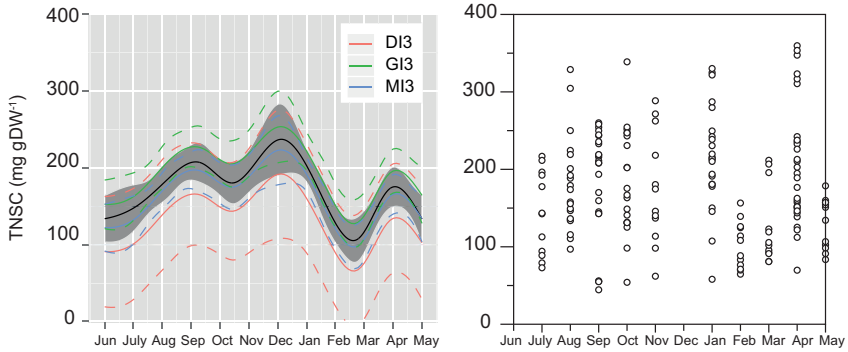

Figure 8: Seasonal patterns of TNSC of *C. serrulata* from all years pooled at all locations (intertidal only) presented as a GAM plot (left), and as raw data (right). Months are ordered according to the MMP reporting year (June-May), which places the late dry season or growing season towards the centre.

2.2.2. Temporal drivers

Storage reserves (total carbohydrate content, TNSC) were significantly ($p<0.001$) affected by condition of the meadow (fixed factor, in decline or recovery) in all models for *H. uninervis* and *T. hemprichii* (but not *C. serrulata*, which also had fewest samples contributing to the analysis) and all locations (Table 4, Table 5, Figure 5, Figure 9, Figure 10). From 2008 to 2011, the meadows were in decline or in a state of loss (including Green Island to some extent, though losses were not as great). Previous research (NERP 5.3) has shown that fewer carbohydrates are directed towards rhizome storage in the presence of elevated nutrients (Collier, et al., 2015), and therefore this cannot be discounted as contributing to lower TNSC pre-2011 when there was elevated terrestrial discharge. Further analysis incorporating nutrients are therefore warranted. During the decline period, meadows were in a depressed state of resilience (low storage reserves), and less able to cope with short-term perturbations compared to meadows during recovery when resilience was higher and they were able to absorb short-term disturbances.

Following 2011, when meadows were in a recovery mode increasing their abundance and area/extent, the TNSC was higher. During recovery, despite investment into new biomass production, reserves were directed to rhizomes as storage. The differences were large, for example at the Magnetic Island intertidal site TNSC of *H. uninervis* was 144 mg gDW^{-1} (or 14% DW) during loss and 312 mg gDW^{-1} during recovery (Figure 9, Figure 10). The mean concentration of TNSC in *H. uninervis* was very similar at the subtidal site (324 mg gDW^{-1}) during its recovery. This demonstrates the sensitivity of carbohydrates to overall seagrass condition. During recovery, there have been periods of very low light, particularly at Magnetic Island, and yet meadows have continued to thrive (McKenzie, et al., 2016). The tolerance of meadows to these short-term events may have been supported by the higher TNSC during recovery.

Interestingly, *H. uninervis*, had the strongest response of TNSC to overall condition compared to other species. This may however be a consequence of the greater sample size for this species. Generally, the storage of TNSC is considered most important to the resilience of structurally large species with large rhizomes (such as *T. hemprichii*), and it is these species that are able to tolerate extreme conditions for the longest owing to their supply of reserves (see functional form model by Walker, et al., 1999). However, *H. uninervis* also has a very large total pool of TNSC due to a large proportion of below-ground biomass (i.e. high below- to above-ground biomass) (Collier, et al., 2009), and it was tolerant of medium-term low light conditions (Collier, et al., In Press). Therefore, it may also have a large reliance on storage reserves for survival.

This is the first demonstration, known to the authors, of links between TNSC to overall changes in seagrass condition and abundance. These findings highlight the importance of storage reserves for overall resilience and demonstrate a potential application as an indicator of meadow trajectory, which existing indicators may not show.

Table 4: General linear model results for total carbohydrate content (TNSC) of *Halodule uninervis* testing for effects of within canopy daily light (1 month prior av), within canopy water temperature (1 month prior av), abundance (z-score transformed, $Abund_Z$), and growth phase (fixed factor) as decline (recovery is the intercept).

	Estimate	Std Err	t value	P	Estimate	Std Err	t value	P
Intertidal, all sites					Intertidal, Magnetic Island			
(Intercept)	84.81	69.542	1.22	0.224	71.69	92.563	0.774	0.443
Light	-4.692	1.029	-4.56	**<0.001**	5.011	3.041	1.648	0.107
Temperature	10.315	2.435	4.236	**<0.001**	6.533	2.585	2.528	**0.015**
$Abund_Z$	20.801	10.448	1.991	**0.048**	32.769	14.999	2.185	**0.035**
Decline	-106.112	11.408	-9.301	**<0.001**	-137.899	15.712	-8.777	**<0.001**
Subtidal, all sites					Subtidal, Magnetic Island			
(Intercept)	5.056	57.404	0.088	0.930	250.968	54.41	4.613	**<0.001**
Light	-3.881	1.536	-2.526	**0.012**	-10.933	4.02	-2.719	**0.008**
Temperature	10.594	2.207	4.801	**<0.001**	2.274	1.846	1.232	0.222
$Abund_Z$	23.251	7.868	2.955	**0.004**	48.467	7.092	6.834	**<0.001**
Decline	-71.887	11.018	-6.524	**<0.001**	-88.937	12.25	-7.26	**<0.001**

Table 5: General linear model results for total carbohydrate content (TNSC) of *Cymodocea serrulata* and *Thalassia hemprichii* testing for effects of canopy light levels (1 month prior average), within canopy water temperature (1 month prior average), abundance (z-score transformed, $Abund_Z$), and growth phase (fixed factor) as recovery (loss is the intercept).

	C. serrulata				*T. hemprichii*			
	Estimate	Std Err	t value	P	Estimate	Std Err	t value	P
Subtidal, all sites					Intertidal, all sites			
(Intercept)	218.871	55.214	3.964	**<0.001**	354.4958	104.644	3.388	**<0.001**
Light	8.147	1.563	5.212	**<0.001**	0.9018	1.5228	0.592	0.555
Temperature	-4.028	2.108	-1.911	0.058	-4.3329	3.6656	-1.182	0.239
$Abund_Z$	-10.94	7.689	-1.423	0.158	41.4094	15.9458	2.597	**0.010**
Decline	-7.372	11.355	-0.649	0.518	-95.1591	18.363	-5.182	**<0.001**

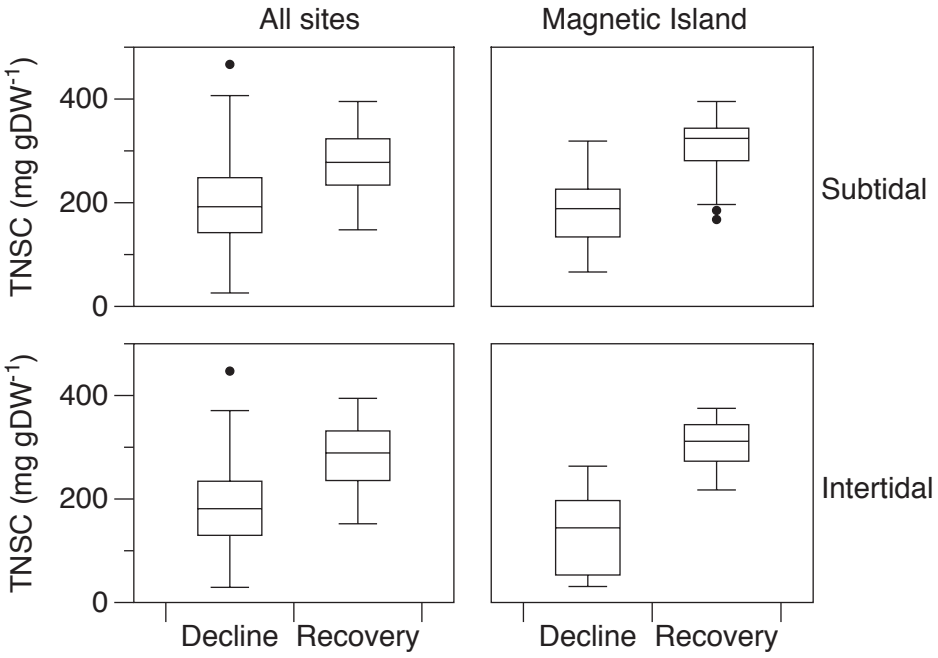

Figure 9: Boxplot of TNSC of *H. uninervis* during the period of seagrass decline (2008-2011), and recovery (2012-2015) at subtidal (top) and intertidal sites (bottom), with all locations pooled (left), and at Magnetic Island only (right).

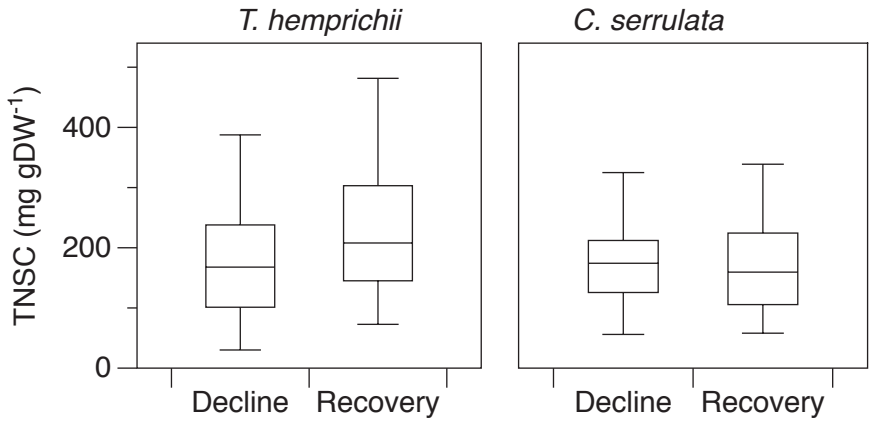

Figure 10: Boxplot of TNSC of *T. hemprichii* during the period of seagrass decline (2008-2011), and recovery (2012-2015) at all intertidal sites.

TNSC of both *H. uninervis* and *T. hemprichii* were significantly related to abundance ($Abund_Z$) at all sites (Table 4, Table 5, Figure 9, Figure 10). The relationship was stronger (lower p-value) at subtidal sites than at intertidal sites (which may have been affected by slightly smaller overall range in abundance), but was statistically significant in all analyses (Table 4, Figure 9). Further, the relationship with $Abund_Z$ was not as strong (smaller slope, and p-value), compared to condition (decline and recovery) effects. The relationship was positive, such that higher abundance corresponded to higher TNSC (Figure 11) in both the decline and recovery periods. Thus, the variable $Abund_Z$ describes the relationship between TNSC and abundance over finer time-scales and highlights that TNSC corresponds to short-term variability in seagrass abundance as well as longer-term trends in condition.

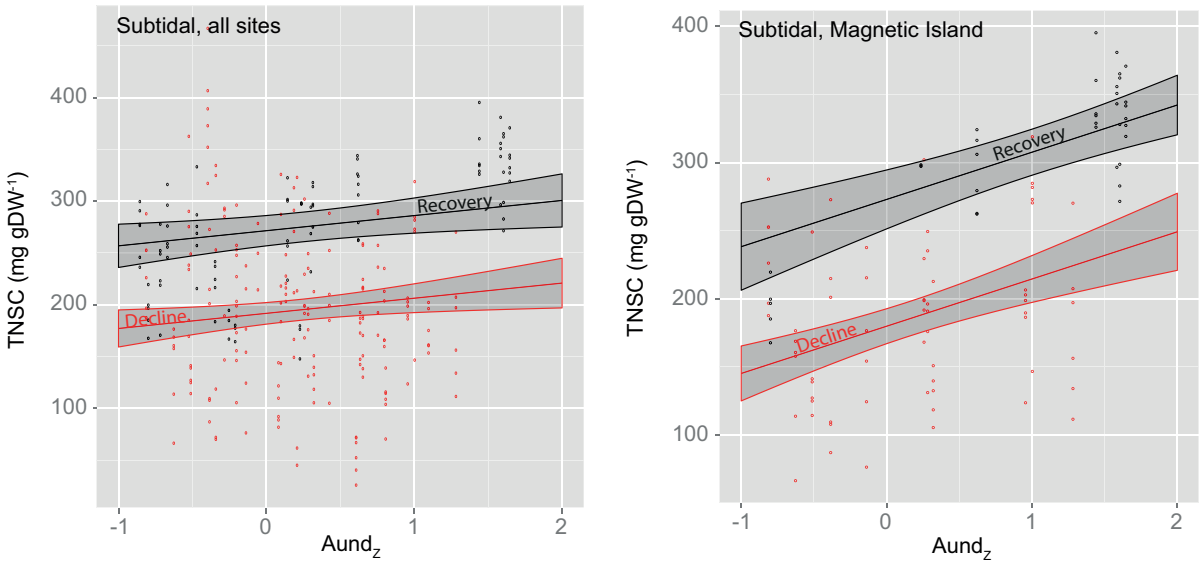

Figure 11: Total carbohydrates (TNSC) of *H. uninervis* rhizomes relative to seagrass abundance (z-score transformed, $Abund_Z$) at all subtidal sites (left) and at the Magnetic Island subtidal site (right) from 2008 – 2015. Red is during loss and black is during recovery.

2.2.3. Environmental drivers

Meadow abundance in the Burdekin and Wet Tropics regions declined (Figure 1 Figure 2) in association with periods of poor water quality and low daily light (Collier, et al., 2012; Devlin, et al., 2012; McKenzie, et al., 2016). We hypothesised that reduced daily light (and low photosynthetic rates) would also drive declines in TNSC; however, this was not the case. Daily light slightly negatively affected TNSC. At intertidal sites, this may be coincidental, as periods of very high light occur at low tide when heat and desiccation stress may have an overriding influence on TNSC (Larkum, et al., 2006). However at subtidal sites, the cause of the slight negative effect of light is not so apparent, but perhaps it is caused by biological processes (e.g. increased allocation to reproduction or rhizome extension) at times of high light.

TNSC of *H. uninervis* increased at higher temperatures (positive slope), with highest water temperature generally occurring in December to March (McKenzie, et al., 2016). However, based on the seasonal trends described above (Figure 6), the highest TNSC occurred from October – December), therefore later wet season values (January – March) may account for some of the variation in responses. Photosynthetic rates and net productivity are also strongly correlated to water temperature, increasing to reach thermal optima, which is above 30ºC for these tropical species (Adams, et al., In Prep; Campbell, et al., 2006; Collier, et al., 2011). Therefore, faster rates of productivity probably drive TNSC accumulation as well. However, it is important to note that these collections were undertaken at a time relatively moderate temperature, with few extremes (McKenzie, et al., 2016). Net productivity rapidly declines at very high temperatures above thermal optima. Similarly, the effect of temperature on TNSC is likely to be non-linear such that TNSC probably declines due to thermal stress at high temperatures.

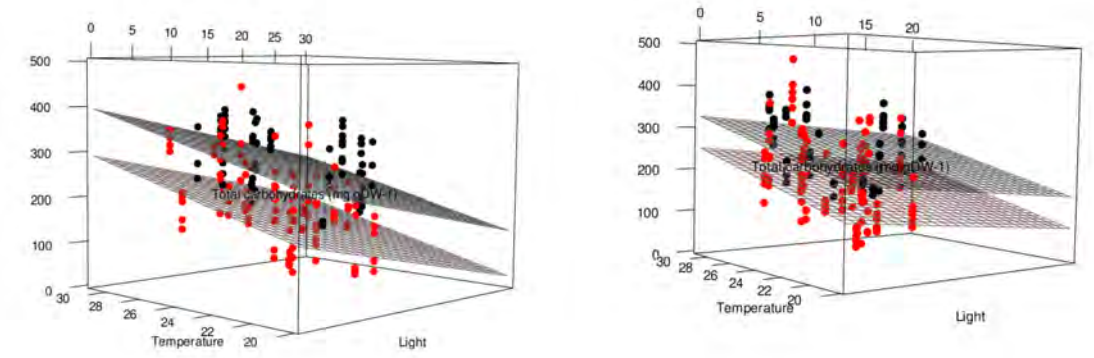

Figure 12: TNSC of *H. uninervis* for all locations pooled at intertidal sites (left) and subtidal sites (right).

In summary, seagrass abundance and meadow condition are integrators of the environment (Waycott and McKenzie, 2010), hence we observed declines in abundance and low TNSC in the years leading up to 2011. However, the environmental drivers acting on seagrass meadows are numerous and include water quality (e.g. turbidity, herbicides, low salinity, high nutrients), as well as physical disturbances (Collier and Waycott, 2009). TNSC was also low at this time (2008 – 2011), and yet this does not appear to relate directly to declines in light. There may however, be an indirect effect that we cannot account for in these models. For example, there may be cumulative environmental effects (no data available for other environmental conditions) or by plant biological processes (e.g. changing carbon allocation strategies) that are complex and unknown.

2.2.4. Spatial analysis

As an additional unplanned component of this project, a *spatial analysis* of TNSC was undertaken on 39 sites across the GBR (a distance >2000 km). However, the data set does not lend itself easily to statistical analysis because the species are not uniformly represented among habitats and regions. Instead, a visual description of results is presented here. There were no apparent differences in the TNSC of rhizomes among habitats for any species (Figure 13, Figure 16, Figure 17, Figure 15). However, between-region differences in TNSC content occurred for both *H. uninervis* and *Z. muelleri*, which were present in 5 of the 6 NRM regions. Specifically, the TNSC of *H. uninervis* was highest in Cape York and Burdekin, followed by the Wet Tropics, Mackay Whitsunday and Fitzroy NRMs and the later grouping were rated as very poor or in poor in condition for abundance. As a preliminary exploration of regional trends, we plotted TNSC of *H. uninervis* against the MMP abundance score for 2014-15 (McKenzie, et al., 2016) which resulted in an overall correlation (R^2 = 0.496), which was not statistically significant (p = 0.169) (Figure 14), but this trend warrants further exploration.

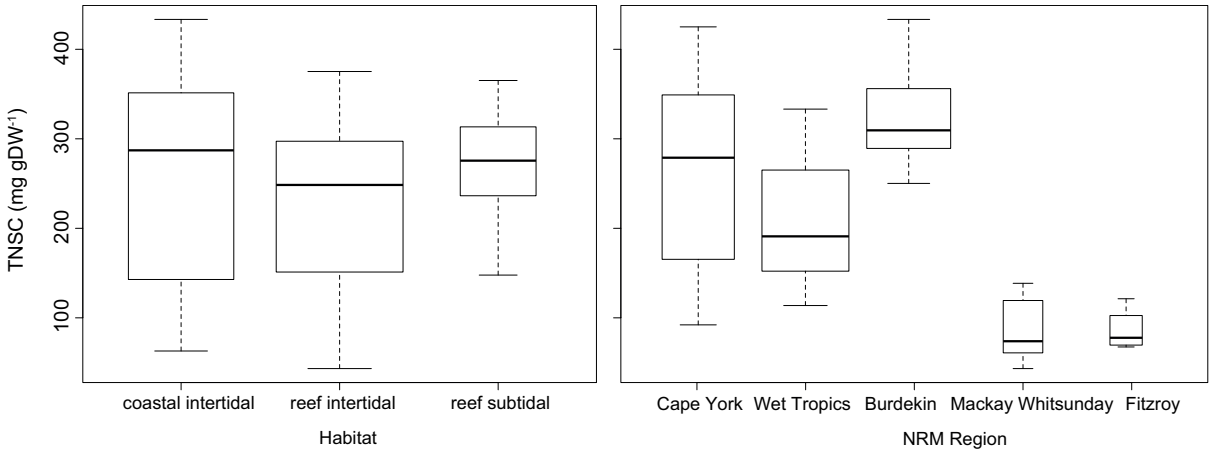

Figure 13: Total non-structural carbohydrates (TNSC) of *H. uninervis* by habitat (left), and NRM region (right) arranged from north to south in the late dry season (Sep-Nov) 2014.

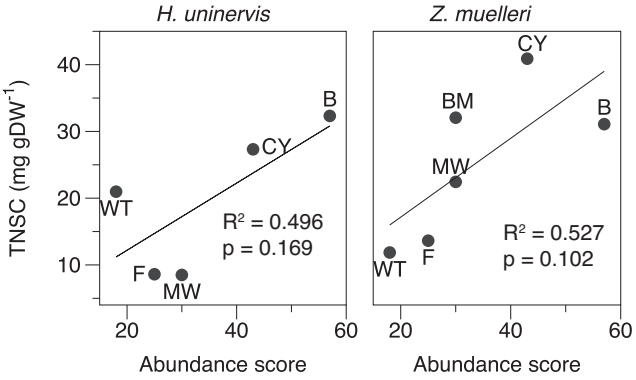

Figure 14: Correlation between mean TNSC of *H. uninervis* (left) and *Z. muelleri* (right) within each region in 2014 and the NRM-wide report card score for total abundance in 2014-15 (McKenzie, et al., 2016).

For *Z. muelleri*, TNSC was highest at far northern sites in Cape York and Burdekin, and in the Burnett Mary in the far south (Figure 14). As for *H. uninervis*, TNSC was lower at Wet Tropics (though only 1 sample was available), Fitzroy and Mackay Whitsunday, which were in poorer condition resulting in a non-significant correlation (R^2 = 0.527, p = 0.102) between the abundance score and TNSC.

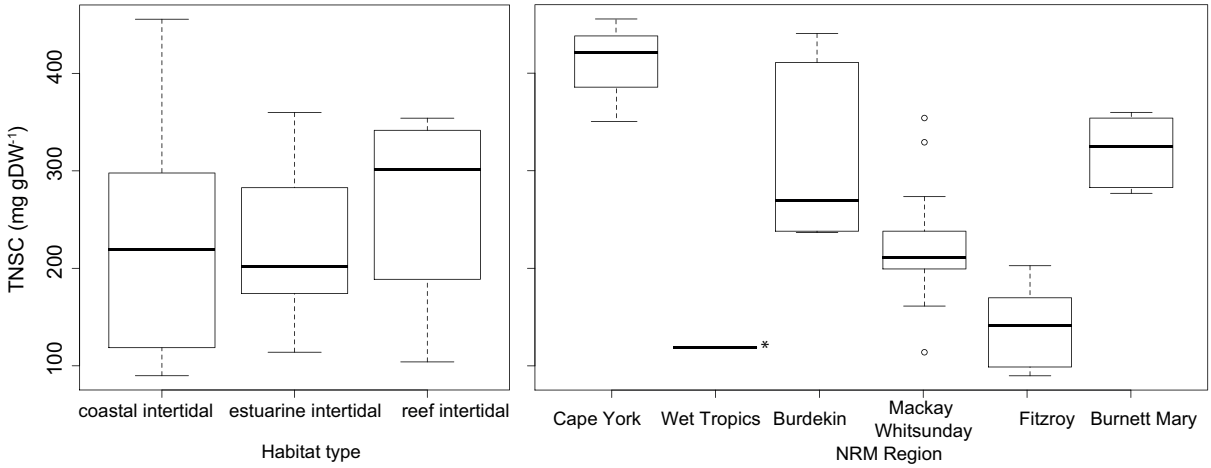

Figure 15: Total non-structural carbohydrates (TNSC) of *Z. muelleri* by habitat (left), and NRM region (right) arranged from north to south in the late dry season (Sep-Nov) 2014. *only one sample has contributed to the Wet Tropics data as *Z. muelleri* is not common at WT MMP sites.

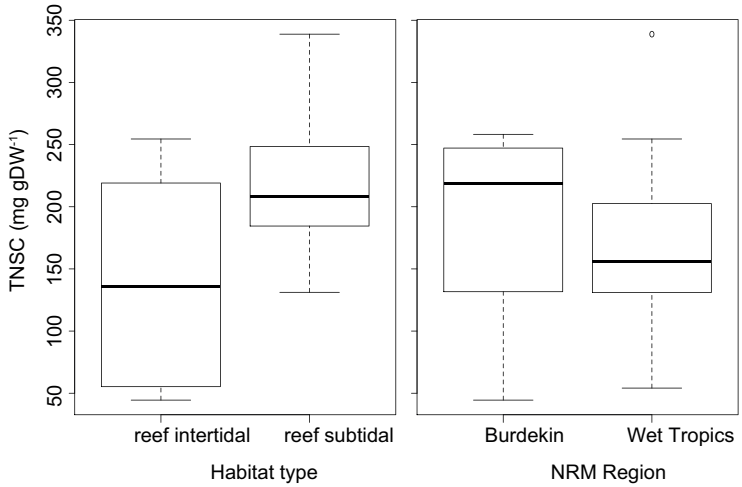

Figure 16: Total non-structural carbohydrates (TNSC) of *C. serrulata* by habitat (left), and NRM region (right) in the late dry season (Sep-Nov) 2014.

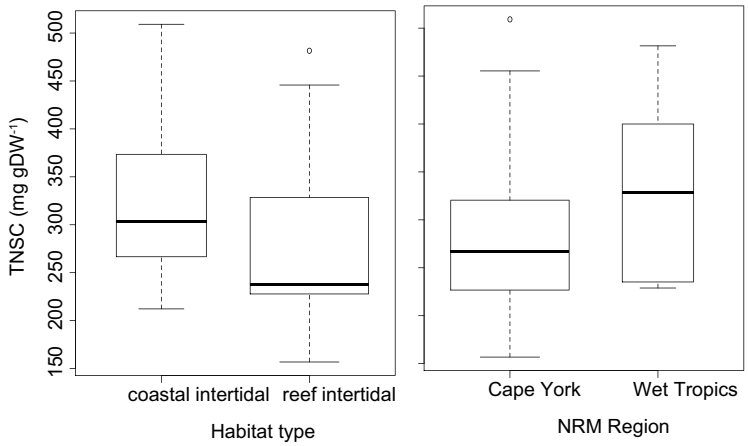

Figure 17: Total non-structural carbohydrates (TNSC) of *T. hemprichii* by habitat (left), and NRM region (right) in the late dry season (Sep-Nov) 2014.

2.2.5. Future research

We tested for the effect of temperature and light as environmental drivers of TNSC. Since 2008 – 2011 was a relatively mild period in terms of water temperature, low light was the principal environmental pressure. The very large in TNSC pre 2011 compared to post 2011 (i.e. decline and recovery), justifies further exploration of environmental pressures on seagrass abundance and TNSC. For example, as described above, nutrient availability (and tissue nutrients), can affect the allocation of carbon to storage reserves (Collier, et al., 2015). This, together with other indicators of cumulative pressures (e.g. water type exposure Devlin, et al., 2015), may improve our model describing trends in TNSC, but was beyond the scope for this project. Furthermore, there may be biological effects on TNSC, such as investment of carbon into reproductive effort and meadow expansion (rhizome extension). We could explore these other factors using *H. uninervis* data, which was the largest data set.

Near-Infrared spectroscopy offers the potential for rapid and cost effective (<1/10th the price) measurement of TNSC (Lawler, et al., 2006). This technique requires time invested into calibration of the reflectance wavelengths against laboratory-analysed samples. The rhizome samples measured in this project using laboratory chemistry procedures were retained,

which enables this calibration to be performed against results without requiring further laboratory analysis. Calibration of NIR against results from this project would take approximately 6 months and requires laboratory (NIR) and statistical analysis time and can be undertaken at the Rapid Assessment Unit at the James Cook University Cairns campus. Once calibrated, this methodology would enable storage reserves (as TNSC of rhizomes) to be included in routine monitoring with minimal additional cost.

Improving the cost-effectiveness of sample analysis would make it more feasible to fill the data gaps needed to further explore the TNSC dynamics. In particular, the TNSC of *T. hemprichii*, *C. serrulata*, and *Z. muelleri* were incomplete data sets leading to inconclusive results in many of the analyses. Increased sampling frequency/number could enable the TNSC of these species to be more thoroughly investigated.

3. BIOMASS CALIBRATION

3.1. Biomass methods

3.1.1. Biomass collection and analysis

Biomass collections were undertaken in conjunction with percent cover estimates according to MMP monitoring protocols (McKenzie, et al., 2010). A 50cm quadrat was placed in a mono-specific meadow. The percent cover of seagrass in the quadrat and canopy height (10 shoots) was measured, a photograph was taken (and subsequently checked) for quality control and then the entire quadrat was harvested, placed in a bag and frozen for later processing. Up to six quadrats were harvested from each meadow, intentionally targeting a gradient of increasing cover (which was constrained by the range in densities present at the site) to enable regression analysis. For very small densities (<3%), cover is counted in number of shoots (McKenzie, et al., 2010). Single shoots were separated out of the biomass samples (10 shoots) to obtain a mean shoot weight. These were used for biomass of 1% (2 – 10 shoots depending on morphology) and 2% (3 – 6 shoots depending on morphology) cover equivalents. Sampling was undertaken at 7 locations as the available budget limited further sampling and analysis. Sampling was performed at low tide for intertidal sites, and by SCUBA for subtidal sites. Sampling was designed to capture the range in morphologies for *H. uninervis* (thin and wide), and *Z. muelleri* (thin, wide, short, tall) as these will also affect the calibration.

In the lab, each sample was washed and separated into shoots (leaves), and below-ground parts (rhizome and roots). Epiphytes were gently scraped from the surface of leaves, dried at 60°C for at least 48 hrs and weighed. This processing is very time-consuming as the entire 50x50cm quadrat is processed, requiring the separation of shoots from rhizomes, and in some samples, shoot count would be in excess of 1000.

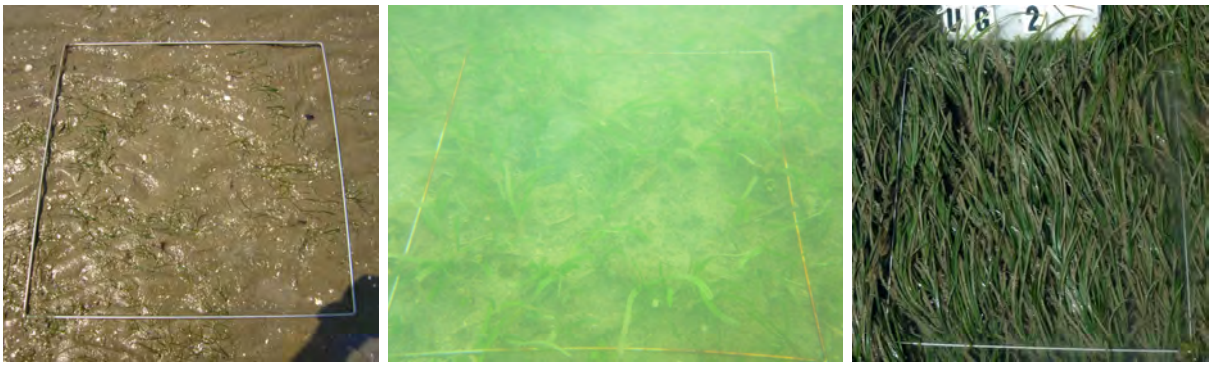

Figure 18: Harvested quadrats for percent cover calibration from (left to right) *Z. muelleri* (narrow leaf) at Shoalwater Bay (RC), *C. serrulata* at Magnetic Island (MI3) and *Z. muelleri* (wide leaf) at Urangan (UG).

3.1.2. Biomass statistical analysis

Leaf biomass was correlated to percent cover using general linear models. An additional analysis correlated biomass to percent cover * canopy height, to account for the contribution that long leaves makes to overall shoot biomass.

The calibration equations is:

$$Bio_{Est} = \beta_0 + \beta_1 \ X_1 \qquad \text{Eq 2}$$

where β_0 is 0, as zero biomass must equal zero percent cover, β_1 is the estimate and X_1 is percent cover (or percent cover $*$ canopy height).

Model performance (with or without canopy height) was compared using Akaike information criterion (AIC). All analyses were performed using R statistical software.

3.2. Biomass results and discussion

3.2.1. Biomass calibration

All linear models fitting above ground biomass and %cover were highly significant ($p<0.001$) (Table 6). The *estimate* generated from the model (which will be used to calibrate percent cover to biomass) was larger for wide leaf populations (and the "large" morphology of *Z. muelleri*), than it was for the narrow leaf populations thus highlighting the importance of morphology-specific calibration values.

In most cases the model fit was better (lower AIC), where canopy height was considered in the analysis than it was without canopy height. The exception was the limited dataset of wide-leafed *H. uninervis* from Green Island, and this anomaly will require additional investigation by testing a greater sample of wide leafed *H. uninervis* populations. Further, the model estimates used to make the conversions were more similar among the three *Z. muelleri* populations (0.0131 – 0.0219, 167%) with canopy height considered than without (0.0527 – 0.2529, 480%). Therefore, consideration of canopy height provides the best prediction and most consistent means to apply the biomass calibration thereby reducing the risk of over or underinflating biomass estimates.

The Estimates in Table 6 can be used to calculate biomass from percent cover for the species/morphologies specified using equation 1.

3.2.2. Ongoing development of biomass calibration

Due to budget constraints, the number of samples, species, habitats and sites measured was limited (sample processing is very time consuming). Therefore, we have developed some preliminary calibration estimates that can be applied as an interim but further work is recommended to refine the calibration for other habitats and seagrass leaf morphologies as well increasing the database for the models presented here.

Table 6: Linear model output for biomass calibration data. Bold type indicates the better model (between %cover only or %cover ∗canopy height) based on the AIC score.

NRM	Habitat	Site	Leaf morphology	Species	Percent cover			Percent cover ∗ canopy height		
					Estimate	p	AIC	Estimate	p	AIC
WT	Reef	GI	*Wide*	*H. uninervis*	**0.1345**	**<0.001**	**28.182**	0.0221	<0.001	32.344
B	Coastal	BB	*Thin*	*H. uninervis*	0.0385	<0.001	-11.177	**0.0057**	**<0.001**	**-16.714**
BM	Estuarine	UG	*Large*	*Z. muelleri*	0.2529	<0.001	37.873	**0.0181**	**<0.001**	**33.309**
MW	Coastal	MP	*Wide, small*	*Z. muelleri*	0.0832	<0.001	17.554	**0.0219**	**<0.001**	**10.736**
F	Coastal	RC	*Thin, small*	*Z. muelleri*	0.0527	<0.001	-3.565	**0.0131**	**<0.001**	**-3.6248**
B	Reef subtidal	MI		*C. serrulata*	0.2772	<0.001	21.858	**0.0345**	**<0.001**	**15.666**
WT	Reef	LI		*H. ovalis*	0.0112	<0.001	-32.552	-	-	-

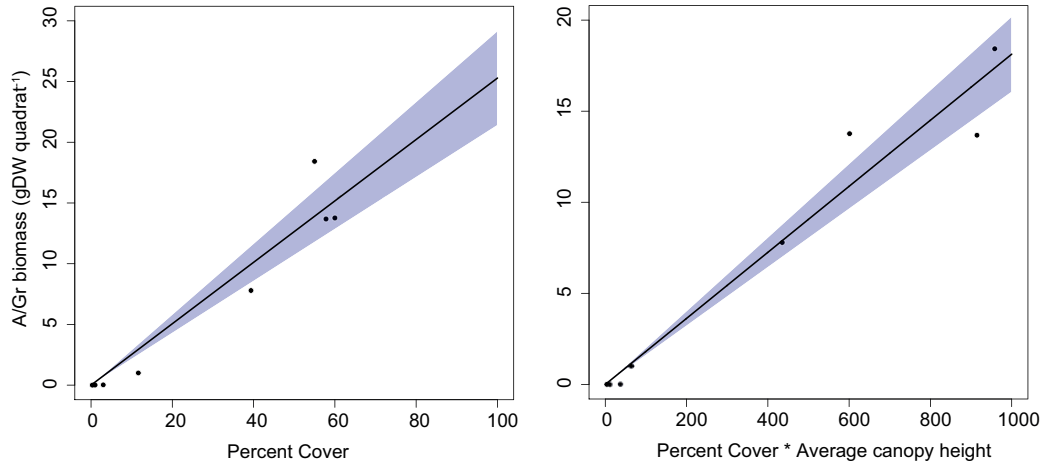

Figure 19: Example of the linear model fits for *Z. muelleri* from Urangan in the Burnett Mary region with percent cover only (left), and with canopy height factored into the calibration (%cover ∗ canopy height, right).

4. RECOMMENDATIONS AND CONCLUSION

Carbohydrate content was affected by seagrass condition, including that TNSC was:

- lower in 2008 – 2011 at a time when climatic conditions drove seagrass decline, compared to 2012 – 2015 when they were in recovery mode (*H. uninervis* and *T. hemprichii*);
- correlated to abundance in the *temporal analysis* (*H. uninervis* and *T. hemprichii*).

The relationship between meadow condition (pre and post 2011) and storage reserves was an unexpected finding, which has not previously been shown to the best of our knowledge. This finding has application: for example, if the condition of a meadow was unknown, TNSC could provide insight into meadow trajectory. In that sense, TNSC can be an early-warning indicator that meadows are in decline or in recovery, which may not be apparent from existing indicators. This discovery was made possible by the long-term data set collected at a time of dynamic meadow changes. These trends were most notable for *H. uninervis*, which had the greatest sample number and occurred across all sites/habitats. However, even for this species, all data from the temporal analysis were based on four reef locations (8 sites) in the Wet Tropics and Burdekin only and the relationship to condition does need to be validated for other regions and species.

Storage reserves do not appear to be a good indicator of low light stress in this analysis and therefore may not be a good indicator within a DPSIR framework where links to environmental pressures are crucial. This is despite repeated recommendation from the literature that storage reserves are good indicators (McMahon, et al., 2013; Roca, et al., 2016). These findings may be constrained by the analysis, including that samples were collected quarterly (and they can respond much faster e.g. Burke, et al., 1996) and that the light metric used was mean daily light over past 30 days when shorter or longer time frames may be more suitable (Adams, et al., 2015). Irrespective of this, TNSC was an indicator of cumulative stress (being correlated to abundance and condition), but the specific pressure(s) could not be identified. Further work exploring linkages between reserves and these other environmental pressures (such as plume exposure/water type, nutrient availability) is therefore warranted. There may also be biological explanations. For example, when light is high, energetic surpluses (i.e. carbon fixed through photosynthesis) may be directed elsewhere including towards sexual reproduction or rhizome extension. Some additional analyses on environmental and biological drivers of storage reserve dynamics can be carried out using this existing data for *H. uninervis*; however, data were limited for other species. Increasing data availability for these can be made possible through routine sample collection (e.g. MMP) if cost-effective analytical protocols can be implemented. Near-Infrared analysis has potential in making routine storage reserve analysis more cost-effective, which could be calibrated using samples already analysed in this study.

In summary, we have had the unique opportunity to explore a long-term data set on storage reserves at a time of dynamic meadow changes and this has yielded some exciting results on their sensitivity to meadow condition and abundance. However, this did not provide conclusive evidence to support the inclusion of TNSC as an indicator in monitoring programs such as the MMP at this stage, because the link to the main environmental pressure tested — light — was not demonstrated by this analysis. However, the strong link to condition and

abundance provides justification for further inquiry into the effect of other pressures, (e.g. nutrients and flood plume exposure), other biological processes (e.g. reproduction and meadow expansion) and to obtain further data on other species, in particular for those known to rely heavily on storage reserves.

Calibration between biomass and percent cover was successful; however estimates varied among sites (even for the same species) therefore, the calibration needs to be expanded to include all dominant species in each habitat type. The variability in calibration values among sites was lowest when canopy height was included in the estimates, and therefore canopy height should be considered for future calibration. Continuing to refine this relationship is vital to the implementation of RIMREP.

Recommendations (in order of priority):

1. Adopt biomass calibrations where applicable.
2. Continue to refine biomass calibrations for other species and habitats.
3. Continue exploring existing storage reserve data for effects of other environmental pressures such as other light indicators (e.g. $H_{sat,}$ or different averaging times), water type (Devlin, et al., 2015) and nutrients and in relation to plant dynamics such as reproduction and meadow expansion. Also explore changes in the proportion of sugars and starch comprising TNSC (~ 6months at 0.5 FTE).
4. Calibrate existing carbohydrate data/samples against NIR to develop a more cost-effective analytical technique for TNSC (~6 months at 0.5 FTE).
5. Consider adoption of carbohydrates as a complimentary indicator of decline or recovery only if cheaper alternatives (NIR) for analysis prove successful.
6. Examine fine temporal scales of change (weeks to months), for application in assessment of acute disturbances.

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