Seagrass beds at Tuapiro Point

Seagrass health monitoring in Tauranga Harbour

Bay of Plenty Regional Council
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Executive summary/Whakarāpopototanga Matua

Seagrass meadows are an integral component of the estuarine ecosystem, providing a range of ecosystem services such as habitat and food provision, sediment stabilisation, carbon storage and the removal of nutrients. Large losses of seagrass cover in the Bay of Plenty occurred between 1959 and 2011 (a total loss of >1192 ha in Tauranga Harbour). A range of human induced stressors may be contributing to the loss of seagrass, including eutrophication, sedimentation, turbidity, water temperatures, waterfowl grazing and physical disturbances.

The Bay of Plenty Regional Council aims to give effect to the Regional Coastal Environment Plan, by preventing the further loss of the quality and extent of rare and threatened habitats such as seagrass. A one-year intertidal seagrass health project was conducted that aimed to firstly quantify the spatial and seasonal variability in various seagrass health indicators, secondly to identify potential stressors to seagrass based on health indicators, and thirdly to identify suitable indicators for the long-term measurement of seagrass health. The key outcome of the seagrass health project is to make recommendations on a new seagrass monitoring programme that is fit for purpose and meets our obligations to protect and enhance marine indigenous ecosystems. The project measured a number of seagrass health metrics at nine intertidal sites across Tauranga Harbour (once for six sites, and quarterly for three sites). The sites were chosen to cover an environmental gradient of mud and organic matter content to provide an assessment of the variability of seagrass health metrics across differing quality of estuarine habitat. Seagrass health metrics included a number of physical parameters (seagrass % cover, leaf length, leaf width, above and below ground biomass), chemical parameters (seagrass leaf C: N ratio, δ^{13}C, δ^{15}N and rhizome non-structural carbohydrate content), and environmental parameters (water light conditions (PAR, LUX), sediment grain size, sediment organic matter content, sediment porosity, sediment median grain size, chlorophyll *a*, phaeopigment).

A clear spatial variance in seagrass physical metrics was observed across the sites. In addition, large seasonal changes were evident at some sites, in relation to changing light conditions and decrease of seagrass coverage over winter. The combination of physical, environmental and chemical metrics of seagrass health have allowed clear site-specific trends to be detected. The effects of increased sediment muddiness, organic matter and corresponding reduction in light conditions had an effect on both the physical and chemical composition of the seagrass. Pahoia, Waiau, Matahui, Te Puna and Ongare responded to increasing muddiness/decreased light conditions with wider leaves and a negative relationship to δ^{13}C. Seagrass leaf C: N ratio was also increased at these sites under increasing eutrophication stress. Sites such as Tuapiro, Otumoetai, Waimapu and Ōmokoroa had narrower leaves and an increased δ^{13}C, indicative of high light conditions. An international seagrass health grading system Ecological Quality Status (EQS) utilising multivariate data was trialled to compile the health results to provide a grade. Waiau and Matahui were graded as poor, indicating higher stress and loss of ecological health occurring at these sites. These results appear to be driven by increased muddiness, organic matter and reduction in light conditions described above. Otumoetai was the only site graded as very good ecological quality.

The use of seagrass health indicators provides an estuarine habitat level assessment of health, which can be used as an integrated response indicator of the overall estuary health in that area. The data collected from a number of representative sites in Tauranga Harbour also provides a baseline against which future monitoring results can be compared to. Additional sites should be added across all Bay of Plenty estuaries, including a number of physical, chemical, disease and habitat quality metrics. A core set of seagrass health metrics have been identified for long-term measurement of seagrass health. These include physical metrics (meadow coverage, % cover, leaf length and width, above- and rhizome biomass, herbivory), chemical early warning metrics (leaf C and N, leaf C: N ratio, leaf δ^{13}C and δ^{15}N, rhizome sucrose), disease/algal stressors (fungal wasting disease, epiphyte and macro algae coverage) and habitat quality metrics (sediment grain size, sediment nutrient content (TOC, TN, TP), sedimentation rates, light conditions (PAR/turbidity)). These measurements should form an annual monitoring programme to be conducted in the mid-late summer period during the seagrass peak growth period. This monitoring programme will provide a set of suggested indicators to support future ecological modelling requirements to sustain healthy seagrass communities and overall estuarine health in Bay of Plenty estuaries.

Contents/Rārangi Upoko

Introduction/Kupu Whakataki

Seagrass ecosystems

Seagrass meadows are a critical component of the estuarine ecosystem, providing ecosystem services including habitat, food, and nursery areas for a range of fish and macroinvertebrates, which increases biodiversity (Morrison *et al.*, 2014). Seagrasses trap sediments and remove nutrients, whilst providing a carbon sink removing CO_2 and sequestering carbon within their sediments (Unsworth *et al.*, 2018). Seagrass ecosystems are estimated to contribute US \$6000 – \$19,000 ha^{-1} $year^{-1}$ of ecosystem services, potentially equal to, or greater than the value provided by salt marsh and mangroves (US \$9990 ha^{-1} $year^{-1}$ (Duarte *et al.*, 2008); based on 1997 values in Costanza *et al.*, 1997). Seagrass ecosystems are under threat globally from a range of human induced stressors including eutrophication, sedimentation/turbidity (reducing light availability), changing water temperatures, grazing by waterfowl and physical disturbance (dredging, marine structures) (Orth *et al.*, 2006, Roca *et al.*, 2016, Unsworth *et al.*, 2018). Part of the lack of action to protect seagrass ecosystems is the lack of awareness of the importance of these ecosystems compared to more charismatic systems (coral reefs, mangroves, saltmarsh) (Duarte *et al.*, 2008).

Seagrasses require high light levels for healthy photosynthetic activity (about 25% incident radiation; Morrison *et al.*, 2014). Increased turbidity in the water decreases light availability, limiting the ability of seagrass to photosynthesise and/or smothering the leaves whilst submerged. During emerged periods, seagrasses have been shown to increase primary productivity in response to increased turbidity during inundation (Drylie *et al.*, 2018). Eutrophication is the excessive enrichment of nutrients, resulting in an excessive growth of algae. Eutrophication can reduce light availability due to increased primary productivity, such as phytoplankton growth and the stimulation of epiphytic algae on the seagrass, killing seagrass or decreasing the amount of CO_2 fixed and stored in the sediments (Jiang *et al.*, 2018). Eutrophication may also indirectly reduce the ability of seagrass beds to sequester sediment organic carbon, by enhancing the growth and input of labile organic carbon enhancing microbial respiration and usage of deposited organic material (Jiang *et al.*, 2018). Seagrasses have low nitrogen loading tolerances, with a range of work showing a threshold N loading rate of 100 kg N ha^{-1} y^{-1} (27.4 mg m^{-2} d^{-1}) (Schallenberg *et al.*, 2017, Park, 2018). After this threshold limit, seagrass is sparse and macro algae and/or phytoplankton communities dominate. However, TN loading thresholds summarised in (Park, 2018) show loss of seagrass can be observed at nitrogen loading rates as low at 10 mg m^{-2} d^{-1}.

Seagrass in the Bay of Plenty

In New Zealand there is one species of seagrass, *Zostera muelleri* (dos Santos & Matheson, 2017), sometimes referred to as eelgrass. A variety of research has been conducted on this species in New Zealand and overseas, and tolerances to environmental conditions and stressors have been empirically tested (Dos Santos *et al.*, 2012, Bulmer *et al.*, 2016, Gladstone-Gallagher *et al.*, 2018, Li *et al.*, 2018). The thresholds of environmental stress could be utilized for management, ensuring conditions within seagrass beds remain favourable to support healthy growth. In the Bay of Plenty, mapping of aerial photography has been used to calculate the coverage of seagrass in estuarine ecosystems (the last survey was conducted in 2011). Tauranga Harbour holds the majority of our region's seagrass (96.4%, 2744.9 ha; (Park, 2016). Large losses of seagrass in Tauranga Harbour occurred between 1959 and 1996 (>1000 ha, 34%; Park, 1999), which predominantly occurred in the southern harbour (50% loss) and its enclosed sub-estuaries. Between 1996 to 2011, the overall loss was 192 ha (6.5% loss between 1996 to 2011, Park, 2016). In Ōhiwa Harbour the seagrass extent showed a loss of around 33 ha from 1945 to 1996, however it appeared to stabilise after this period up to 2011. In Maketū and Waihī estuaries, almost all seagrass has disappeared due to the degrading health (Park, 2018).

The impacts of epiphyte growth on seagrass at some sites in Tauranga Harbour have been observed, such as an example from Katikati at a sediment monitoring plate site, where seagrass was completely absent at the site in the year following the observed epiphytic algae growth (Figure 1). Further visual observations of a larger seagrass bed near this site showed filamentous algae growing in patches across the entire seagrass bed. A large bloom of green filamentous algae (*Cladophora* sp.) was observed further north in Tauranga Harbour, at Bowentown this past summer 2018/2019 (Figure 2), which will likely have impacts on the seagrass beds it covered during this period.

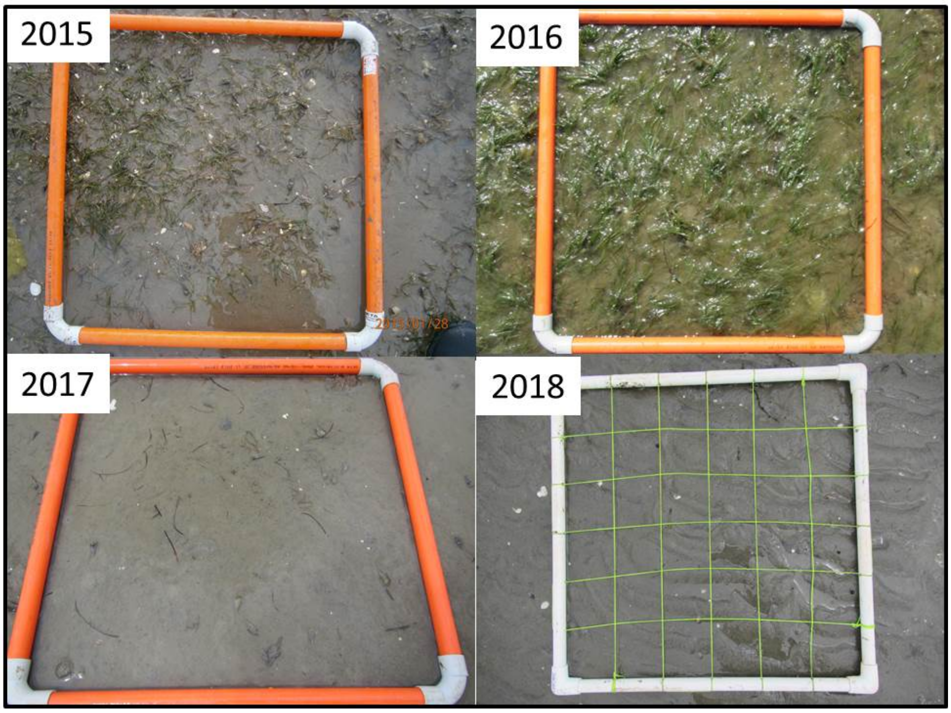

Figure 1 *Quadrat photos taken at sediment plate site 49 in Katikati. A large amount of epiphyte growth was observed on the seagrass in 2016, and in the following years seagrass was not observed at the site.*

Figure 2 *A large bloom of the green filamentous algae Cladophora sp. over a seagrass bed at Bowentown, northern Tauranga harbour.*

Regulatory framework

Various pieces of documentation exist in government to protect the health of estuarine ecosystems, which include provisions for seagrass. The New Zealand Coastal Policy Statement (2010) requires Councils: *"To protect indigenous biological biodiversity in the coastal environment"* and specifically to "*avoid significant adverse effects and avoid, remedy or mitigate other adverse effects of activities on: (iii) indigenous ecosystems and habitats that are only found in the coastal environment and are particularly vulnerable to modification including……eelgrass…"* (page 16).

The Bay of Plenty Regional Council (BOPRC) Regional Coastal Environment Plan, came into effect on the 3 December 2019. Seagrass loss is identified as a key issue (Issue 8) and a specific objective has been included to take action to prevent further loss (Objective 4): "*Prevent the further loss of the quality and extent of rare and threatened habitats in the coastal environment of the region. These include ….seagrass beds…"* (page 19). Seagrass health monitoring was funded in the science Long Term Plan (LTP) due to it being identified as a research gap in (Lawton, 2017), giving action to the LTP outcome "Freshwater for life: Good decision making is supported through improving knowledge of our water resources".

The National Policy Statement for Freshwater Management 2020 directs Regional Councils to recognise the interactions between freshwater, land, and sensitive receiving environments, including estuaries. This includes setting target attribute states for freshwater bodies, with regard to the connection to estuaries. Understanding the health of sensitive receiving environments and ecosystems within them (such as seagrass) will allow further evidence to support sustainable nutrient and sediment limits to ensure these ecosystems can thrive in the future.

Monitoring seagrass

Bioindicators can be used to complement snapshot environmental data collected in monitoring programmes, providing a time-integrated component, which reflects both past and current environmental conditions (McMahon *et al.*, 2013). A range of seagrass health indicators have been developed for use in environmental management (McMahon *et al.*, 2013, Roca *et al.*, 2016). Seagrass health has been widely studied, and a vast range of literature exists that can be used to indicate thresholds of stressors on seagrass health (further detail in selection of indicators below).

A list of robust indicators have been collated to identify seagrass stress responses to light limitation, nutrient enrichment, burial, organic matter and hypersalinity (Roca *et al.*, 2016), and the differences in timeframes that light stress begins to show in morphological indicators (McMahon *et al.*, 2013). Catastrophic losses of seagrass beds have been reported worldwide (Orth *et al.*, 2006), often occurring suddenly and without warning (Connell *et al.*, 2017). This sort of change indicates an ecological threshold or "tipping point", where a small change in environmental conditions can elicit a large response (Foley *et al.*, 2015, Selkoe *et al.*, 2015, Hewitt & Thrush, 2019). This threshold response has been experimentally tested in a seagrass ecosystem, where increasing nutrient levels past a certain concentration resulted in a switch from net seagrass production to net seagrass loss (Connell *et al.*, 2017).

These studies have identified a range of key morphometric indicators to measure seagrass health and resilience to environmental conditions, including percentage cover, leaf length and width, and above and below ground biomass. Simple chemical measurements of the seagrass leaves and rhizomes can provide additional information on nutrient/sediment stress and potential resilience (leaf C: N ratio and rhizome sucrose concentrations). A selection of these indicators were utilised in the current study, and are discussed in the methods section below.

Purpose/Take

The purpose of this study was to trial a one-year project focussing on intertidal seagrass health in Tauranga Harbour, linked closely with a BOPRC funded PhD project at University of Waikato. Specifically, the objectives of this seagrass health survey were to:

- Investigate spatial variability in seagrass health indicators both within and between seagrass sites;
- Investigate seasonal variability in seagrass health indicators (quarterly);
- Identify potential stressors to seagrass based on health indicators;
- Identify future threats to seagrass and research gaps; and
- Identify suitable indicators for the long-term measurement of seagrass health.

The key aim of the seagrass health project is to provide knowledge for the implementation of a new seagrass monitoring programme for the Bay of Plenty that is fit for purpose and meets our obligations to protect and enhance marine indigenous ecosystems under our Regional Coastal Policy Statement.

Methodology/Huarahi

Site Selection

Nine study sites were selected to represent a mud/eutrophication gradient across Tauranga Harbour. These sites aligned with a BOPRC funded PhD study, which is investigating the role of benthic photosynthetic communities (microphytobenthos, seagrass) in supporting benthic ecosystem function. A range of seagrass metrics and potential sediment stressors were collated to investigate the current seagrass and environmental conditions at each of the identified sites.

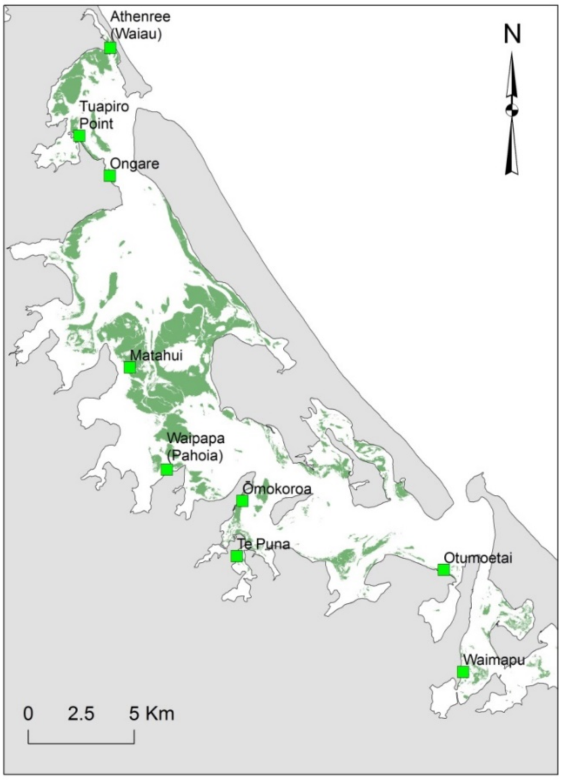

Figure 3 Site locations for the seagrass health project. Dark green colour within the harbour shows the seagrass cover recorded in 2011 (Park, 2016).

Table 1 Site locations for the BOPRC-funded PhD project and linked seagrass health project. Asterisk (*) indicates which sites were measured quarterly to investigate seasonal variability in seagrass health indicators. Co-ordinates are in NZTM.

Site name	Northing	Easting	Date sampled
Ōmokoroa	1868882	5829894	24/01/2019
Te Puna	1868619	5827291	25/01/2019
Otumoetai	1878380	5826642	29/03/2019
Matahui	1863569	5836189	25/02/2019
Pahoia	1865317	5831373	26/02/2019
Ongare	1862579	5845264	29/03/2019
Waimapu*	1879321	5821956	Summer 31/01/2019 Autumn 06/05/2019 Winter 15/07/2019 Spring 09/10/2019
Waiau*	1862677	5851173	Summer 31/01/2019 Autumn 06/05/2019 Winter 15/07/2019 Spring 09/10/2019
Tuapiro Point*	1861188	5847123	Summer 31/01/2019 Autumn 06/05/2019 Winter 15/07/2019 Spring 09/10/2019

Environmental characteristics

At the three quarterly sites, light/temperature loggers (Hobo MX Light/Temp) were deployed for one-week periods over each seasonal sampling to investigate the light and temperature environment. Loggers were set to sample every five minutes over the deployment period. The light data was then cleaned to remove periods of night (based on sunrise and sunset times). Sediment and light characteristics were available from the PhD project summer sampling. Light intensity (LUX) was recorded over four hour periods with a Hobo MX Light logger positioned 10 cm above the sediment surface, during the PhD project experiments. The loggers were set to record 10 minute average light intensity over the deployment period. The light conditions were collected for a different purpose over a short period of time, therefore must be interpreted with caution due to some varying weather conditions, and disturbance of muddy sediments by chamber insertion. Light intensity measurements were converted to PAR (umol m^{-2} s^{-1}) (Thimijan & Heins, 1983). Sediment mud content and sediment organic matter were quantified from five sediment cores (2.6 cm diameter to 2 cm depth) collected from each seagrass chamber (x 10). Porosity was calculated as sediment weight difference before and after drying at 60°C. Organic matter was quantified as the weight lost after furnace combustion (550°C for 4 hours). Sediment grain size was determined by laser diffraction (Malvern Mastersizer 3000) following the digestion of organic matter using 10% hydrogen peroxide. Chlorophyll *a* and phaeopigment content was determined using buffered 90% acetone to extract pigments followed by flurometric measurement on a Turne 10-AU flurometer, before and after acidification with hydrochloric acid (following methods by Arar & Collins (1997)).

Seagrass sampling

Sites were sampled during the peak summer growth period (January – February 2019). Additionally, quarterly measurements were taken at the three quarterly sites (Waimapu, Tuapiro and Waiau) (Table 1).

A range of seagrass health metrics were selected for this project and are detailed in Table 2.

Table 2 Summary of selected seagrass health indicators.

Seagrass physical morphometrics	Description of indicator	Response to degradation
Percentage cover (%)	Percentage seagrass cover is a population level indicator of seagrass health (Martínez-Crego *et al.*, 2008), and can respond to light stress over the timescale of months (McMahon *et al.*, 2013). Seagrass cover is expected to decrease with increasing anthropogenic stressors (Martínez-Crego *et al.*, 2008, Guimarães *et al.*, 2012).	Decrease
Leaf length	Seagrass leaf length is an individual level descriptor of plant health, and generally seagrass will decrease in length in response to a range of stressors (Cabaço *et al.*, 2008, Martínez-Crego *et al.*, 2008).	Decrease
Leaf width	Seagrass leaf width will generally decrease in response to light stress, and also alters the photosynthetic performance of seagrass (Collier *et al.*, 2012, Bertelli & Unsworth, 2018). However, due to the cost of loss of photosynthetic efficiency, some seagrasses show an increase in width to boost light adsorption (Ralph *et al.*, 2007, Collier *et al.*, 2012).In particular, the New Zealand species of seagrass (*Z.muelleri*) retains high leaf width under higher light limitation scenarios (Collier *et al.*, 2012). Thus, in our study, we consider wider seagrass leaves to be indicative of potential light stress following this research and personal observations in the field.	Increase
Above-ground biomass (leaves)	High above ground biomass indicates high stocks of seagrass leaves available for energy production, which is influenced by the percentage cover and leaf morphometrics (McMahon *et al.*, 2013). Above ground biomass is expected to decrease under stress (Leston *et al.*, 2008, García-Marín *et al.*, 2013). Biomass is a useful measure because it responds to perturbation quickly and in a sufficient amount to be detected statistically (Duarte & Kirkman, 2001).	Decrease
Below-ground biomass (rhizomes)	The rhizomes of the seagrass are the carbon energy stores for overwintering, and used in time of light or nutrient limitations. Higher rhizome biomass can indicate higher health and is expected to decrease under stress (Leston *et al.*, 2008, García-Marín *et al.*, 2013).	Decrease
Above: below ground ratio	Above/below ground ratio will increase when rhizome biomass is decreasing in comparison to above ground biomass, indicating periods of stress (Plus *et al.*, 2001, García-Marín *et al.*, 2013).	Increase
Seagrass nutrient storage		
Leaf nitrogen content	The leaf nitrogen content indicates the availability of nitrogen in the water column, and is expected to increase when there is an abundance of nitrogen availability (Martínez-Crego *et al.*, 2008). Leaf nitrogen can also increase in response to shading (Fernandez *et al.*, 2001, Cabaço *et al.*, 2008, Roca *et al.*, 2016).	Increase
Leaf $\delta^{15}N$ content	The leaf $\delta^{15}N$ can provide an assessment of the source of nitrogen in the water, due to the high microbial processing resulting in an excess of the heavier ^{15}N isotope. Higher $\delta^{15}N$ values can be indicative of human wastewater source, and lower values indicative of a nutrient source free of human influence (Cabaço *et al.*, 2008). It can also be indicative of high sediment nutrient processing (Vidon & Hill, 2005, Sebilo *et al.*, 2006), thus must be interpreted with care.	Increase
Leaf $\delta^{13}C$ content	Leaf $\delta^{13}C$ is a strong indicator of shading in plants (Roca *et al.*, 2016). Decreasing $\delta^{13}C$ values (more negative) indicate decreased light conditions and photosynthesis (Campbell & Fourqurean, 2009, Roca *et al.*, 2016) result in higher uptake of the $\delta^{13}C$ isotope and decreasing (more negative values).	Decrease
Leaf carbon/nitrogen ratio	Seagrass leaf C:N ratio is expected to be lower when seagrass increases nitrogen uptake and storage in the tissues (Burkholder *et al.*,	Decrease

	1994). The C:N ratio also decreases when light conditions decrease (McMahon *et al.*, 2013).	
Non-structural carbohydrates	Indicates the amount of underground food stores available to the seagrass in times of light limitation (overwintering, increased turbidity, sediment deposition) (Sørenson *et al.*, 2018). Sucrose stores can be decreased in periods of light limitation (Brun *et al.*, 2008). Previous work by Sørenson (in prep) has shown higher rhizome sucrose concentrations can enhance seasonal survival of seagrass in Tauranga Harbour.	Decrease

A representative site location was selected from within each seagrass bed on the seaward edge to align with the PhD sampling programme. Samples were haphazardly collected within a 50 x 20 m block. At each site, 10 photo quadrats (50 x 50 cm) were taken to assess percentage cover (Duarte & Kirkman, 2001) (Photo analysis of 50 points using Coral Point Count).

One sediment core (15 cm deep, 13 cm diameter) was taken from within each quadrat for collection of seagrass biomass on a 1 mm mesh sieve (10 sediment cores per site). Each seagrass sample was checked for epiphytes – if epiphytes were present these were scraped off and dry biomass measured. Seagrass samples were cleaned in freshwater to remove sediment, debris and dead tissues and frozen until analysis. Five of the ten seagrass core samples were measured for leaf characteristics. 10 seagrass leaves from five cores were haphazardly selected targeting the mature leaves, and measured for length and width with digital callipers resulting in 50 leaf measurements from each site. Each of the 10 seagrass samples were separated into above and below ground biomass, and the dry weight measured (dried to a constant weight at 60°C) (Duarte & Kirkman, 2001). Due to the difficulty in distinguishing between living and dead root biomass, only the rhizomes were measured and utilised as a proxy for below ground biomass (Li *et al.*, 2019).

A subsample of the dried leaves were ground to a powder using a coffee grinder and a subset from each site were sent for analysis of leaf carbon and nitrogen (including stable isotopes ($\delta^{13}C$ & $\delta^{15}N$; Isotrace, University of Otago). Additionally, a subset of the rhizome samples were analysed for rhizome non-structural carbohydrates (NSC; University of Waikato) following the protocols of (Sørensen *et al.*, 2018).

Data analysis/Ngā Tātaritanga Raraunga

One-way analysis of variance was used to investigate significant site differences in seagrass health metrics using the ‘aov’ function. Two-way analysis of variance was used for investigating seasonal changes in seagrass health, using site and season as fixed factors. Where significant interaction terms were found, sites were analysed for seasonal changes separately. ANOVA assumptions were tested using the ‘car’ package in RStudio with the Levenes test (homogeneity of variance) and Shapiro tests (normality of residuals), and transformed where necessary. Posthoc tukey tests were used to calculate pair-wise comparison between the means of the seagrass sites using ‘tukeyHSD’ or ‘hsdtest’ (‘agricolae’ package). Graphs and statistical processing were undertaken using R Studio 1.1.463 (RStudio Team 2016) with R base 3.6.0 (R Core Team 2019). Bar graphs were produced using package ‘ggplot2’ and ‘ggpubr’.

Pearsons correlation tables were used to investigate pair-wise relationships between seagrass health metrics and the environmental variables, using the ‘corstars’ function in R. Statistically significant relationships between variables were marked in bold and flagged with stars according to the level of significance ($p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***).

Multivariate metrics can be used to derive a qualitative measure of Ecological Quality Status (EQS) for seagrass beds (Romero *et al.*, 2007, García-Marín *et al.*, 2013). In this study the basis of the EQS was utilised with different seagrass health variables to create a metric for Tauranga Harbour seagrass. These rely on observed changes in health factors showing a positive or negative relationship to degradation, and deriving an “optimal” and “worst” seagrass condition factor for each variable. Artificial optimal and worst sites were established by averaging the top and bottom three measurements of each health variable, and these were added into the principal component as supplementary sites. This therefore means that if seagrass sites are chosen based on environmental gradients other than mud, the results may be variable and will need to be considered in future surveys. Principal component analysis (PCA) was used to combine the values of all the seagrass health metrics into a scale of ecological health. Seagrass health metrics were included which provided the best fit along PC1, to ensure the health grading was established based on a continuous gradient. The best fit included the physical metrics percentage cover, rhizome biomass, leaf biomass, and total biomass, and the chemical metrics leaf nitrogen and leaf C:N ratio.

The seagrass health survey sampling sizes were based off previous studies of seagrass metrics in Tauranga Harbour. However, many of these studies were designed to test for effects between treatment sites, rather than investigating long-term trends across sites. Therefore as part of this study, a power analysis was conducted to estimate the sample size to increase the power of the study to detect significant changes in seagrass health. The 'pwr' function in R was used to estimate the power of the current study, and investigate required sample size to reach 80% power at alpha 0.05.

The PCA was computed and visualised using the ‘factoextra’ package, which includes normalisation of the variables. Variables were square root transformed prior to analysis. The score of each sampling site on the first principal component is considered an estimate of its ecological health. The ecological quality ratio of each site (EQR'_x) was calculated as:

$$EQR'_x = (PC_x - PC_{worst}) / (PC_{optimal} - PC_{worst})$$

Where PC stands for principal component score on the first component. To enable easy to understand banding to be established, five health rankings were established based on García-Marín *et al.* (2013) and colour coded in line with BOPRC science reporting. These bands are described in Table 3.

The very poor grading has been set from 0 – 0.09 and only applies if no seagrass is present at a site, as we know many sites have lost seagrass across the Bay of Plenty (Park, 1999), however these sites were excluded from this study.

Table 3. The health grading and site banding of the seagrass Ecological Quality Rating (EQR) (Romero et al., 2007, García-Marín et al., 2013).

Health grade	EQR	Description
Very good	0.775 - 1	High seagrass cover, high quality health across indicators
Good	0.550 – 0.774	High seagrass cover, some high grading in some variables
Fair	0.325 – 0.549	Medium seagrass cover, decreased grading in many variables
Poor	0.1 – 0.324	Low cover seagrass, low grading in many variables
Very poor	0 – 0.09	No seagrass present

The “pwr” package in RStudio was used to determine the sample sizes required to detect an effect of a given size with a given degree of confidence. The group means, standard deviation and within group variance were calculated for each seagrass health metric using the seagrass health pilot study data. Power was set at 0.8, reflecting the test has an 80% probability of rejecting the null hypothesis (no site differences) with a 5% significance level.

Results/Ngā Otinga

Site environmental characteristics

Data were collated from the various monitoring programmes in estuary zones within Tauranga Harbour, based on the aerial seagrass monitoring programme (Park, 2016). The current seagrass extents and change over time, predicted NIWA sedimentation rates, actual sediment accumulation rates, and sediment mud content were collated to identify a range of intertidal seagrass sites covering the harbour representing a range of these indicators (Table 4). Mud content (%) is taken from the nearest site in 2019 prior to seagrass survey (Lawton & Conroy, 2019). % of ENSC is the current percentage of Estimated Natural State Cover of seagrass (Park, 2016) in each subestuary zone in 2011. Long term SAR is the Sediment Accumulation Rate at the nearest site to the survey, calculated from the BOPRC sediment plate monitoring programme (data up to 2018/19 sampling season). NIWA sediment model is the modelled sediment inputs to the sub-estuaries in the southern harbour (Green, 2010). % seagrass change is the change in seagrass cover between 1996 and 2011, and 2011 seagrass cover is the area of mapped seagrass cover in hectares (Park, 2016).

Table 4 The identified seagrass health project sites and associated characteristics. NA denotes there is no data available for that site.

Site	Mud %[a]	% of ENSC (2011)[b]	Long term SAR (mm/yr)[c]	NIWA sediment model (mm/yr)[d]	% seagrass change[b]	2011 seagrass cover (ha)[b]
Tuapiro	3.3	65.6	NA	NA	-17.44	254
Otumoetai	9.27	32.7	-0.2	0.0	-44.46	18
Waimapu	9.5	74.7	5.4	0.0	-2.07	45
Ōmokoroa	13.37	96.1	0.4	0.3	89.99	27
Ongare	14.07	90.5	-12.6	NA	90.06	9
Pahoia	17.8	43.9	4.8	0.0	11.77	50
Te Puna	18.2	70.1	2.9	0.7	74.02	18
Waiau	18.5	51.4	4.2	NA	-41.93	14
Matahui	34.2	66.6	NA	NA	-25.69	213

[a] ***Lawton & Conroy, 2019***
[b] ***Park, 2016***
[c] ***Unpublished BOPRC data***
[d] ***Green, 2010***

Sediment mud content, organic matter and water column light conditions were recorded at each seagrass health site to compare against the seagrass health metrics (Table 5). The seagrass sites varied greatly with their sediment composition. Mud content was significantly variable across sites (ANOVA, $F_{(8, 36)} = 54$, $p<0.001$). The muddiest site was at Te Puna with 19% mud content, and the sandiest site was at Tuapiro Point with 4.5% mud. Organic matter was variable across sites (ANOVA, $F_{(8, 36)} = 8$, $p<0.001$).The most organic rich sediments were at Te Puna and Pahoia (4.5% organic matter) and the least organic was at Waimapu (2.1% OM). Chlorophyll *a* concentrations were variable across sites (ANOVA, $F_{(8, 93)} = 63$, $p<0.001$). The highest concentration of chl *a* was recorded at Pahoia (33 ug g^{-1}), with the lowest concentration at Tuapiro (13 ug g^{-1}). Phaeopigment content was also variable across sites (ANOVA, $F_{(8, 35)} = 101$, $p<0.001$). Similar to chl *a*, the phaeopigment content was highest at Pahoia (20 ug g^{-1}), and lowest at Tuapiro (6 ug g^{-1}).

Light measured within chambers were significantly variable (ANOVA, $F_{(8, 93)} = 55$, $p<0.001$).The lowest average light conditions were recorded at Ongare (40 PAR) and the greatest at Tuapiro (908 PAR). Previous work in Tauranga Harbour sediments has shown that the sediment mud content is strongly related to increased concentrations of total organic carbon, total nitrogen, total phosphorus, and the heavy metals arsenic, copper and lead (Appendix A).

Table 5 *Average sediment and water column characteristics at the seagrass health sites in Tauranga Harbour in summer 2019 ordered from northern harbour to southern harbour sites. Average chamber light condition and site light condition reports the light intensity at each seagrass site over a 4 hour period. Error shows standard deviation. Grain size/mud content/water content n=10, organic matter n=5, chl a/phaeopigment n=10, chamber light n=5, site light n=1. Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

Site	Median grain size	% mud content	Water content (%)	% organic matter	Chl *a* (ug g^{-1})	Phaeopigment (ug g^{-1})	Porosity (0-2 cm)	Average chamber light conditions (PAR)	Site light conditions (PAR)
Waiau	217 ± 12^{c}	17.6 ± 1.8^{b}	26 ± 1.1^{ac}	2.4 ± 0.3^{ab}	16 ± 1.2^{bc}	9 ± 1.1^{bd}	28 ± 2.3^{ab}	441 ± 29^{b}	678
Tuapiro	176 ± 8^{b}	5.9 ± 1.0^{a}	27 ± 1.5^{ab}	2.7 ± 0.3^{ab}	13 ± 1.4^{ab}	6 ± 0.8^{a}	28 ± 4.3^{ab}	1030 ± 35^{d}	908
Ongare	135 ± 10^{a}	23.7 ±1.7^{cd}	26 ± 0.8^{bc}	2.9 ± 0.2^{bc}	18 ± 1.5^{c}	11 ± 2.0^{d}	30 ± 2.9^{bc}	334 ± 13^{b}	40
Matahui	172 ± 7^{bc}	19.4 ± 1.5^{d}	22 ± 0.8^{cd}	1.7 ± 0.2^{c}	12 ± 1.4^{e}	8 ± 1.7^{f}	30 ± 1.7^{c}	145 ± 8^{a}	293
Pahoia	204 ± 42^{b}	26.9 ± 7.0^{bc}	25 ± 0.3^{ac}	2.8 ± 0.1^{ab}	33 ± 6.6^{a}	20 ± 4.7^{bc}	33 ± 4.8^{bc}	370 ± 28^{b}	229
Ōmokoroa	122 ± 5^{a}	22.3 ± 2.2^{bd}	32 ± 1.8^{bc}	3.5 ± 0.2^{ac}	16 ± 1.5^{bc}	10 ± 1.4^{cd}	30 ± 1.9^{bc}	328 ± 18^{b}	84
Te Puna	113 ± 6^{a}	32.8 ± 1.2^{e}	32 ± 0.7^{d}	4.6 ± 0.2^{c}	22 ± 1.8^{d}	16 ± 2.7^{e}	33 ± 1.3^{c}	418 ± 42^{b}	578
Otumoetai	198 ± 14^{bc}	9.6 ± 1.6^{a}	25 ± 1.8^{ac}	2.0 ± 0.3^{ac}	21 ± 3.3^{d}	10 ± 1.1^{cd}	28 ± 1.4^{ab}	785 ± 18^{c}	895
Waimapu	251 ± 8^{d}	9.5 ± 0.8^{a}	22 ± 1.2^{a}	2.1 ± 0.2^{a}	15 ± 0.7^{ac}	7 ± 0.5^{ab}	25 ± 0.7^{a}	356 ± 24^{b}	644

Spatial variability in seagrass health indicators

Seagrass physical metrics

Seagrass percentage cover

Average seagrass cover within quadrats was highly variable across the nine monitored sites in Tauranga Harbour (Figure 4). Seagrass cover can be highly patchy and variable at the site scale. The lowest average seagrass cover was recorded at Waiau with an average cover of 34% (and a range of 14 – 56% cover) (Figure 5). The greatest average seagrass cover was recorded at Otumoetai with an average cover of 84 % (and a range of 66 – 100%) (Figure 5). Significant differences in cover were recorded between the different sites (ANOVA, $F_{(8, 79)} = 11.7$, $p<0.001$).

Figure 4. Example of seagrass quadrat coverage in summer from the three quarterly monitoring sites in summer 2019 (Tuapiro Point, Waiau, Waimapu).

Seagrass leaf length

Large physical differences in seagrass morphology were evident across the monitored sites (Figure 5). Average seagrass length was quite similar across many of the monitored sites in Tauranga Harbour. The lowest average leaf length was recorded at Omokoroa with an average leaf length of 48 mm (and a range from 4 – 74 mm). The greatest average leaf length was recorded at Waiau with an average length of 70 mm (and a range of 25 – 128 mm). Significant differences in leaf length were recorded between the different sites (ANOVA, $F_{(8, 41)} = 3.589$, $p<0.01$).

Seagrass leaf width

The average seagrass width was variable among sites (Figure 5). The smallest average seagrass width was recorded at Tuapiro Point (1.5 mm, range 0.93 – 2.27 mm) indicative of high water light conditions. The largest average seagrass width was recorded at Te Puna (3.0 mm, range 1.91 – 4.0 mm), suggesting decreased light conditions. Significant differences in leaf width were recorded among sites (ANOVA, $F_{(8, 41)} = 27.95$, $p<0.001$).

Seagrass above-ground biomass

The average seagrass above-ground biomass was variable among sites (Figure 6). The lowest average above-ground biomass was recorded at Ōmokoroa (287 g DW m^{-2}, range 171 – 422 g DW m^{-2}). The largest average above-ground biomass was recorded at Otumoetai (786 g DW m^{-2}, range 191 – 1090 g DW m^{-2}). Significant differences in above-ground biomass were recorded among sites (ANOVA, $F_{(8, 81)} = 7.676$, $p<0.001$).

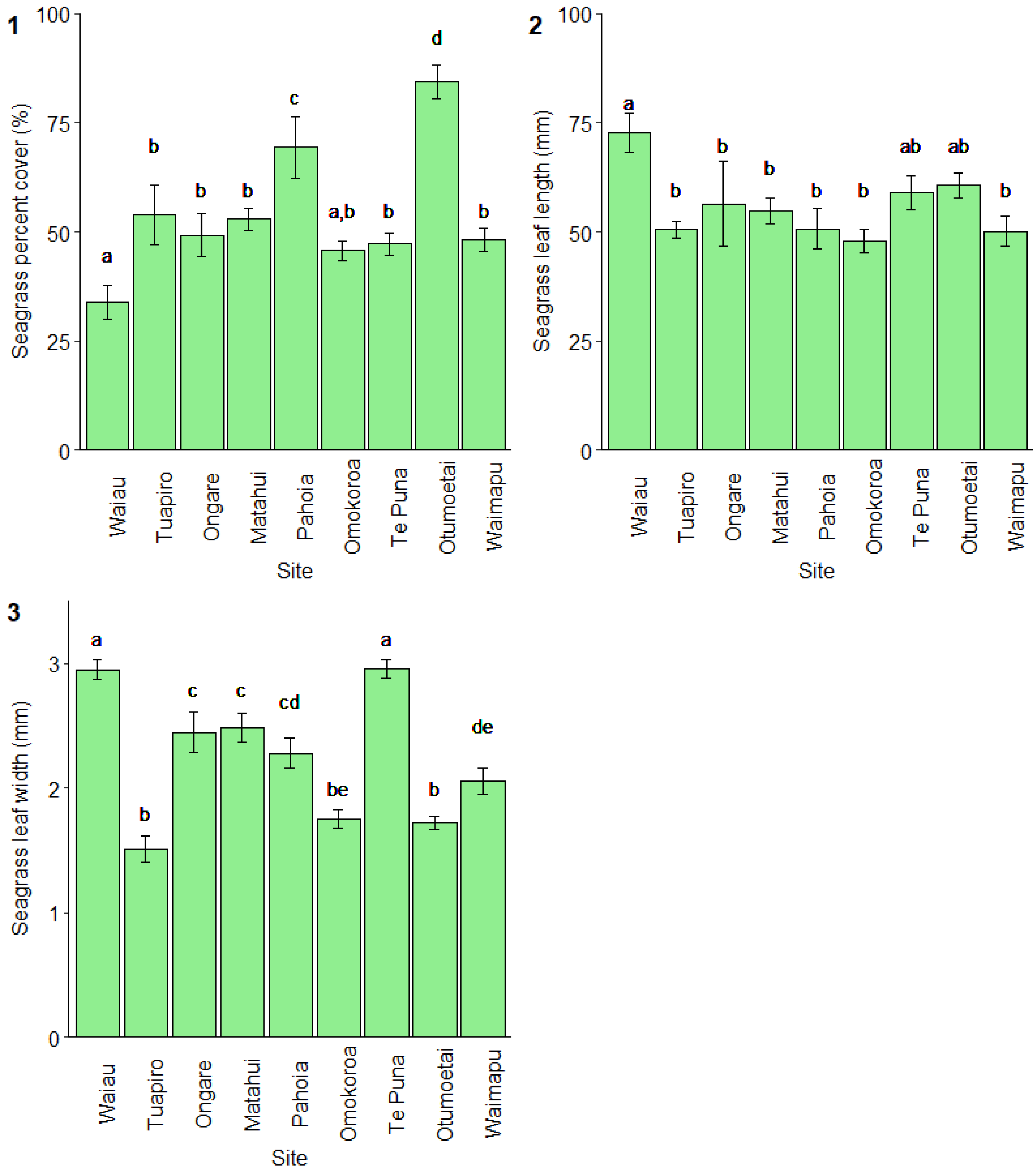

Figure 5 *Average seagrass metrics recorded in 0.25 m² quadrats in Tauranga Harbour in summer 2019. 1 = Seagrass percent cover (%). 2 = Seagrass leaf length (mm). 3 = Seagrass leaf width (mm). Error bars show standard error (seagrass cover, n = 10; leaf length and width, n = 50). Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

Seagrass rhizome biomass

The average rhizome biomass was variable among sites (Figure 6). The lowest average rhizome biomass was recorded at Matahui (85 g DW m^{-2}, range 25 – 231 g DW m^{-2}). The largest average rhizome biomass was recorded at Ongare (423 g DW m^{-2}, range 131- 583 g DW m^{-2}). Significant differences in rhizome biomass were recorded among sites (ANOVA, $F = _{(8, 81)} = 7.825$, $p<0.001$).

Seagrass above/below ground ratio

The average seagrass above- to below-ground biomass ratio was variable among sites (Figure 6). The lowest average ratio was recorded at Ōmokoroa (1.6 g, range: 0.7 – 3.5 g). The largest average above- to below-ground ratio was recorded at Matahui (6.4 g, range: 1.9 – 14.1 g). Significant differences in below-ground biomass were recorded among sites (ANOVA, $F = _{(8, 80)} = 4.082$, $p<0.001$).

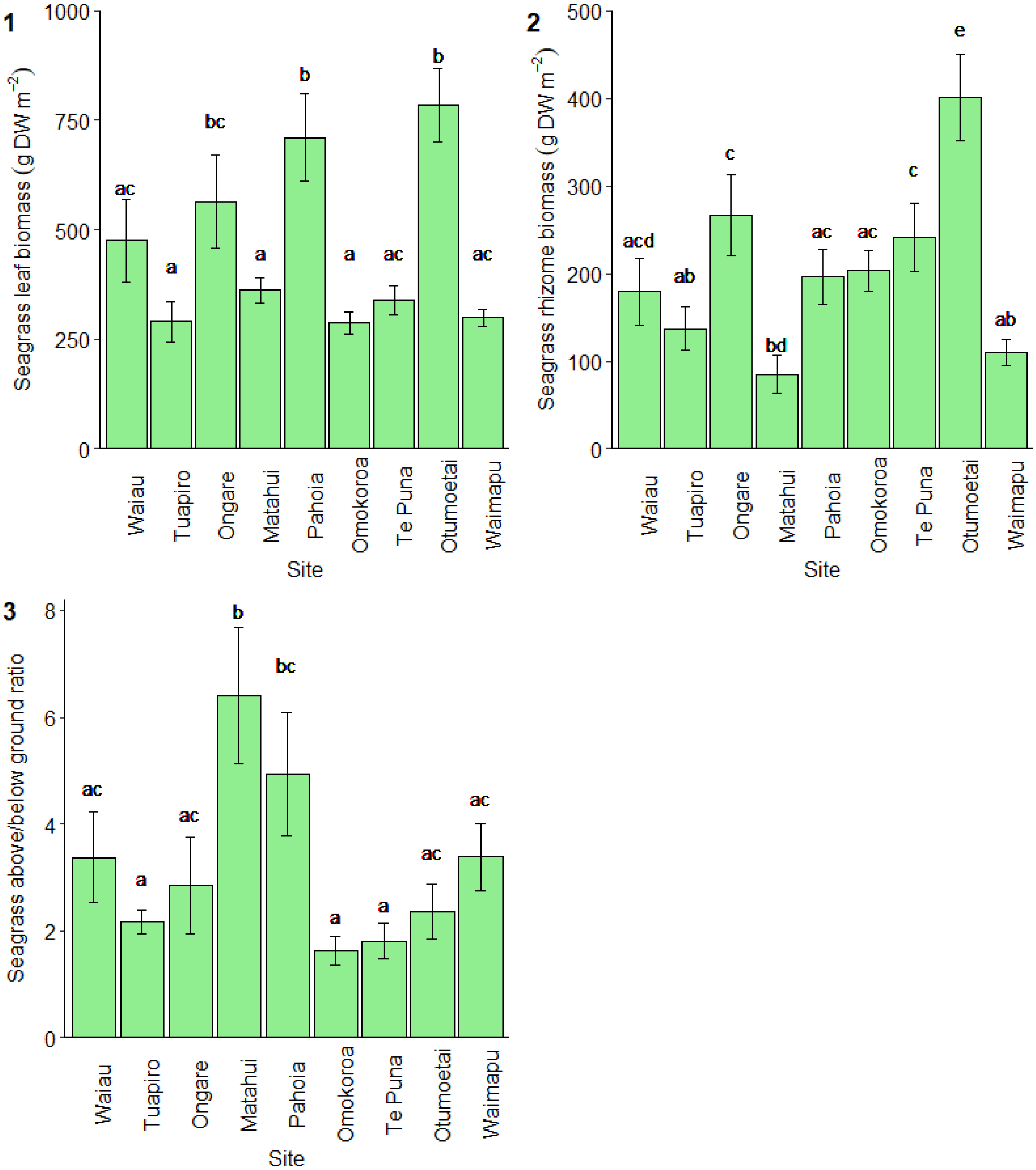

Figure 6 *Average seagrass metrics recorded in 13 cm wide cores in Tauranga Harbour in summer 2019. 1 = Seagrass leaf biomass (g DW m^{-2}). 2 = Seagrass rhizome biomass (g DW m^{-2}). 3 = Seagrass above/below ground ratio. Error bars show standard error (n = 10). Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

Seagrass nutrient storage

Carbon to nitrogen ratio

The average seagrass C: N ratio (mol: mol) was variable among sites (Figure 7). The lowest average ratio was recorded at Tuapiro Point (24.3, range 23.37 – 25.73). The greatest average C: N ratio was recorded at Waiau (32.4, range 31.7 – 33.92). Significant differences in C: N ratio were recorded among sites (ANOVA, $F = _{(8, 18)} = 8.396$, $p<0.001$).

Leaf nitrogen content

The average seagrass leaf nitrogen was not highly variable between sites (Figure 7). The lowest average nitrogen content was recorded at Waiau (1.22%, range 1.17 – 1.29%). The greatest leaf nitrogen was recorded at Otumoetai (1.67%, range 1.50 – 1.85%). The only significant differences were observed between Waiau and Otumoetai (ANOVA, $F = _{(8, 18)} = 3.245$, $p<0.05$).

Leaf carbon content

The average leaf carbon content had minimal variability between sites (Figure 7). The lowest average site carbon content was at Tuapiro (31%, range 26.97 – 34.45%) and the greatest carbon content at Otumoetai (35.4%, range 34.35 – 36.17%). No significant differences in leaf carbon were reported between sites (ANOVA, $F = _{(8, 18)} = 1.855$, $p>0.05$).

Leaf $\delta^{15}N$ content

There was a lot of variability observed between sites (Figure 7). The lowest site average $\delta^{15}N$ was recorded at Pahoia (4.6‰, range 4.1‰ – 5.5‰) and the greatest at Waimapu (6.9‰, range 6.5‰ – 7.4‰). There were significant differences between sites (ANOVA, $F = _{(8, 18)} = 10.5$, $p<0.001$).

Leaf $\delta^{13}C$ content

High seagrass $\delta^{13}C$ variability was recorded between sites (Figure 7). Pahoia had the most negative $\delta^{13}C$ value (-12‰, range -11.5‰ to -12.6‰) and Tuapiro the least negative (-8.4‰, range -7‰ to -10‰). Significant differences in $\delta^{13}C$ were reported between sites (ANOVA, $F = _{(8, 18)} = 6.5$, $p<0.001$).

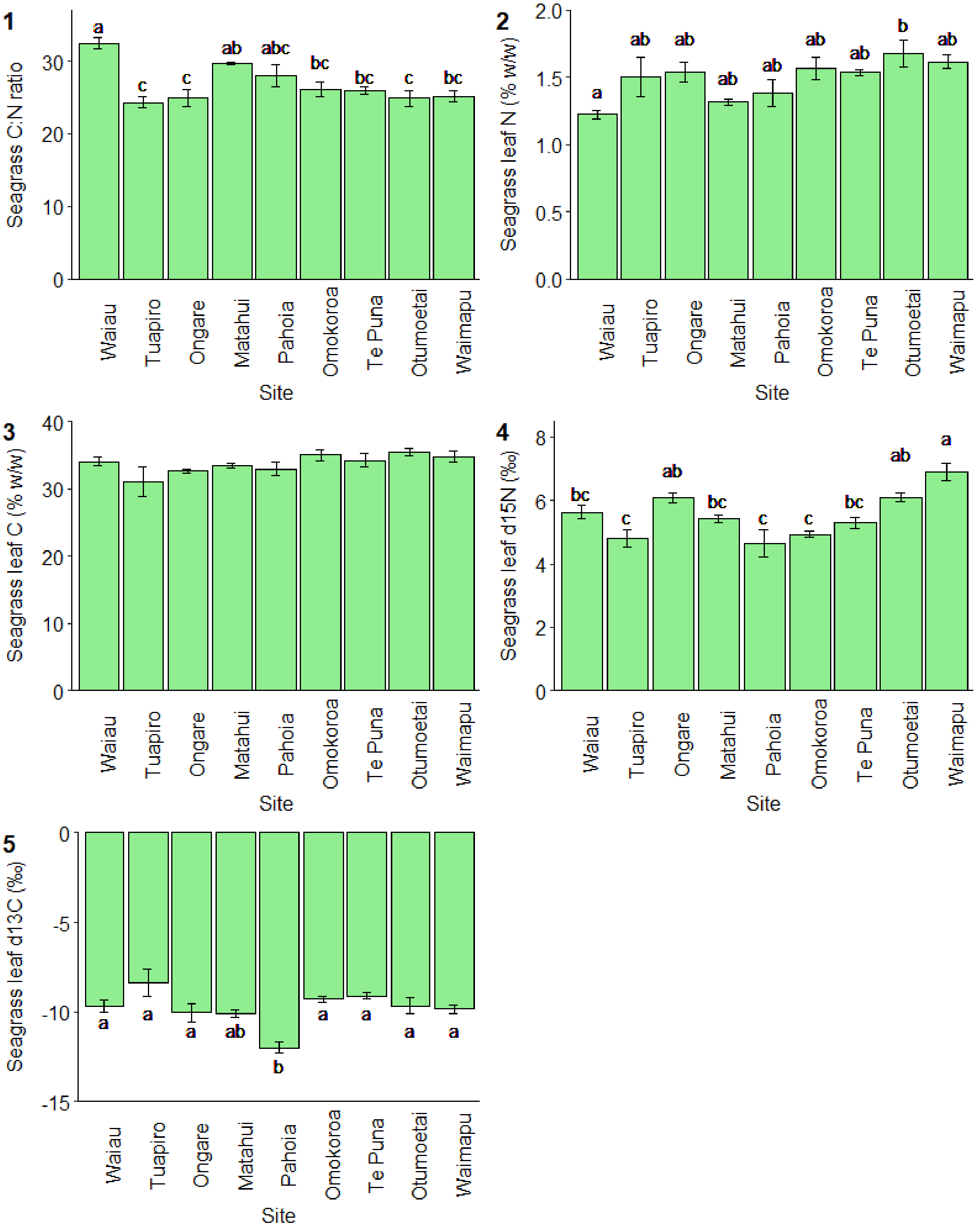

Figure 7 *Average seagrass leaf nutrient metrics recorded in 13 cm wide cores in Tauranga Harbour in summer 2019. 1 = seagrass leaf C: N ratio. 2 = seagrass leaf N content (%). 3 = seagrass leaf C content (%). 4 = seagrass leaf $d^{15}N$ (‰). 5 = seagrass leaf $d^{13}C$ (‰). Error shows standard error (n=3). Tukey posthoc test results are shown where significant variation was observed between sites – the same letter notes the sites are not significantly different from each other.*

Seagrass non-structural carbohydrates

Three sites had rhizomes analysed for non-structural carbohydrates (NSC) (Figure 8).

Significant differences in total soluble NSC were recorded across the measured sites (ANOVA, $F = _{(2, 6)} = 7.353$, $p<0.05$), with greater NSC storage in Tuapiro compared to Waimapu. Significant differences in sucrose content were also recorded (ANOVA, $F = _{(2, 6)} = 7.371$, $p<0.05$), with Tukey posthoc test results showing a significant difference between Waimapu and Tuapiro sucrose content. No significant differences in fructose content (ANOVA, $F = _{(2, 6)} = 2.936$, $p>0.05$), glucose content (ANOVA, $F = _{(2, 6)} = 0.784$, $p>0.05$), or starch content were observed (ANOVA, $F = _{(2, 6)} = 0.58$, $p>0.05$).

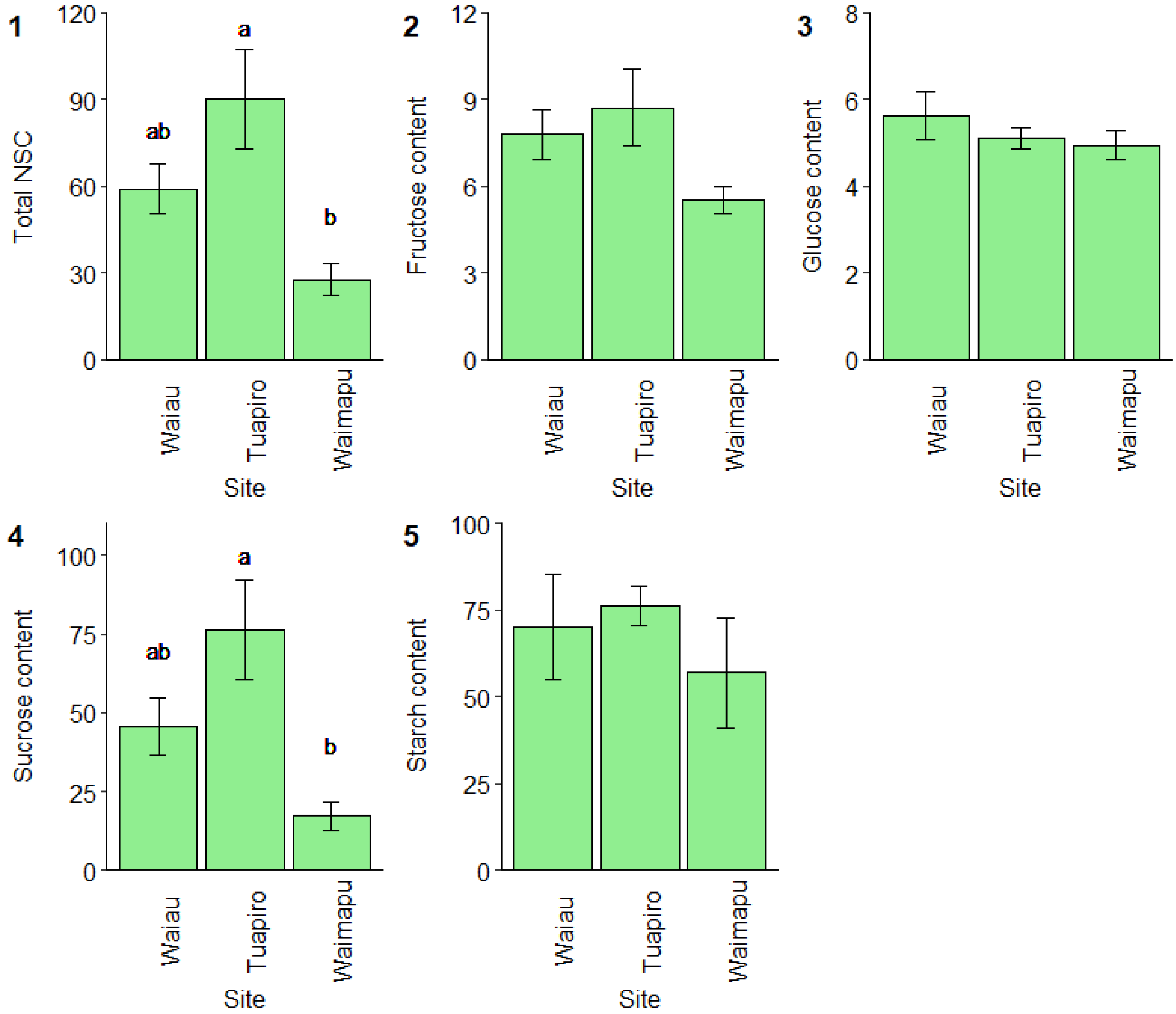

Figure 8 *Average seagrass rhizome non-structural carbohydrates (NSC) recorded in 13 cm wide cores in Tauranga Harbour in summer 2019. 1 = total non-structural carbohydrates (mg/g rhizome DW). 2 = fructose content (mg/g rhizome DW). 3 = glucose content (mg/g rhizome DW). 4 = sucrose content (mg/g rhizome DW). 5 = starch content (mg/g rhizome DW). Error bars show standard error (n=3). Tukey posthoc test results are shown where significant variation was observed between sites – the same letter notes the sites are not significantly different from each other.*

Seasonal variability in seagrass health indicators

Seagrass coverage was variable across both the seasons (ANOVA, $F = _{(3, 108)} = 33.8$, $p<0.001$) and sites measured (ANOVA, $F = _{(2, 108)} = 15.34$, $p<0.001$) (Figure 9). There was also an interactive effect between season and site (ANOVA, $F = _{(2, 108)} = 15.34$, $p<0.001$), indicating that the seasonal differences in seagrass percent cover is dependent on the site investigated. At all sites summer seagrass percentage cover was higher than winter and spring. Waimapu also had significantly higher seagrass coverage in autumn compared to winter and spring.

Changes in leaf length were evident across seasons (ANOVA, $F = _{(3, 53)} = 4.4$, $p<0.01$) and sites (ANOVA, $F = _{(2, 53)} = 61.7$, $p<0.001$) (Figure 9). There was also a significant interaction effect between site and season, indicating seasonal changes in leaf length were dependant on site location (ANOVA, $F = _{(6, 53)} = 3.4$, $p<0.01$). At Waiau, leaf length was significantly shorter in summer compared to spring. At Waimapu, leaf length was significantly shorter in summer compared to winter. At Tuapiro, leaf length was shortest in autumn compared to winter and spring.

Leaf width was stable at the sites seasonally, with no changes in leaf width across seasons (ANOVA, $F = _{(3, 53)} = 1.4$, $p>0.05$) (Figure 9).

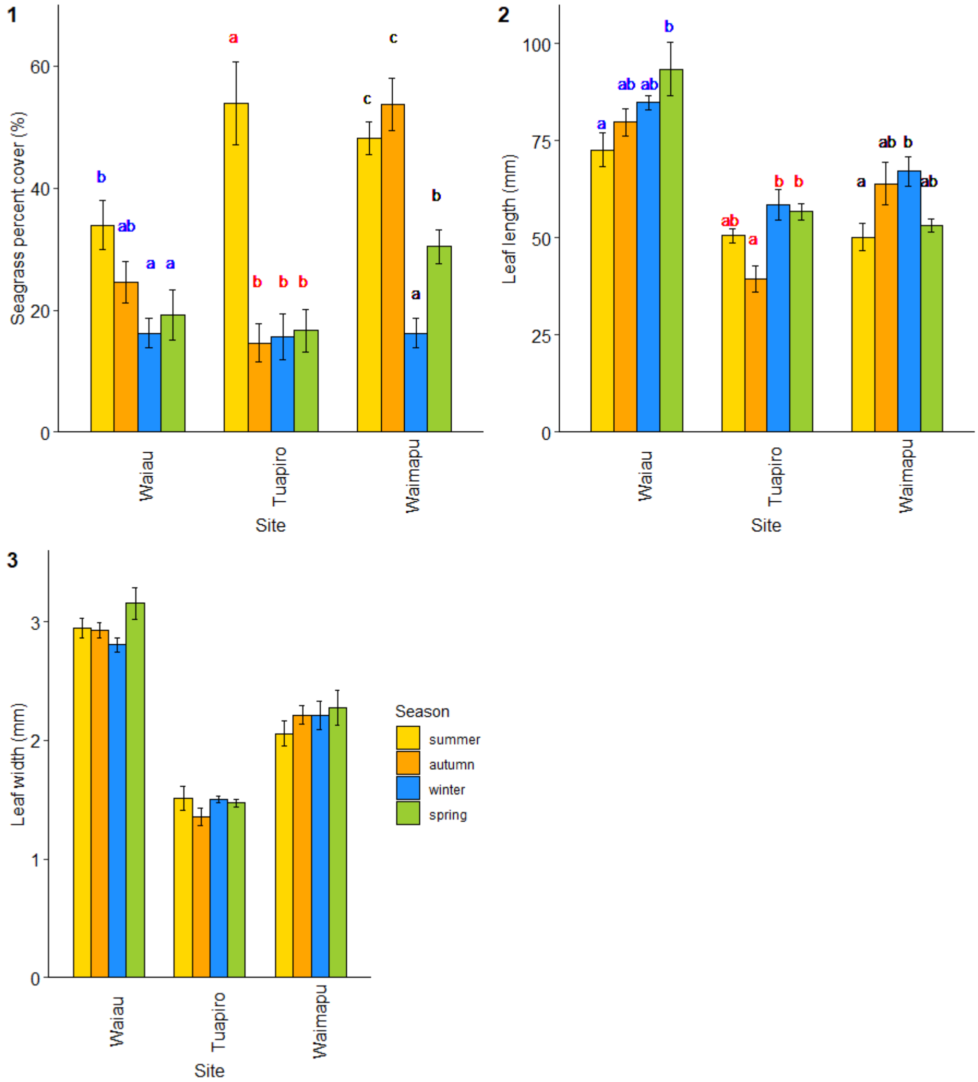

Figure 9 *Average seagrass metrics recorded in 0.25 m² quadrats in Tauranga Harbour in across seasons in 2019. 1 = Seagrass percent cover (%). 2 = Seagrass leaf length (mm). 3 = Seagrass leaf width (mm). Error bars show standard error (seagrass cover, n = 10; leaf length and width, n = 50). Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

Leaf biomass changed slightly between seasons (ANOVA, $F_{(3, 108)} = 3.9$, $p<0.05$), with high variability between sites (ANOVA, $F_{(2, 108)} = 22.3$, $p<0.001$), and a strong interaction effect (ANOVA, $F_{(6, 108)} = 4.6$, $p<0.001$) (Figure 10). Seasonal trends were highly variable across the sites. At Waiau, peak biomass occurred summer compared to winter. At Tuapiro, peak leaf biomass occurred in summer compared to autumn and spring. At Waimapu, autumn had higher leaf biomass compared to summer and spring.

The rhizome biomass changed across seasons (ANOVA, $F_{(3, 108)} = 6.7$, $p<0.001$) (Figure 10), sites (ANOVA, $F_{(2, 108)} = 7.8$, $p<0.001$), and the interaction term (ANOVA, $F_{(6, 108)} = 4.3$, $p<0.001$). No significant rhizome biomass changes were evident at Waiau or Tuapiro. At Waimapu, rhizome biomass was significantly higher in winter and spring, compared to summer and autumn.

The above/below ground ratio changed greatly across the seasons (ANOVA, $F_{(3, 107)} = 20.3$, $p<0.001$) (Figure 10), sites (ANOVA, $F_{(2, 107)} = 7.3$, $p<0.01$), and the interaction term (ANOVA, $F_{(6, 107)} = 2.3$, $p<0.05$). At both Tuapiro and Waimapu, above/below ground ratio was higher in summer and autumn, compared to winter and spring. At Waiau, above/below ground ratio was greatest in autumn compared to winter.

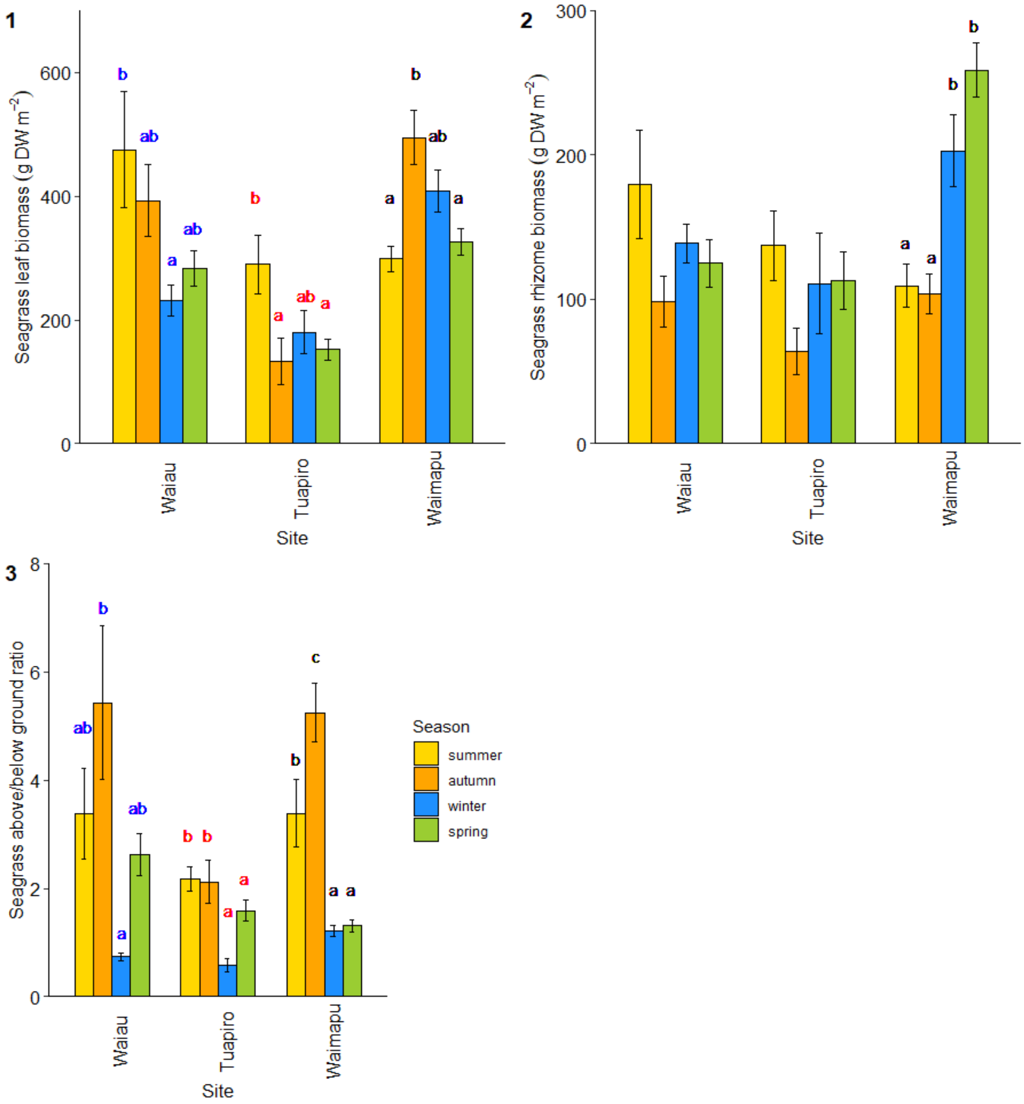

Figure 10 *Average seagrass metrics recorded in 13 cm wide cores in Tauranga Harbour across seasons in 2019. 1 = Seagrass leaf biomass (g DW m^{-2}). 2 = Seagrass rhizome biomass (g DW m^{-2}). 3 = Seagrass above/below ground ratio. Error bars show standard error (n = 10). Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

There was no significant seasonal variance in the leaf C: N ratio with the combined analysis (ANOVA, $F_{(3, 24)} = 2.7$, $p>0.05$), however there was significant site (ANOVA, $F_{(2, 24)} = 8.1$, $p<0.01$), and interaction terms (ANOVA, $F_{(6, 24)} = 29.8$, $p<0.001$) (Figure 11). When sites were analysed for seasonal trends individually, significant seasonal changes were evident. At Waiau, the winter and spring C: N ratio was higher than summer and autumn. The opposite trend was observed at Waimapu, with the highest C: N ratio occurring in summer and autumn compared to the lower values in winter and spring. At Tuapiro, the highest C: N ratio occurred in winter, compared to the lowest in autumn.

Similar to the leaf C: N ratio, the leaf nitrogen content showed no significant seasonal variance in the combined analysis (ANOVA, $F_{(3, 24)} = 0.51$, $p>0.05$), however site (ANOVA, $F_{(2, 24)} = 4.6$, $p<0.05$), and interaction trends were evident (ANOVA, $F_{(6, 24)} = 11.6$, $p<0.001$) (Figure 11). No seasonal trends were evident at Tuapiro. At Waiau, leaf nitrogen was significantly higher in summer compared to all other seasons. At Waimapu, winter and spring had significantly higher nitrogen compared to summer and autumn.

No significant seasonal trends were evident in the leaf carbon content (ANOVA, $F_{(3, 24)} = 0.1$, $p>0.05$), or interaction term (ANOVA, $F_{(6, 24)} = 0.3$, $p>0.05$), however there was site differences evident (ANOVA, $F_{(2, 24)} = 6.7$, $p<0.01$) (Figure 11).

There were differences observed in the leaf $\delta^{15}N$ across seasons (ANOVA, $F_{(3, 24)} = 19.3$, $p<0.001$), sites (ANOVA, $F_{(2, 24)} = 18.6$, $p<0.001$), and the interaction term (ANOVA, $F_{(6, 24)} = 3.6$, $p<0.05$) (Figure 11). No significant $\delta^{15}N$ changes were observed at Tuapiro. At both Waimapu and Waiau, $\delta^{15}N$ was higher in winter and spring compared to summer and autumn.

Significant differences were observed in the leaf $\delta^{13}C$ across seasons (ANOVA, $F_{(3, 24)} = 14.1$, $p<0.001$), sites (ANOVA, $F_{(2, 24)} = 20.9$, $p<0.001$), and the interaction term (ANOVA, $F_{(6, 24)} = 3.9$, $p<0.01$) (Figure 11). No significant differences in $\delta^{13}C$ were observed at Waimapu. At Waiau, $\delta^{13}C$ was more negative in winter and spring compared to summer and autumn. At Tuapiro, $\delta^{13}C$ was most negative in spring compared to autumn.

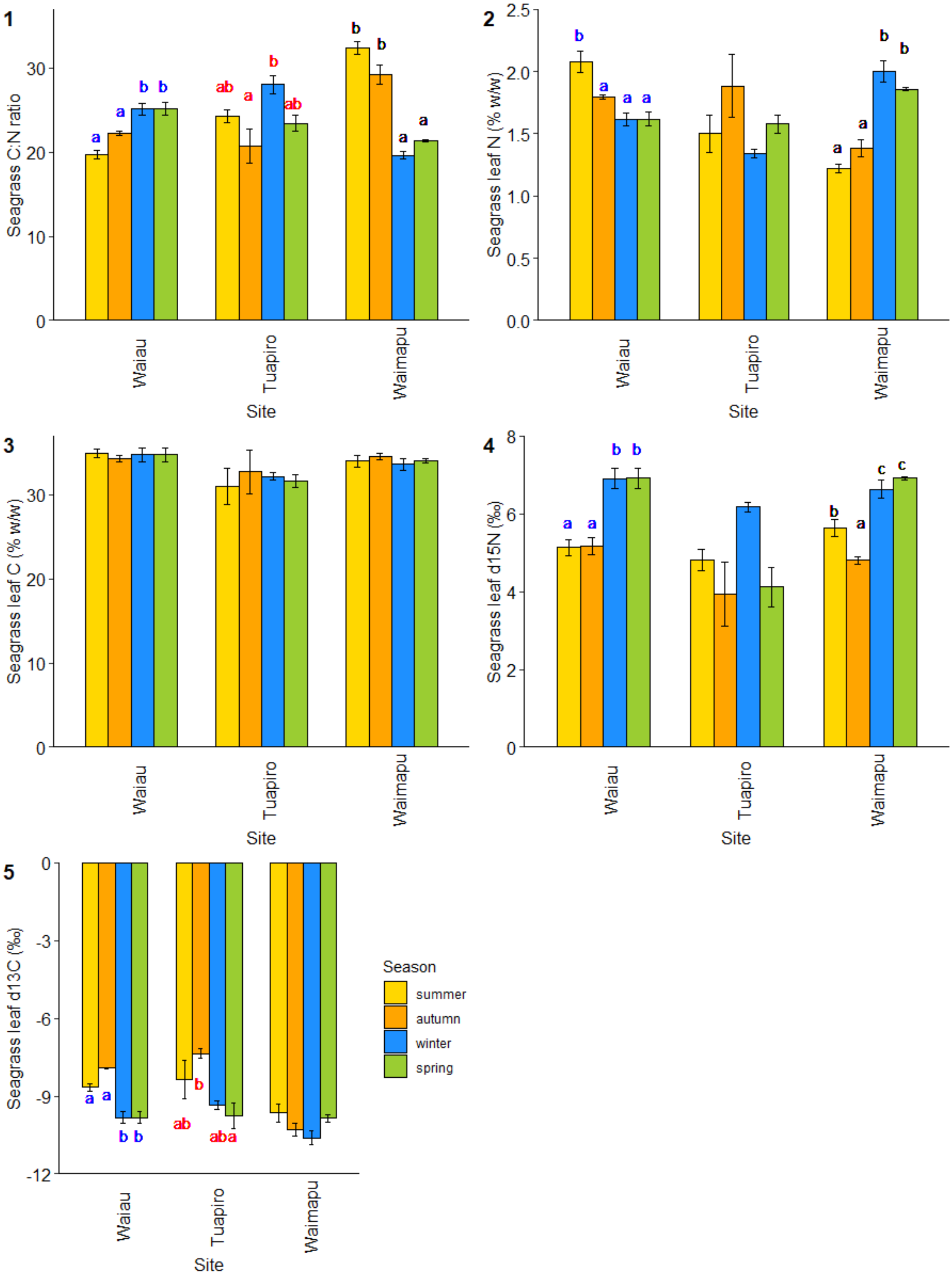

Figure 11 *Average seagrass leaf nutrient metrics recorded in 13 cm wide cores in Tauranga Harbour across seasons in 2019 (n=3). 1 = Seagrass C: N ratio. 2 = Seagrass leaf nitrogen (% w/w). 3 = Seagrass leaf carbon (% w/w). 4 = Seagrass leaf δ15N (‰). 5 = Seagrass leaf δ13C (‰). Error bars show standard error. Tukey posthoc test results are shown where significant variation was observed between sites with the same letter indicating sites that are not significantly different from each other.*

Light environment

Water light conditions changed significantly over the seasonal period (ANOVA, $F_{(3, 66)} = 14.8$, $p<0.001$), with no site (ANOVA, $F_{(2, 66)} = 2.6$, $p>0.05$), or interaction effects (ANOVA, $F_{(6, 66)} = 0.2$, $p>0.05$) (Figure 12). Summer had significantly greater light irradiance compared to autumn, winter, and spring ($p<0.001$). The light climate was highly variable during the spring, with some large periods of both low light and high light occurring. Autumn and winter had the lowest average light conditions.

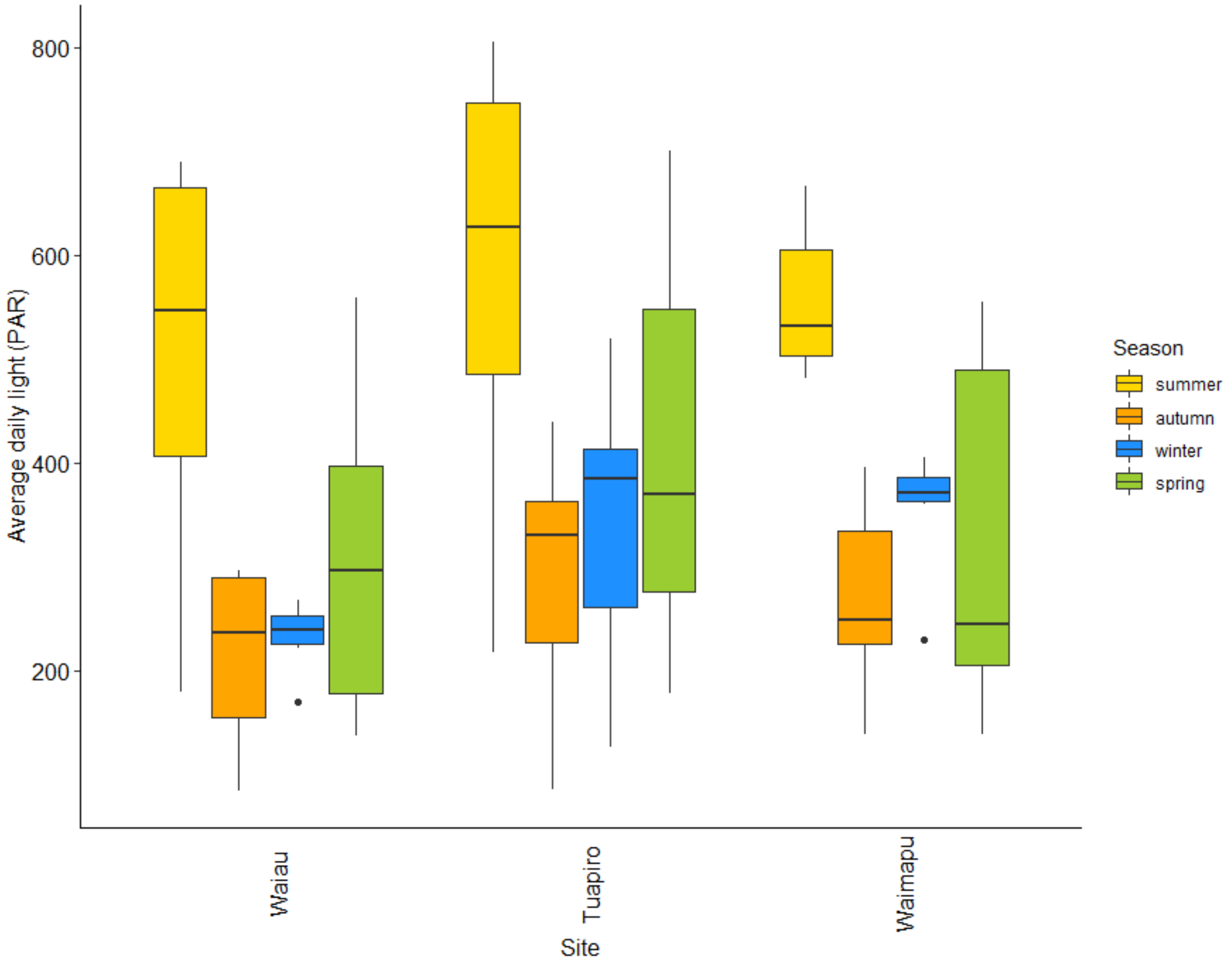

Figure 12 Average daily light (PAR) at the three seagrass sites across seasons, showing the spread of light availability over a seven day period.

Stressors of seagrass health based on health indicators

Seagrass metrics correlations

Strong relationships were evident between a number of the seagrass health metrics (Table 6). Seagrass cover was positively related to total dry biomass, leaf biomass, and rhizome biomass ($p<0.001$), with a negative relationship to leaf width ($p<0.01$). Seagrass cover also showed relationships with some of the leaf nutrient storage measurements, including a positive relationship to leaf nitrogen content ($p<0.01$), and negative relationships to $d^{13}C$ and C: N ratio ($p<0.01$). Leaf length showed positive relationships to the leaf width ($p<0.001$), leaf biomass and total dry biomass ($p<0.01$). Leaf length also had relationships to the nutrient storage measurements, including a positive relationship to leaf C: N ratio ($p<0.01$) and a negative relationship to leaf nitrogen ($p<0.05$). Leaf width had similar relationships to leaf length, including a positive relationship to C: N ratio ($p<0.001$), and negative relationships to leaf nitrogen ($p<0.001$) and $d^{13}C$ ($p<0.05$). Leaf biomass was positively related to the rhizome biomass and total dry biomass ($p<0.001$), and the A/B ratio ($p<0.01$). It had a negative relationship to the $d^{13}C$ ($p<0.001$). The rhizome biomass showed positive relationships to the total dry biomass ($p<0.001$) and leaf nitrogen ($p<0.01$), and negative relationships to A/B ratio ($p<0.001$) and leaf C: N ratio ($p<0.05$). Total dry biomass had negative relationships to $d^{13}C$ ($p<0.01$).

The A/B ratio showed negative relationships with leaf nitrogen and $d^{13}C$ ($p<0.01$), and positive relationships to the C: N ratio.

Environmental health gradient

The sampling sites were characterised from poor to high ecological quality utilising a PCA to create a single gradient of mud/eutrophication, utilising mud content, organic matter and light (Table 7, Figure 13). These environmental variables were the best predictive variables of poorer seagrass health, as the chlorophyll *a* and phaeopigment metrics increased positively with the seagrass health metrics. The CI scores indicate an "environmental health gradient", where lower CI values reflect poorer health with increasing mud and organic matter, and decreasing light availability (Table 8, Figure 14). The environmental CI scores showed clear correlations with each of the environmental variables in the principal component (Figure 14). The first PC explained 66.6% of the observed variance and is proposed as a good fit for assigning environmental health gradients to the seagrass sites. An arbitrary three scale grade was applied to the environmental health gradient, resulting in four sites graded as high (Waiau, Tuapiro, Otumoetai, Waimapu), one as intermediate (Matahui), and four as low (Ongare, Pahoia, Ōmokoroa, Te Puna) (Table 8).

*Table 6. Pearsons correlation maxtrix showing pair-wise relationships between seagrass health metrics. Significant results are displayed in bold. Significance levels are shown with stars ($p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***).*

	Seagrass cover	Leaf length	Leaf width	Leaf biomass	Rhizome biomass	Total dry biomass	A/B ratio	$d^{15}N$	Nitrogen %	$d^{13}C$	Carbon%
Leaf length	0.03										
Leaf width	**-0.28****	**0.52*****									
Leaf biomass	**0.57*****	**0.31****	0.07								
Rhizome biomass	**0.47*****	0.15	-0.06	**0.45*****							
Total dry biomass	**0.60*****	**0.29****	0.01	**0.89*****	**0.68*****						
A/B ratio	-0.01	0.09	0.19	**0.34****	**-0.62*****	0.04					
$d^{15}N$	-0.03	0.14	0.1	0.02	0.1	0.03	-0.05				
Nitrogen%	**0.31****	**-0.23***	**-0.48*****	0.02	**0.28****	0.17	**-0.29****	**0.29****			
$d^{13}C$	**-0.27****	0.03	**-0.24***	**-0.37*****	-0.03	**-0.28****	**-0.30****	0.08	0.2		
Carbon%	0.05	0.13	0.08	0.07	0.16	0.13	-0.04	**0.35*****	**0.42*****	-0.1	
C:N ratio	**0.32****	**-0.33****	**-0.56*****	-0.02	**0.23***	0.12	**-0.29****	0.14	**0.90*****	**0.27****	-0.02

Table 7 *The percentage of variance explained and PCA loadings environmental characteristics used to determine the environmental health gradient.*

Variable	PC1	PC2
Explained variance	66.6%	25.4%
Environmental characteristics		
OM	-0.748	0.624
Mud	-0.929	-0.006
Light	0.758	0.610

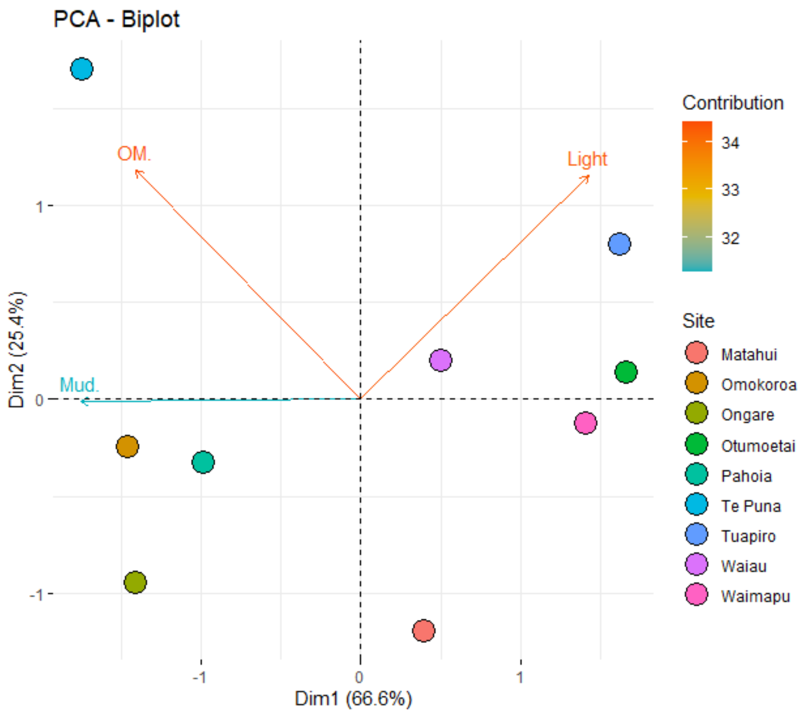

Figure 13 *Environmental factor PCA used to determine the best environmental gradient for assessment of effectiveness of Ecological Quality Ratio (EQR). The more negative PC values indicate declining environmental health.*

Table 8. *Environmental characterization of sites based on the measured sediment mud and organic matter content, and the site light characteristics. Site scores of the first PCA component (CI score) and the arbitrary three-scale grade of health.*

Site	Mud %	OM %	Light	CI Score	Grade
Waiau	17.6 ± 1.8	2.4 ± 0.3	36630	1.664	High
Tuapiro	5.9 ± 1.0	2.7 ± 0.3	49052	1.628	High
Ongare	23.7 ±1.7	2.9 ± 0.2	2141	-1.410	Low
Matahui	19.4 ± 1.5	1.7 ± 0.2	15825	0.399	Intermediate
Pahoia	26.9 ± 7.0	2.8 ± 0.1	12347	-0.988	Low
Omokoroa	22.3 ± 2.2	3.5 ± 0.2	4525	-1.460	Low
Te Puna	32.8 ± 1.2	4.6 ± 0.2	31238	-1.749	Low
Otumoetai	9.6 ± 1.6	2.0 ± 0.3	48307	1.664	High
Waimapu	9.5 ± 0.8	2.1 ± 0.2	34770	1.412	High

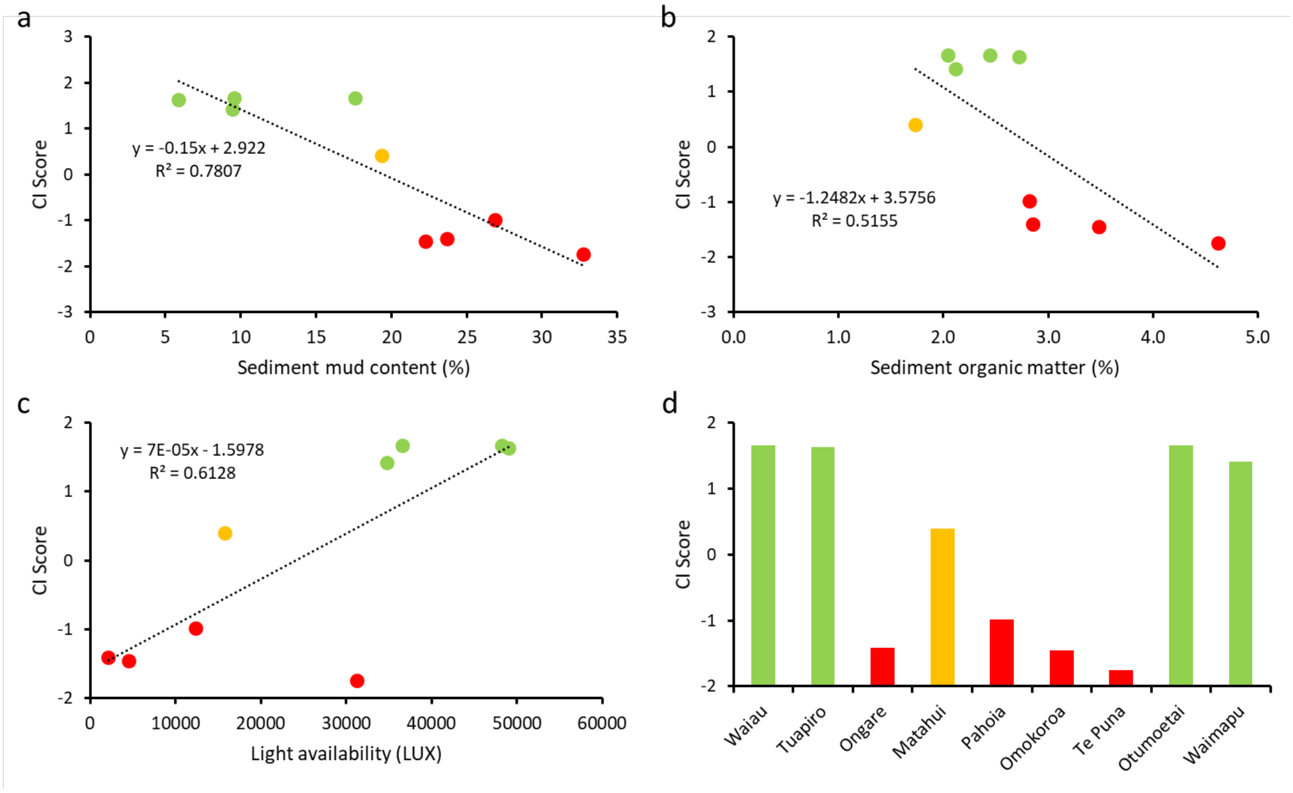

Figure 14 *Site environmental quality gradient (CI) as identified by PC1 in Figure 11 correlated against the environmental predictors mud content (a), organic matter (b) , and light availability (c), and the site names and scores (d). A more negative CI score indicates increasing eutrophication gradient.*

Seagrass correlations with environmental gradients

A Pearsons correlation table was used to investigate the relationships between the sediment predictor variables (including the new environmental CI score (EnvPCA)) against the seagrass health metrics (Table 9). Two variables were directly correlated with the EnvPCA gradient, including leaf width (negative) and leaf $d^{15}N$ (positive), which would be two expected relationships to an increasing eutrophication gradient. A number of the leaf nutrient heath metrics showed strong negative relationships to mud including $d^{13}C$, nitrogen% and $d^{15}N$, whilst the leaf C: N ratio increased with mud content. Organic matter showed positive relationships to leaf width, however negative relationships to above/below ground ratio, $d^{15}N$, and leaf C: N ratio. The light availability showed a negative relationship to leaf length, but a positive relationship to $d^{13}C$. Chl *a* and phaeopigment showed positive relationships to a number of seagrass health metrics, including seagrass cover, leaf and rhizome biomass, and leaf width.

*Table 9. Pearsons correlation maxtrix showing pair-wise relationships between seagrass health metrics and environmental predictor variables. Significant results are displayed in bold. Significance levels are shown with stars ($p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***).*

Seagrass health metrics	Median Grain	Mud	Water Content	OM	Chl *a*	Phaeo	Porosity	Light LUX	EnvPCA
Seagrass cover	0.11	-0.13	-0.17	-0.19	**0.35*** **	0.2	0.1	0.11	0.08
Leaf length	0.11	0.08	-0.01	-0.07	-0.01	0.01	-0.04	**0.25***	0.2
Leaf width	-0.12	**0.66*****	0.03	**0.22***	0.17	**0.40*****	**0.38*****	-0.15	**-0.31****
Leaf biomass	0.15	0.1	-0.17	-0.16	**0.45*****	**0.33****	0.16	-0.03	0.03
Rhizome biomass	-0.18	0.13	**0.25***	0.19	**0.34****	**0.27***	0.15	-0.02	-0.17
A/B Ratio	**0.27****	0.02	**-0.43*****	**-0.37*****	0	0.02	0.02	-0.05	0.16
d^{15}N	**0.39*****	**-0.31****	**-0.48*****	**-0.40*****	**-0.27***	**-0.38*****	**-0.66*****	0.16	**0.33****
Nitrogen%	-0.18	**-0.24***	0.19	0.19	0.06	-0.06	-0.2	0.05	-0.13
d^{13}C	**-0.31****	**-0.40*****	**0.39*****	0.2	**-0.67*****	**-0.62*****	**-0.36*****	**0.39*****	0.18
Carbon%	0.06	0.11	0.08	-0.02	0.1	0.08	-0.14	0.04	-0.03
C:N ratio	**-0.24***	**-0.30****	0.19	**0.24***	0.02	-0.1	-0.14	0.03	-0.16

Ecological quality status

A principal component was used to create a single metric of seagrass health using both physical and nutrient status indicators (Figure 15, Table 10). The derived “optimal” and “worst” sites are visualised over the PCA for comparison. The first component shows strong scores from the selected physical seagrass health metrics (percentage cover, rhizome biomass, leaf biomass, total biomass), and positive relationships with leaf nitrogen, and explains 57.4% of the observed variance. The leaf C: N ratio is negatively correlated along this axis. Positive scores along PC1 indicate increasing seagrass health. PC2 is driven by the leaf C: N ratio and leaf nitrogen content, which are negatively related to each other. This axis explains 30.7% of the observed variance. Sites spread positively along PC1 therefore had higher EQR scores such as Otumoetai, Ongare and Te Puna. Those ranked negatively to PC1 had lower EQR scores such as Matahui and Waiau.
The calculation of ecological quality ratio (EQR) resulted in one site being ranked as very good, two sites as good, four sites as fair, and two sites as poor (Table 11, Figure 16).

The EQR has shown some clear responses to the environmental gradient targeted by this survey (from seagrass at muddy, organic rich sites through to sandy, low organic sites). However unlike other research that targeted water column nutrients to develop the environmental health scores, this study utilised sedimentary variables, and did not include any nutrient metrics. The seagrass EQR showed two distinct patterns when correlated against the environmental gradient (CI) (Figure 17). The expected pattern was for seagrass health EQR to increase with an increasing quality of the environmental gradient. Five sites clearly showed this pattern (Otumoetai, Pahoia, Ongare, Ōmokoroa and Te Puna). However a second subset of sites emerged, where the environmental health was classified in the higher quality categories, however seagrass health gradings were in the fair to poor grade. Sites in this category included Waimapu, Tuapiro, Waiau and Matahui. Matahui was characterised by having the lowest average rhizome biomass and highest above/below ground ratio, and relatively low leaf C: N ratio and $d^{13}C$ compared to the other sites. Waiau was the second poorest site in terms of seagrass health, characterised by the lowest percentage coverage, longest blade length and relatively thick blades. The leaves also had the highest C: N ratio, and lowest leaf nitrogen content. Waimapu and Tuapiro were similar in their percentage cover, leaf length, and leaf biomass. Waimapu was different to Tuapiro in a few variables such as wider leaves, lower rhizome biomass, higher $d^{15}N$, higher nitrogen, higher C: N ratio and lower $d^{13}C$.

Table 10 The percentage of variance explained and PCA loadings of the seagrass health metrics used to determine the seagrass health index.

Variable	PC1	PC2
Explained variance	57.4%	30.7%
Physical characteristics		
Percent cover	0.827	-0.024
Rhizome Biomass	0.866	0.080
Total Biomass	0.903	0.400
Leaf Biomass	0.789	0.582
Nutrient storage		
C:N ratio	-0.498	0.838
Leaf nitrogen %	0.570	-0.796

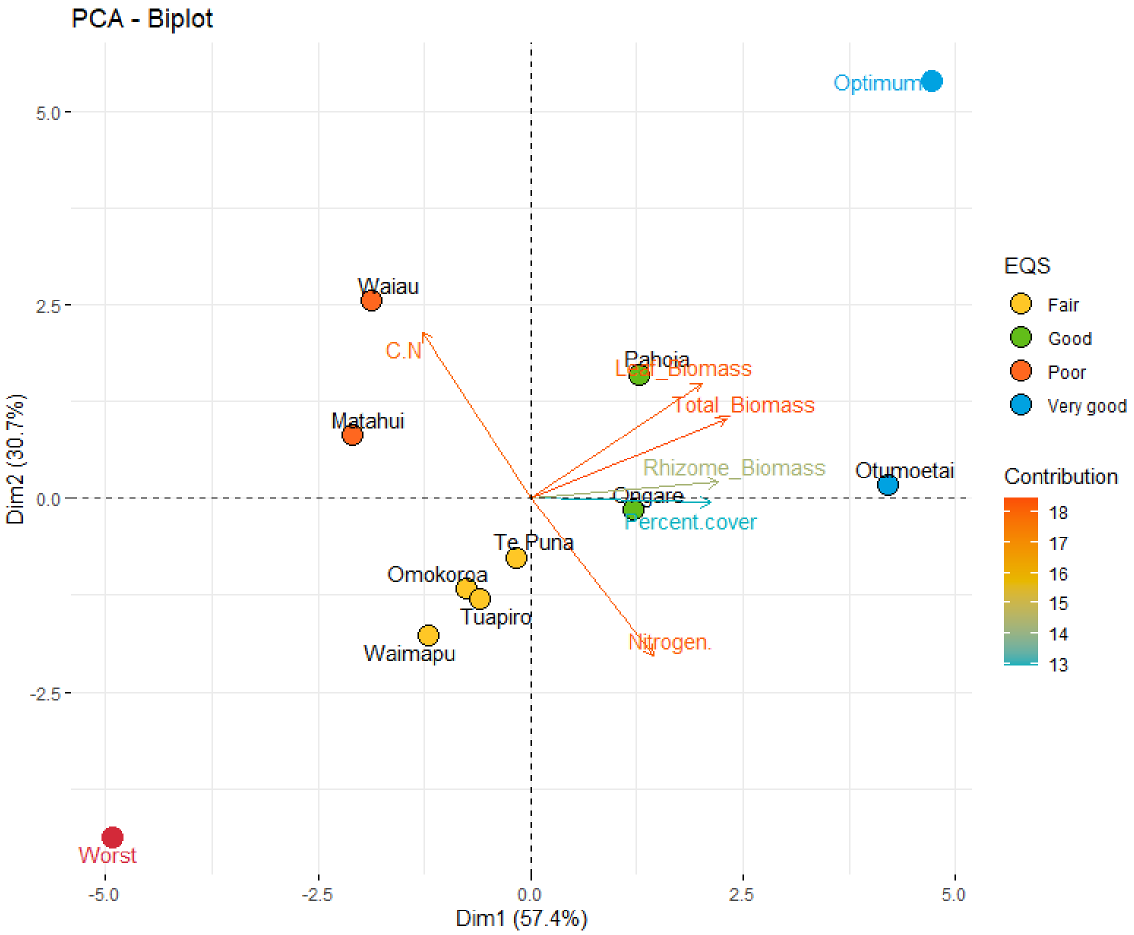

Figure 15 *Principal component analysis to calculate the ecological quality status for seagrass health. Optimum and worst sites are overlaid but not included in the PC.*

Table 11 *Scores of the seagrass health PCA first component (CI) obtained from the seagrass health metric PCA, ecological quality ratio (EQR) values calculated for each site, and the ecological quality status (EQS) obtained according to Table 3.*

Site	CI	EQR	EQS
Waiau	-1.8699	0.317	Poor
Tuapiro	-0.6037	0.448	Fair
Ongare	1.2135	0.637	Good
Matahui	-2.0928	0.293	Poor
Pahoia	1.2849	0.644	Good
Ōmokoroa	-0.7486	0.433	Fair
Te Puna	-0.1773	0.492	Fair
Otumoetai	4.1960	0.946	Very good
Waimapu	-1.2021	0.386	Fair
Optimum	4.7130		
Worst	-4.9192		

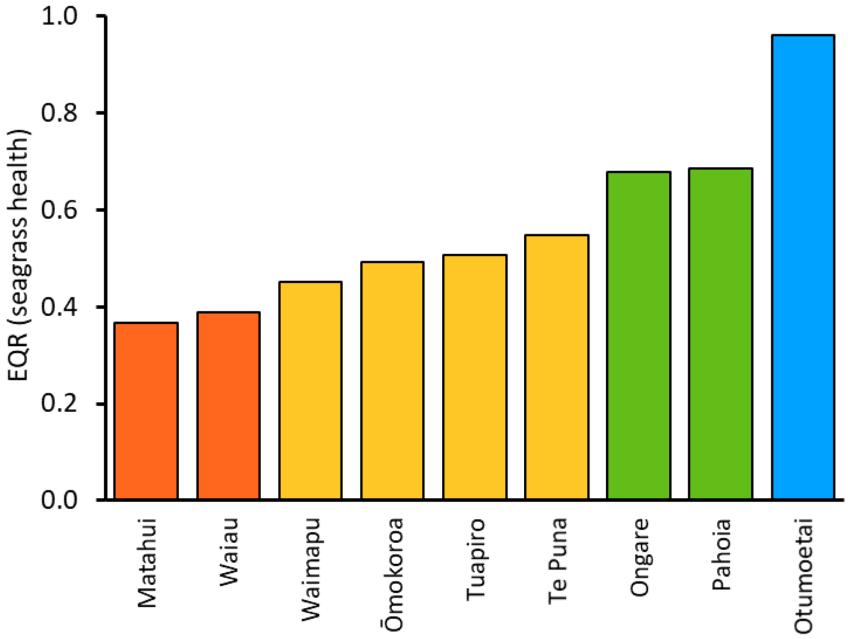

Figure 16 *Seagrass health grading (EQR) from low to high.*

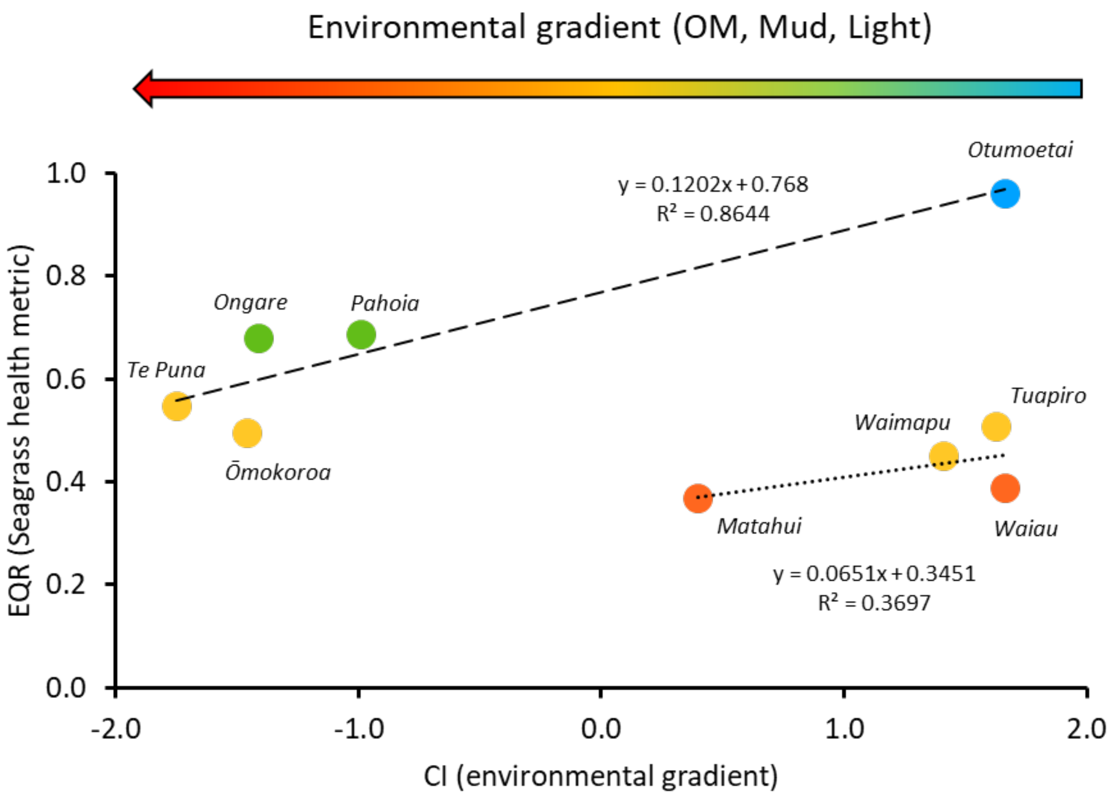

Figure 17 *Relationship between the seagrass health EQR and the environmental gradient CI scores. More negative CI scores indicate a lower environmental quality indicative of eutrophication. Site colour coding represents EQS (ecological quality status).*

Power analysis

The highest power in this study was for the leaf length and width, with 99% power, due to the higher number of replicate sub-samples taken from each core and averaged across the site replicates (Table 12). It estimated future studies will only require 12-15 leaf width/length measurements to have a high power. There was high variability in the seagrass cover, which was in part due to the sampling method of including all sample quadrats although some were affected by patchiness. To reduce the error in future surveys the photo quadrat number should be increased from 10 to 20 quadrats, and the quadrat should be re-thrown if it lands in a bare patch of sediment (Duarte & Kirkman, 2001). Leaf and rhizome biomass were inherently variable, due to the smaller size of the subsample, indicating only 43% power in the current study. To increase power to 80% sample size should be increased to 20. The chemical metrics had lower variability, and estimated sample size should increase from three to seven to increase power. Non-structural carbohydrates were measured at only three sites, with three replicates due to the high processing cost, thus had the lowest power of all the investigated metrics. It is estimated that the samples should increase to 16 to reach higher statistical power.

Table 12 Power analysis using seagrass health survey data across nine sites in Tauranga Harbour. Sample size (current study) is the sample size for the health report. Between variation is the between group variance, within variation is the within group variance, sample size (n) is the estimated sample size required for 80% power at alpha 0.05, and current study power is the current power of the study.

Seagrass health metric	Sample size (current study)	Between variation	Within variation	Sample size (n)	Current study power
Seagrass cover	10	217	2105	19	0.45
Leaf length	50	58	428	15	0.99
Leaf width	50	0.27	1.60	12	0.99
Leaf biomass	10	0.14	1.43	20	0.43
Rhizome biomass	10	0.04	0.36	20	0.43
Leaf d^{15}N	3	0.53	1.6	7	0.33
Leaf d^{13}C	3	0.98	2.93	7	0.33
Leaf nitrogen	3	0.02	0.06	7	0.33
Rhizome sucrose	3	871.9	2615.8	16	0.15

Discussion/Matapakitanga

Spatial variability in seagrass health indicators

Seagrass coverage was highly variable at both the meadow level, and within the sampled quadrats across all sites in Tauranga Harbour, which has been a noted feature of seagrass meadows in various studies across the world (Duarte & Kirkman, 2001). At some sites there are gradients in seagrass coverage evident from the high to low tide line, and also some patchiness where seagrass has been eroded or fed upon by herbivores (Dos Santos *et al.*, 2012). The coverage of seagrass was also highly variable between the studied sites in Tauranga Harbour, with some clear distinctions evident. Leaf length was highly variable, and it was difficult to draw distinctions between sites based on length alone, with the exception of Waiau which had blades up to 20 mm longer than the other sites. Although power for the seagrass leaf length was high, high variability was still reported within sites. Leaf width was a clearer metric than seagrass length, with groupings able to be distinguished between sites. The leaf biomass and rhizome biomass had some clear distinctions between sites, although there was quite high variability between subsamples. Often sites with higher leaf biomass also had higher rhizome biomass. The addition of nutrient storage metrics of the seagrass leaves added another layer to the seagrass physical metrics, and some clear distinctions were evident between sites even with the lower replication number. Higher levels of replication would likely enhance this metric as a useful indication of nutrient availability and storage of the seagrass, as indicated by the power analysis. In particular, the sites with likely higher nutrient inputs had increased leaf C: N ratios, and the $d^{13}C$ was more negative in sites that were muddy and likely to have a lower light environment. There was a trend between light availability and $d^{13}C$ ($r = 0.39$, $p<0.001$). Non-structural carbohydrates (NSC) were measured as a health indicator metric, however only at a smaller subset of sites. Sucrose content was the only non-structural carbohydrate metric to show significant site differences, where seagrass at Tuapiro would be most resilient to acute disturbances due to the positive link between sucrose and survival, whereas seagrass at Waimapu would appear to exist in the least favourable conditions (Sørenson *et al.*, 2018). $\delta^{15}N$ values can indicate the potential source of nitrogen, with higher $\delta^{15}N$ values typically associated to higher nitrogen enrichment (such as sewage or effluent inputs) (Barr *et al.*, 2013, Andrisoa *et al.*, 2019). Seagrass leaf $\delta^{15}N$ was correlated to the leaf total nitrogen in our study ($r=0.29$, $p<0.01$). Seagrass leaf $\delta^{15}N$ values measured in our survey are in the lower range reported in other *Zostera* species (3.15 – 20.16‰: (Jones *et al.*, 2018)), and are not highly enriched by nitrogen.

The spatial survey was designed to target a number of representative seagrass sites covering a sediment mud/eutrophication gradient, to provide an overview of seagrass health in Tauranga Harbour. A number of seagrass variables were selected for this assessment based on recommendations in the literature (McMahon *et al.*, 2013, Roca *et al.*, 2016). The combination of physical, environmental and chemical metrics of seagrass health have allowed some site-specific trends to be extracted. The effects of increased muddiness, and corresponding reduction in light conditions may have had an effect on both the physical and chemical composition of the seagrass. Sites such as Tuapiro, Otumoetai, Waimapu and Ōmokoroa had narrower seagrass leaves and an increased $\delta^{13}C$ indicative of high light conditions. Comparatively, Pahoia, Waiau, Matahui, Te Puna and Ongare responded to increasing muddiness/decreased light conditions with wider leaves and a negative relationship to $\delta^{13}C$. Ongare and Pahoia both had relatively thick leaves, and a corresponding high biomass. Te Puna had the thickest leaves of all the sites, and was also present in the muddiest sediment conditions. Thicker leaves in *Zostera* spp. can be a response to low light levels, by increasing the surface area available for light collection (Ralph *et al.*, 2007, Collier *et al.*, 2012). This metric may prove a useful tool for indicating light limitation for seagrass, and could be further refined through site light measurements aligned with leaf $d^{13}C$ sampling times.

Future steps for seagrass monitoring need to take into account these varying spatial scales, and include various methods for assessing the spatial patchiness. Additional replication may be required to ensure significant differences can be distinguished, and estimates of replication based on this survey are outlined by the power analysis.

Seasonal variability in seagrass health indicators

Seasonal changes were monitored at three sites over four seasons. Large differences were clearly evident in all the measured metrics, with peak seagrass coverage and biomass evident in summer. Leaf length increased at a number of sites between spring and autumn, when light conditions were reduced compared to summer. This trend was also evident in the $d^{13}C$, where it became more negative during winter and spring, again when light conditions were lower. This shows the clear seasonal differences in light availability, as when light becomes limiting, seagrass will utilize the lighter carbon isotope preferentially, thus reducing the $\delta^{13}C$ value (McMahon *et al.*, 2013). The seagrass above ground to belowground ratio was greatly enhanced during summer and autumn at all sites, with reductions over winter when the leaf biomass decreased. Rhizome biomass was generally the lowest in autumn, indicating high usage of nutrient stores, and these nutrient stores generally increased again into winter and spring. However, the only statistically significant change in rhizome stores occurred at Waimapu.

Leaf nutrient statuses were quite variable across the sites and showed different site specific trends. At Waimapu, there was higher leaf nitrogen during winter and spring, whilst the highest leaf N at Waiau was during summer, and in autumn for Tuapiro. This indicates nutrient availability across the harbour is quite variable across seasons, with potential implications for seagrass growth. The leaf C: N ratio generally followed the opposite trend to leaf nitrogen. The leaf $d^{15}N$ was highest at all sites in winter, and equally high in spring at Waiau and Waimapu, which may indicate increased human influences during these seasons, such as stormwater runoff.

Seasonal differences were pronounced in many health indicators across the monitored sites, however these were often highly site specific. Future monitoring programmes should be targeted to a particular season, to reduce the potential for seasonal variability to influence results. Any future seasonal sampling would need to be intended for site specific responses, and results may not be easily extrapolated across the harbour.

Stressors to seagrass in Tauranga Harbour

The utilisation of PCA and previous studies on multimetric indicators of seagrass health allowed a grade of health to be established for both the seagrass environment and the seagrass health indicators (García-Marín *et al.*, 2013). The environmental health gradient was composed of the sediment mud and organic matter content, and the water light availability, providing a comprehensive indicator of a mud/potential eutrophication gradient. The environmental gradient also provided a "reality check" against the seagrass health gradings that were computed from the seagrass health indicators. The calculated environmental gradient differed from previous studies, in that we used sediment characteristics rather than water quality parameters to characterise the seagrass environment. This may mean we miss the capture of the full environmental conditions the seagrass experiences, such as nutrient inputs from the water, sediment nutrient recycling or turbidity. The seagrass health EQR grading provided a multivariate approach to combining a range of health indicators into one overall score. Sites with higher seagrass coverage, leaf biomass, rhizome biomass and total biomass, higher % nitrogen, and higher leaf C: N ratio were determined to be in a higher ecological state. This study utilised some different metrics to previous studies (García-Marín *et al.*, 2013) due to the availability of data. Compared to the García-Marín *et al.* (2013) study, our first component PCA explained a higher % of variance (57% vs 47%), however the artificial optimal and worst sites did not fit as well along the first axis. The PCA split out the sites well by indicating sites that had higher physical metrics, as well as those that had higher storage of nitrogen in the leaves, and C: N ratios. The resulting seagrass health EQR grades showed a good fit to the environmental gradient for some sites, whereas a subset of the sites showed an alternate pattern. The expected pattern was for seagrass health EQR to increase with an increasing quality of the environmental gradient. Five sites clearly showed this pattern (Otumoetai, Pahoia, Ongare, Ōmokoroa and Te Puna). However a second set of sites emerged, where seagrass health gradings were relatively lower than other sites, although being graded with a higher quality of environmental health. Sites in this category included Waimapu, Tuapiro, Waiau and Matahui. Seagrass species, in particular *Zostera* spp. are known to show high plasticity when it comes to changing environments, and can show quite fast physical changes in response to environmental stressors (Roca *et al.*, 2016), which is what makes them an effective environmental indicator. However, this can make comparing across sites difficult, in particular when the sites cover quite a large environmental health gradient. Other environmental metrics not measured in this study include nutrient stressors, grazing, and smothering by macroalgae. Some of these metrics may be responsible for the lower seagrass health gradings and lower relationship to the calculated environmental gradient.

As leaf nitrogen increases, it is assumed that this is due to over enrichment of nitrogen in the system (Martínez-Crego et al., 2008), thus being a negative indicator of seagrass health ((Cabaço *et al.*, 2008, García-Marín *et al.*, 2013). The poor fitting artificial worst and optimal sites appears to be explained by the inclusion of leaf nitrogen. Nitrogen concentrations in Tauranga Harbour water is generally quite low (Scholes, 2015), thus there could be the potential that increased nitrogen content in seagrass leaves is due to nutrient storage for times of nutrient shortage. To trial this theory, we changed the nitrogen content of the artificial optimal and worst sites and re-ran the PCA, which resulted in the worst and optimal sites fitting closely to the first component, however this resulted in odd EQR scores. This may indicate some of the seagrasses are affected by nitrogen enrichment, whilst other EQR scores were higher due to storage of nitrogen to use in times of scarsity. The seagrass samples were also collected over a two-month period, and the differences could be due to changing nitrogen availability over that period, which was indicated to be quite variable across our seasonal sampling sites.

Waiau and Matahui were graded in the poor category for seagrass health, due to having quite low or high characteristics in a number of the health indicators. Matahui, graded as the poorest health seagrass site, had the lowest average rhizome biomass and quite low leaf C: N ratio and $d^{13}C$ compared to other sites. This can indicate a combination of nutrient enrichment at the site (Gladstone-Gallagher *et al.*, 2018), and potentially lower light conditions (Campbell & Fourqurean, 2009; Roca et al., 2016). Large rhizomes are used for storage of carbon, for utilisation in times of light or nutrient limitation (Leston *et al.*, 2008, García-Marín *et al.*, 2013), thus small rhizome stores indicate the seagrass bed is unable to put away extra stores for overwintering. Matahui was in the mid-range for the light availability, however it is noted that the light data was only collected over a 4 hour period, and represents calm wind conditions that were required for the PhD experiments. Longer light data collections may elucidate the long term light availability at this site. Waiau was graded as the second poorest site, likely due to having the lowest percentage seagrass cover, however it also had the longest blade length, and relatively thick blades, indicating some level of potential light limitations. It had the highest leaf C:N ratio and lowest leaf nitrogen content, possibly indicating nutrient poor conditions, as leaf nitrogen has been shown to increase with the availability of nitrogen in the environment (Martínez-Crego *et al.*, 2008), or in response to shading (Fernandez *et al.*, 2001, Cabaço *et al.*, 2008, Roca *et al.*, 2016). Tuapiro and Waimapu were graded as fair health, although having some of the highest environmental health grades. Both sites were characterised by sandy sediments and high light conditions, however coverage of seagrass was only around 50%, with relatively short leaves and low leaf and rhizome biomass. Both sites also had low C: N ratios, however leaf nitrogen was in the middle range compared to other sites. The seagrass at Tuapiro had lots of patches across it, due to grazing by a large waterfowl population at that end of the harbour. There is also the occasional occurrence of a filamentous algae across the seagrass bed. Waimapu seagrass is often subjected to macro algae coverage (particularly sea lettuce) in the spring and summer. It may be possible other external factors have resulted in lower health gradings for these sites that were not quantified in this survey.

Waiau and Matahui were graded as poor indicating higher seagrass stress and loss of ecological health occurring at these sites. These results appear to be driven by increased sedimentation and reduction in light conditions described above. The recent BOPRC Tauranga Moana State of the Environment Report (Lawton & Conroy, 2019) has shown that fine sediments are accumulating at a higher rate than background levels at 59% of sites monitored. The Waiau seagrass site at the end of the Waiau River and is almost entirely filled with mangroves. Mangroves are known to expand when increased fine sediments are being discharged from the catchment, which has been reported on previously in Tauranga Harbour (Park, 2004, Win *et al.*, 2015). Sedimentation plates in Waiau estuary show sedimentation rates up to 12 mm/year (BOPRC unpublished data), which likely have a detrimental impact on the seagrass beds, with issues in both light reduction and smothering possible. Pahoia was graded as good, however it had similar characteristics to Waiau and Matahui. The Pahoia seagrass had the second highest percentage coverage, with short leaves and a high above/below ground ratio. The leaves had the lowest recorded $d^{13}C$ across the sites, and an intermediate environmental health grading – although being comprised of high mud, and relatively low light conditions. Both Matahui and Pahoia seagrass sites are nearby large mangrove stands, and likely also have large amounts of sediment moving past. Seagrasses slow hydrodynamics and enhance the settlement of fine sediments, thus if there are increased fines in the water it is likely some will accumulate in the seagrass beds greatly changing the quality of the habitat. The impacts of increasing muddiness on seagrass prevalence has previously been reported in Tauranga Harbour, with seagrass found to be absent above a sediment silt content of 13% (Park & Donald, 1994). Our study found seagrass presence in a range of 6 – 33% mud content, however we note these were based on different methods of grain size analysis (our study using laser diffraction, with a higher certainty around the smaller grain size fractions such as mud content). The seagrass located at the muddiest sites appeared to show a level of localised adaptation to the muddier environment, showing larger leaf width and lower $d^{13}C$ indicative of lower light conditions. Similarly, Park and Donald (1994) indicated that the sites with better water quality (lower turbidity) also showed greater seagrass biomass. Seagrass beds under stress from high water column turbidity have been shown to increase primary productivity during periods of emergence, due to the decreasing ability of the seagrass to photosynthesise during submerged periods (Drylie *et al.*, 2018).

Locally in Tauranga Harbour a range of seagrass experiments have occurred investigating thresholds of stressors on seagrass, local adaptations and indicators of health (Gladstone-Gallagher *et al.*, 2018, Li *et al.*, 2018, Sørenson *et al.*, 2018). These experiments have laid out baseline knowledge of seagrass response to stressors in Tauranga harbour. Sørenson *et al.* (2018) found that seagrasses showed local adaptations to sedimentation, and some morphometric characteristics gave the seagrass some resilience to sedimentation (increased number of blades, wider and longer leaves, and greater percentage coverage). Non-adapted seagrass sites had lower tolerance to sedimentation, and showed longer recovery times post-sedimentation. The findings of this work support the results of the seagrass health EQR, which showed a number of sites with thicker and longer leaves to have a high tolerance for muddier environments. Seagrasses with greater rhizome carbon storage (sucrose) had greater seasonal survival. Tuapiro had the greatest sucrose stores compared to Waiau and Waimapu, and had the higher seagrass health grading of the three sites. Higher spatial coverage and replication is required to draw any further conclusions about the rhizome carbohydrates storage and seasonal survival.

Gladstone-Gallagher *et al.* (2018) experimentally elevated porewater nitrogen concentrations (ammonium) across six seagrass sites in Tauranga Harbour. An integrative multivariate health index was created using the morphometric characteristics of percentage cover, leaf length and width, and above and below ground biomass. The C: N ratio of seagrass leaves clearly showed a decrease across the nitrogen enrichment treatments. The C: N ratios in our study were a scale higher than reported in this study (our study site average range 32 - 24, Gladstone-Gallagher average range ~22-16 for control, ~18-13 enriched). Monitoring over time will provide the best estimate of changing nutrient conditions to be comparable with this study. Below a total seagrass biomass of 140 g DW m^{-2}, there appeared to be low resilience to nitrogen enrichment, indicated by highly significant treatment effects on seagrass morphology (Gladstone-Gallagher *et al.*, 2018). When total biomass was 270-285 g DW m^{-2} the effects were variable. Above 400 g DW m^{-2} the seagrass appeared resilient to nitrogen enrichment. A number of our monitored sites show high biomass well above the 400 g DW m^{-2} threshold, in particular Waiau, Ongare, Pahoia, and Otumoetai. These sites were characterised across a range of mud/organic sediment enrichment, and were graded across three of the EQR (Table 14). Sediment nitrogen content was not measured in this study, however given the higher organic matter enrichment at a number of these sites the higher biomass may be providing some resilience as reported by Gladstone-Gallagher *et al.* (2018). For the sites with a lower above ground biomass, increases in sediment nutrient conditions may result in a loss of seagrass.

Many of the stressors discussed in this report have been studied in isolation, however multiple stressors (e.g. mud content and nutrient enrichment) are known to have interactive effects on seagrass health. Li *et al.* (2018) elevated porewater nitrogen concentrations (ammonium) across three sites in Tauranga Harbour over a mud content gradient. The integrative multivariate health index (Gladstone-Gallagher et al. 2018) declined significantly in the treatment sites, predominantly driven by decreasing seagrass percentage cover. Nutrient porewater thresholds were identified, however these thresholds varied based on the initial sediment mud content. At the sandiest site (12% mud content), seagrass began to decline from ammonium porewater concentrations of 10 mmol. In the sand-mud site (17% mud content) seagrass decline was evident just under 50 mmol, and in the muddiest site (23% mud content) seagrass did not begin declining until porewater concentrations were more than 100 mmol. This indicates it will be important to consider multiple stressors and seagrass location specific tolerances when assessing the impact of stressors on seagrass health.

Future threats to seagrass in the Bay of Plenty region

The growth of urban development is rapidly increasing in the Tauranga region where the population is projected to almost double by 2063 (TCC, 2018). This population growth will bring increased anthropogenic stressors especially in land use changes (increased sedimentation/nutrient inputs), and utilization of the coast for recreational activities (such as boating/anchoring). Strong policies and land use planning are required to ensure sustainable utilization of our coasts and include provisions for the protection of seagrass habitats.

Climate change is a key project for the Bay of Plenty Regional Council, with a Climate Emergency declared in 2019. Future forecasting by the Ministry for the Environment suggest that air temperatures could increase by up to 1.1°C by 2040, an increase in hot days above 25°C, a potential for sea level rise of 0.7 m by 2070, and up to 1.6 m sea level rise by 2130 (BOPRC, 2020). Sea temperatures in the Bay of Plenty have been shown to be increasing at a rate of 0.12 – 0.14°C/decade (between 1981 and 2018) (Sutton & Bowen, 2019). In addition to temperature increases, climate change will increase the frequency of storm events, and corresponding increases in freshwater runoff – with the potential to cause physical damage, decreased water salinities and increased turbidity. *Z.muelleri* has been shown to germinate best at salinities less than 8, or at higher salinities with pulses of fresh water (Stafford-Bell *et al.*, 2016). Anecdotal reports around the Bay of Plenty have shown small patches of seagrasses surviving in adverse conditions near large freshwater inflows, where the remainder of seagrass has disappeared (such as Waihī estuary (Russell, 2020)). This indicates that reduced salinity from increased freshwater runoff may be beneficial to seagrass populations in the Bay of Plenty. Increasing atmospheric carbon dioxide is elevating the concentrations of CO_2 in coastal waters, increasing ocean acidity. Research indicates seagrass may provide a strong buffer to ocean acidification in coastal waters, uptaking CO_2 and supporting higher growth rates (Short *et al.*, 2016). The most commonly studied temperate seagrass species, *Zostera marina,* has shown that water temperatures above 25°C can result in growth reduction and a decline in net primary production (Short *et al.*, 2016). Increased temperatures can increase stress on the seagrass during low tide exposure, and possibly result in the retreat of seagrass to deeper waters where temperatures are more stable. This however will rely on water quality being suitable, and the last subtidal seagrass survey found little to no submersed seagrass habitat remains in Tauranga Harbour (Park, 1999). In addition, sea level rise may limit the potential for seagrass beds to retreat into shallower waters due to anthropogenic alteration of the shoreline, causing a coastal squeeze (Doody, 2004). A strong focus on ensuring water clarity is maintained or enhanced in Tauranga Harbour will aid the survival of this critical habitat in a changing climate.

Seagrass grazing by swans (*Cygnus atratus*) has previously been examined in Tauranga Harbour (Dos Santos *et al.*, 2012), and found that swans consume an average of 394 g dry weight of seagrass per day. In intense areas of grazing (annual removal of 19-20% average seagrass biomass), there was a substantial decline (43-69%) in plant biomass the following growing season. Fish and Game conduct annual counts of the black swans. At the time of the grazing study (2008-2010), Fish and Game estimates there was a population of 3520-5100 birds present in Tauranga Harbour (Pers. Comm, M. McDougal). Current estimates (2019) show the population has increased to 6258 birds. Thus the increased grazing pressure of swans may constitute a significant impact on seagrass abundances, especially in popular swan feeding areas. This may pose some confounding results on sites subjected to high swan grazing, resulting in lower biomass measurements by grazing.

The fungal wasting disease *Labyrinthula zosterae* is a marine slime mould, and may act as a seagrass pathogen, infecting the living seagrass leaf cells, causing the death of chloroplasts and creating black lesions (Trevathan-Tackett *et al.*, 2018). It has been reported worldwide and around New Zealand (Clark & Crossett, 2019). Blooms of the wasting disease can occur when conditions are favourable (low light, warm temperatures, high salinity) (Ralph & Short, 2002). The prevalence and severity of infection at two sites in the Waikato region has recently been assessed, with 35-38% prevalence in the Slipper and Mercury Islands (Clark & Crossett, 2019).

Research gaps

There are still many unknown factors in relation to seagrass health and habitat in the Bay of Plenty. A number of potential research projects are identified below, in no particular order of importance:

- Cultural values of seagrass should be examined in collaboration with local hapū/iwi, such as identifying the values they hold of seagrass and supporting the development of cultural health metrics.
- Mapping of suitable seagrass habitat as derived from modelling all principal drivers and stressors in the current seagrass habitat for current and future climate change scenarios. This will provide potential areas for restoration or translocation of habitats under climate change scenarios.
- Little is known about the habitat utilization of seagrass locally, and investigations into the benthic invertebrate, epibenthos community, and fish/stingray habitat utilization would provide additional knowledge to understand the importance of the habitat. Some information may be able to be derived from the University of Waikato's baited remote underwater video surveys conducted in subtidal channels over the past few years. Pipefish have been observed in subtidal seagrass beds of Tauranga Harbour (Pers Comm, P. Ross).
- The genetic variability of seagrass is still unknown in Tauranga Harbour, and whether sites in the more degraded areas are actually a subspecies/genetic variant of the *Zostera* sp. Personal observations have shown at some sites with better water quality (Tuapiro, Athenree) the seagrass is short and sparse, whereas in some occasionally highly muddy/turbid sites (Waiau, Rangataua Bay) seagrass is very long and wide with high biomass.
- The feasibility of seagrass transplants to restore seagrass in areas where it has been lost, once land management interventions have taken place to increase the health of the habitat. Initial trials in New Zealand had mixed results (Schwarz *et al.*, 2005, Matheson *et al.*, 2017) and success of the trials will be reliant on issues that caused the initial decline being absent.

Monitoring programme recommendations

This study identified that strong spatial and seasonal patterns in seagrass health metrics are present in Tauranga Harbour seagrass. Spatially, we identified a wide range of seagrass health evident across an environmental gradient of mud, organic matter, and light availability. Seasonally, there are quite significant changes in a range of indicators, thus making it important that the surveys are conducted within tight timeframes, targeting the summer period when percentage cover and biomass is generally at its peak. The seagrass health project was conducted at the same nine BOPRC funded PhD sites to utilize a range of ecosystem response metrics (denitrification, nutrient fluxes) which were outside the original scope of this project. At a future date this information will provide highly valuable information on seagrass nutrient processing across different seagrass health ratings.
The use of the established EQR provided an additional level of detail of the overall seagrass health based on both physical and chemical metrics, however this is highly dependent upon the use of artificial optimal and worst sites. Further work is required to ensure this approach can be replicated with certainty in future programmes, including identifying sites that are potentially a better representation of an optimal and worst seagrass bed which may need to be estuary specific.

The use of seagrass health indicators provides a detailed assessment of seagrass health, which can also be used as an integrated response indicator of the overall estuary health in that area. This may be useful for tracking the success of land management interventions to reduce sediment loading and turbidity. The data collected from a number of representative intertidal sites in Tauranga Harbour provides a baseline against which future seagrass health can be compared. From this survey and the literature, a number of metrics covering a range of variables (physical, chemical, disease, and habitat) are recommended to initiate a monitoring programme across the Bay of Plenty estuaries (Table 13. A baseline survey of a number of sites across all Bay of Plenty estuaries should be conducted, and ongoing monitoring of these sites continued annually for a period of five years before the timeframes are reviewed.

Table 13. Summary of the intertidal seagrass health metrics recommended for regular monitoring programme.

Physical Health Metric	Chemical Health Metric	Disease/algae	Habitat quality
Meadow extent (drone/satellite/plane)	Leaf C and N	Fungal wasting disease	Sediment grain size
Seagrass percentage cover	Leaf C: N ratio	Epiphyte coverage	Sediment nutrient content (TN, TP, TOC)
Leaf biomass	Leaf d^{13}C and d^{15}N	Macroalgae coverage	Sedimentation rates
Leaf length and width	Rhizome sucrose		Light conditions (PAR, Turbidity)
Rhizome biomass			
Ratio of above and below ground biomass			
Herbivory			

Intertidal seagrass mapping using aerial photography has been the predominant standard for monitoring seagrass health in the Bay of Plenty and around New Zealand with high success (Park, 2016). However, the caveats of this form of monitoring include the availability of suitable aerial photography and that it requires a large time commitment for processing large areas. Current research is working to develop automated classification techniques and machine learning to automatically classify seagrass habitat from aerial/satellite imagery, which may be a promising future technique (Thang *et al.*, 2020). Additionally, the use of drones is increasing in habitat monitoring due to the decreasing cost and increased capabilities (Duffy *et al.*, 2018, Tait *et al.*, 2019, Schenone, 2020). Mapping of the extent of seagrass areas is useful to show large scale changes over time, however it does not provide an assessment of the direct causes of seagrass decline, or provide opportunity for management interventions to prevent seagrass loss unless mapping is done at a high frequency, such as annually.

Given the technological developments this will become an achievable goal in the near future. Finer scale measurements of physical health metrics will provide further detail on site specific seagrass health. Given the high disturbance of collecting biomass measurements, it is recommended that visual biomass assessments could be trialled, as reported on in Clark & Crossett (2019). Some quantification of herbivory at sites should be noted given the potential for high density swan feeding in Tauranga Harbour (and more recently Ōhiwa Harbour). Historical harbour wide seagrass mapping combined with localised intensive environmental monitoring of sediment and water quality can be used to identify areas to focus monitoring efforts. Drones can be utilized for mapping small scale patch dynamics of seagrass, particularly in areas where significant declines have been observed, or to track the success of land management interventions to increase estuarine habitat quality. This will complement the existing wide-scale aerial mapping programme.

The seagrass beds within the Bay of Plenty can be patchy, due to bare areas appearing through overgrazing or hydrodynamics. It is recommended that if a quadrat is thrown into an area of bare sediment, that the quadrat is re-thrown (Duarte & Kirkman, 2001). Regular monitoring of chemical indicators (leaf C: N, d^{13}C, and rhizome sucrose) may provide faster early warning signals of seagrass stress (McMahon *et al.*, 2013, Roca *et al.*, 2016). These metrics showed correlations to the derived environmental gradient (mud, organic matter, light), suggesting they are a good indicator for future monitoring programmes. The coverage of seagrass by macro algae (in particular *Ulva* spp.) or filamentous algae was evident during the monitoring period. Future monitoring should assess the cover of such algal species which may be a key driver of localised seagrass decline due to smothering, changing of oxygen and nutrient conditions, and reduction of light. In addition, monitoring of the fungal wasting disease (*Labyrinthula zosterae*) should be included given its reported prevalence elsewhere in New Zealand, and observed at a number of sites in Tauranga Harbour. A semi-quantitative scale of assessing wasting disease and epiphyte cover has been developed by Burdick *et al.* (1993) and utilised in practice by Clark & Crossett (2019).

The quality of the seagrass habitat has been shown to have clear relationships with the seagrass health indicators. Measurements at monitoring sites should include sediment grain size, sediment nutrient conditions (TOC, TN, TP) and sedimentation rates. The BOPRC coastal and estuarine ecology monitoring programme currently focuses primarily on non-vegetated sediments. This report has clearly identified sedimentation as a stressor on seagrass beds, and the addition of a number of sediment monitoring plates into representative seagrass beds would complement the existing monitoring programme. This should also include monitoring of the benthic invertebrate community and potentially underwater video to investigate the utilization of seagrass habitats by fish and megafauna (e.g. stingrays). Regular monitoring of water clarity (PAR, turbidity) would provide complementary information environmental information to assist with interpretation of the seagrass health metrics. The current PhD research being undertaken by Georgina Flowers (University of Waikato) will provide a detailed assessment of seagrass light requirements for productivity, which can be compared with seagrass restoration potential studies by (Matheson & Wadhwa, 2012). The existing estuarine water quality programme can provide an overview of physiochemical changes in Tauranga Harbour.

Power analysis utilising the results of this survey indicated higher replication will be required to ensure changes in seagrass health can be detected. For the physical metrics, it is suggested that seagrass cover and biomass metrics be assessed with 20 sub-samples at each location. High power was recorded for the leaf length and width, indicating that the replication can be conducted with five leaves measured from 10 cores, resulting in 50 measurements per site. Although power for the seagrass leaf length was high, variability was still reported within sites. A more targeted approach for analysing leaf length may need to be implemented, such as targeting the five oldest, or five longest leaves within a core. Nutrient metrics were trialled with low replication in this survey, and should be increased to quantify a minimum of 10 cores per site.

The value in the seagrass health monitoring will be evident over time, allowing site specific changes to be monitored. Many of the measured health variables are non-destructive, and provide robust techniques for community groups and local hapū/iwi to monitor seagrass patches in their rohe. This could be an an important way to collaborate with tangata whenua monitoring the current state, and response of seagrass beds to various restoration efforts in the catchment of Tauranga Harbour.

Future management of seagrass in the Bay of Plenty

The National Policy Statement for Freshwater Management requires Regional Councils to set robust contaminant limits to protect the health of freshwater ecosystems and receiving environments. For management, the next steps are to identify freshwater sediment and nutrient loads that are sustainable for seagrass health, and develop protection measures to protect these valuable estuarine ecosystems. Further investigations to support this study will include identification of sites where seagrass once existed, and when it was no longer present, to compare with changes in the sedimentary and water environment and identify potential thresholds where seagrass loss has occurred. This will also provide opportunity to identify sites where these thresholds may be close to being exceeded. Examples of limit setting for desired ecological seagrass states has been completed elsewhere in the world (Collier *et al.*, 2020), providing aspirational targets for management decisions. More frequent monitoring on seagrass presence/absence and other desired seagrass characteristics (such as above-ground biomass and extent) will provide datasets required to set such limits, and provide information on seagrass condition and resilience.

The use of modelling tools is required to develop ecological models to investigate potential nutrient and sediment reductions required to achieve a healthy state for our estuarine environments. An ongoing seagrass monitoring programme will provide vital evidence to show how various key stressors are impacting the health and functioning of seagrass ecosystems. Ecological modelling work is currently being completed in Waihī Estuary, which will provide a framework for setting ecologically relevant limits to support seagrass communities. The work can be further extrapolated across the region, or detailed modelling also initiated in the other estuaries of the Bay of Plenty.

Ongoing work is required globally to increase the societal awareness of the importance of seagrass to marine ecosystems (Unsworth *et al.*, 2018). The value of seagrass is regularly overlooked by the public eye, and further work is required to share knowledge on seagrass and the value it can bring to our region. Some of the core ecosystem services provided by seagrass are not visible to the human eye (such as nutrient recycling, food and habitat provision), thus further communications work will be valuable in sharing the value of seagrass habitats. Increased opportunities for tangata whenua and community groups to be actively engaged in developing and conducting monitoring programmes will be crucial steps in this space to create a sense of ownership and longevity of community engagement (Tan *et al.*, 2020).

Finally, there has been some local community interest in the feasibility of seagrass transplants to restore seagrass beds following management improvements of estuary health. In New Zealand, seagrass restoration via transplants have shown variable success, however transplants in Whangarei harbour (Matheson *et al.*, 2017) have shown good success up to 24 months post-transplant if the transplant site is carefully selected. The transplant site must have conditions suitable for growth, and if transplanting to a region previously hosting seagrass beds, the cause of decline needs to be addressed and remediation measures implemented to restore the health of the seagrass habitat (Schwarz *et al.*, 2005). A number of research insights are detailed in Tan *et al.* (2020), including consideration of resourcing requirements and strategic prioritisation of areas for restoration work, given the manually intensive methods involved in transplanting seagrass. Similar to seagrasses, shellfish beds are degraded globally, and utilising cross-ecosystem restoration approaches has shown positive successes (Tan *et al.*, 2020). Widescale seed restoration programmes has lead to the recovery of seagrass beds, but also restoration of associated ecosystem services (Orth *et al.*, 2020), such as improved water quality, carbon storage and habitat provisioning for key food species. A range of evidence exists to support the development of seagrass restoration programmes, and it is an area for consideration in collaboration with tangata whenua following water/sediment quality improvements.

Appendices/Ngā Āpitihanga

Appendix A:

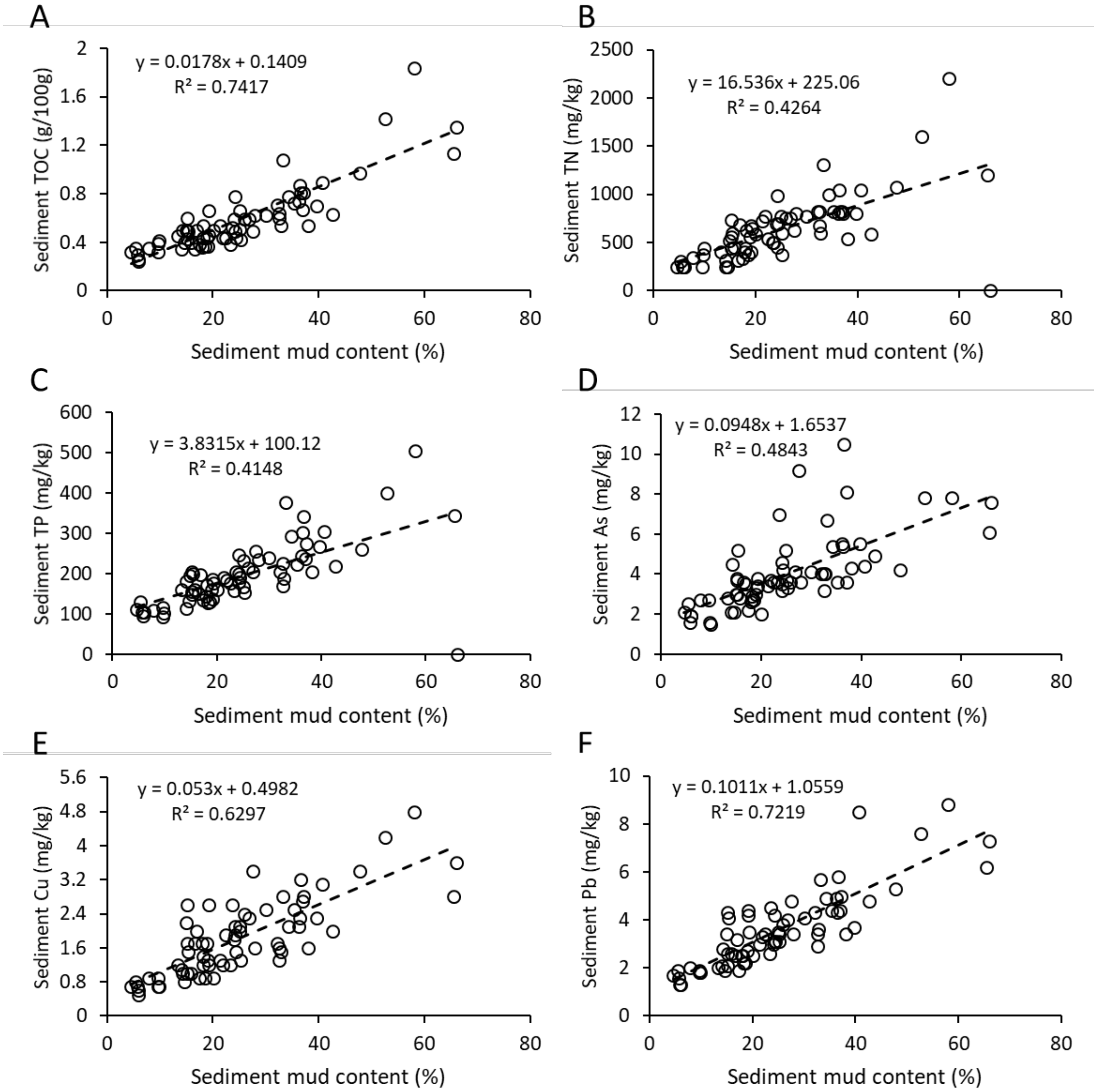

Figure 1. *Linear relationships between sediment mud content (%) and A: sediment total organic carbon (TOC, g/100g), B: sediment total nitrogen (TN, mg/kg), C: sediment total phosphorus (TP, mg/kg), D: sediment arsenic (As, mg/kg), E: sediment copper (Cu, mg/kg), F: sediment lead (Pb, mg/kg). Based on unpublished BOPRC data using 5 yearly sediment averages from 60 sites in Tauranga Harbour.*

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