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Seagrass indicator validation and refinement

Swan-Canning estuary

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Cover photograph: *Halophila ovalis* at extremely low tide at site HTH with views of the Perth city

Disclaimer

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Summary

Seagrasses are a highly valued component of estuary ecosystems since they provide habitat, provide a food source for waterbirds and improve water quality by reducing sediment resuspension and removing nutrients. Seagrasses respond to changes in their physical and chemical environment, and worldwide human-impacts have unfortunately led to the loss of much seagrass habitat. Ensuring that seagrass communities remain viable and resilient is an important component of effective estuary management.

Since 2010, the Department of Water and Environmental Regulation (DWER) has worked to develop metrics for understanding estuarine seagrass health within south-west Western Australian estuaries. In the Swan-Canning, DWER partners with the Department of Biodiversity, Conservation and Attractions (DBCA). Under DBCA's River Protection Strategy, Objective 4 highlights the desire to protect, manage and enhance biodiversity, while Objective 3 seeks to ensure management decisions are based on appropriate knowledge. Information on seagrass is required annually for reporting.

The principle of hierarchical monitoring design has been deliberately included in the development of DWER's seagrass monitoring programs, as described by Neckles *et al.* (2012). The indicator suite chosen was also designed to be suitable for colonising species (Kilminster et al. 2015), and inform on seagrass performance in a given year, and key pressures on seagrass condition. Our proposed indicators span a range of response times and spatial scales. This is important both for providing early-warning indicators, but in the future the suite of indicators chosen may also be able to inform on seagrass resilience. This takes into account the current understanding that the integration of monitoring efforts at different scales is critical to understand seagrass resilience (O'Brien et al. 2017).

The current seagrass monitoring program evolved from a comprehensive 8 month study of seagrass in the Swan-Canning estuary (October 2011 to May 2012: see Kilminster & Forbes 2014). The purpose of this report is to consider data collected in the Swan-Canning estuary for the summer sampling period of 2011-12[1], 2013-14, 2014-15, 2015-16 and 2016-17, to:

1) describe the natural variability of seagrass cover, species composition and other seagrass meadow characteristics as related to changes in key climatic conditions,
2) understand the sensitivity of seagrass metrics in response to anthropogenic stressors such as water quality and sediment stress,
3) refine and validate the seagrass indicators proposed, and
4) provide protocols for the use of these indicators.

Climate is shown within this report to be a significant driver of seagrass condition. Tide heights were unusually high in the first year of sampling (2011-12) over the seagrass growing period (December to March) compared to the other years of monitoring, and compared to the preceding ~20 years. Across the six years of seagrass data collection, there was up to a 25

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[1] A subset of the data was collected in 2012-13, and where possible will be included in the analysis

cm difference in the average tide observed which is a substantial increase in water depth for these shallow seagrasses, reducing the light reaching the seagrass canopy. Additionally, these study years had some of the hottest years, and an unusual summer rainfall event which decreased light, salinity and temperature, and elevated nutrients in the system. These conditions provided variability in environmental conditions for seagrass growth, where we suggest that:

- 2011-12 and 2016-17 had the poorest climate for seagrass performance
- 2014-15 and 2015-16 had average climate conditions for seagrass performance
- 2012-13 and 2013-14 were considered good climates for seagrass performance

Following evaluation of the seagrass indicator suite, minor adjustments were made to how we scored **seagrass performance**. In particular, the percentage cover categories were relaxed slightly. Other changes were made to rationalise field and laboratory processing effort (approximately halving the staff time) required to produce an annual score of seagrass performance at each site that could be used for annual reporting. The table below shows the final assessment of seagrass performance for all sites, in all years of analysis (note 2012-13 only includes a subset of data).

ANNUAL SEAGRASS PERFORMANCE

	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	3.5	3.25	3.25	3.5	3.25	2.25
2012-13[2]	4	4	4	4	4	2
2013-14	3.5	3.5	3.25	3.75	2.75	2.75
2014-15	3.75	3.5	2.75	3.75	3.75	2.5
2015-16	3.75	3.5	3	3.25	3.75	3
2016-17	3.5	3	2.5	3	3.5	2.25

Generally seagrass performance is good across the Swan-Canning sites. Condition at site CAN (at the upper extent of the distribution), shows poorer performance than other sites and site LUB has shown significant evidence of stress and reduced performance over time (likely related to eutrophication pressure). 2016-17 had lower performance generally than all other sites and if the flood had occurred a few weeks earlier (e.g. before sites were surveyed), the effect on the overall performance would have been more notable.

The **key pressure** indicator suite also performed generally well. Adjustments were made to several of the indicators as explained in the report. Considering these key pressures together indicates that overall seagrasses in the Swan-Canning are under moderate stress. Site LUB was generally the most stressed, followed by PPT and HTH, then CAN, while RCK and DLK

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[2] Scored for seagrass presence and cover only

were the least stressed. This seems consistent with our observations and intuitive understanding of the system across the five years of study.

Overall, we recommend that the principles of hierarchical monitoring are maintained within the program. Specifically, this means including the broad-scale mapping of the whole estuary at 3-5 year intervals and measuring seagrass performance at multiple scales. The last time that an estuary-wide map was produced (by DWER in 2010) it was in with close to the highest tide for the last ~25 years. Understandably, a reduction in area of seagrass was noted compared to previous maps. It is recommended that estuary-wide mapping of seagrass is carried out again (preferably during a year when tide heights are more average).

Collecting data annually to support the generation of a seagrass performance score at six sites would support the information needed for annual reporting. Key pressures metrics are recommended at a 1-3 year interval, depending on ease of data collection, cost and annual variability.

Monitoring annual seagrass performance is also in line with current understanding of how best to monitor seagrass resilience. We include measures that target resistance (abundance) and recovery (growth and reproduction) attributes of the seagrass at two-scales (meadow and plant).

1 Context and program design

Seagrasses are flowering marine plants and are a vital component of many estuarine (and marine) shallow-water environments. Internationally, seagrasses are recognised as an excellent indicator of estuarine health. They have been referred to as the ‘canary of the estuary’ as they require both good water quality and good sediment quality in order to thrive. Unfortunately seagrasses are among the most threatened habitats worldwide with loss of seagrass meadows accelerating ((Waycott et al. 2009)).

These plants evolved from land plants, from four distinct lineages, and have adapted to live underwater in estuaries and the coastal ocean. Seagrasses are vital to estuary ecology – providing habitat, sediment stabilisation, food for waterbirds (e.g. Black Swans) and take up nutrients (making them unavailable for unfavourable algal blooms). The seagrasses found in the Swan-Canning estuary are primarily considered *colonising*, characterised by short turnover times of ramets, sexual maturity is reached quickly and high investment in sexual reproduction to produce dormant seeds. Colonising species also have an ability to build up a seed bank, even a short-lived one. Colonising species have low physiological resistance to disturbances but may recover rapidly (Kilminster et al. 2015). We focus our seagrass monitoring efforts on the seagrass *Halophila ovalis* as it is by far the most dominant seagrass present in the Swan-Canning system.

The initial focus of the seagrass monitoring program was to use seagrasses as general indicators of estuary health. A secondary aim, was the development of specific functional-level indicators to provide insight to management as to how to improve seagrass condition. Significant new understanding about seagrass in the Swan-Canning has been generated since this program began, both as a result of the monitoring program, and the collaborative student and research projects that have been supported by the DWER project team (Appendix A).

More recently, we have been considering how our long-term data might be utilised to better understand the resilience of seagrass. Understanding resilience for management in the context of seagrasses relates to their ability to resist pressures or recover from loss. Resilience is inherently a cross-scale attribute ((O'Brien et al. 2017)) and thus monitoring designs that explicitly examine seagrass attributes at a range of temporal and spatial scales are desirable.

In the Swan-Canning, DWER partners with the Department of Biodiversity, Conservation and Attractions (DBCA). Under DBCA’s River Protection Strategy, Objective 4 highlights the desire to protect, manage and enhance biodiversity, while Objective 3 seeks to ensure management decisions are based on appropriate knowledge. Information on seagrass is required annually for reporting. Ensuring viable and resilient seagrass communities is an important component of effective estuary management.

1.1 Sampling: methods and sites

A comprehensive methods manual has been provided to DBCA for the seagrass monitoring program from 2011-17. This section aims to provide an overview without specific detail.

The benefits of a hierarchical monitoring framework in meeting seagrass conservation objectives at a range of scales was reported for estuaries in north-eastern USA in 2012 (Neckles et al. 2012). We embedded these principles of hierarchical monitoring in seagrass assessments in the study of seagrass within the Swan-Canning estuary, along with metrics tailored for colonising seagrass species (see Table 1). This report focuses on the meadow-scale and plant-scale as these have been the focus of the collaborative project with DBCA, monitored since 2011.

Table 1 *Overview of the hierarchical monitoring program adopted for the Swan-Canning estuary.*

Scale	What is monitored	When	Why
System-scale	Low-resolution estimates of seagrass across whole estuary using underwater camera. • Seagrass presence/absence • Species ID • Seagrass cover	Ideally monitored every ~5 years. A system-wide assessment of seagrass was completed by DWER in 2010 (prior to the commencement of the collaborative project with DBCA). Has not been undertaken since although DWER have investigated potential of remote sensing approaches (see Appendix A)	Provides a distributional extent of seagrass throughout the whole estuary. Useful to have current maps for spatial planning and management decisions. Should not be used for seagrass change estimates, due to lack of resolution in data collection points increasing uncertainty.
Meadow-scale	High-resolution observations of seagrass at selected sites (up to 1000 quadrat observations, along 10 x 100 m transects) • Seagrass presence/absence • Species ID • Seagrass cover	Monitored at the likely seasonal peak in seagrass abundance (late February most commonly – see Appendix B)	Sufficient observational resolution to provide a robust estimate of change between years. Valuable to see variability in different seagrass species present in the estuary.

Scale	What is monitored	When	Why
	Camera observations used to extend the information at four sites where seagrass extends beyond the transects		
Meadow-scale	Measurements of photosynthetically active radiation were collected during the Swan and Canning estuary routine water quality program from sites BLA, ARM, HEA, NAR, NIL, SCB2 and SAL.	Monitored fortnightly across seagrass 'summer' sampling period (when instruments working)	Information of euphotic depth (i.e. $E_{d\ 10\%}$ - water depth required to reduce PAR to 10% of surface irradiance) can be used to infer variations in light climate reaching seagrass – related to water quality as turbidity and phytoplankton blooms would reduce the $E_{d\ 10\%}$.
Plant-scale	Focuses only on *Halophila ovalis* – data from quadrat observations, cores and individual shoots. • Seagrass presence/absence, cover (quadrats) • Macroalgal cover (quadrats) • Productivity (tagging individual shoots) • Seagrass reproduction (cores) • Seagrass meadow characteristics (cores) • Seagrass chemical constituents (%C,	Monitored for different aspects of the program between November and March (see Appendix B)	Plant-scale measures provide the fastest-response indicators. These measures are primarily those used to generate the overall indicators of seagrass for both seagrass performance and also indicators of key pressures.

Scale	What is monitored	When	Why
	N, P, S and isotopes) • In-situ conditions (Photosynthetically active radiation and temperature)		

Seagrass monitoring at the meadow- and plant-scale was conducted at six sites in the Swan-Canning from 2011-12 to 2016-17 (Figure 1). These sites were selected in 2011 as representative of *Halophila ovalis* dominant habitats, distributed across much of the spatial range of seagrass in the estuary as a whole. Two sites fall within the Swan Estuary Marine Park (i.e. near Pelican Point adjacent to the University of Western Australia, PPT, and Lucky Bay within the Alfred Cove location, LUB). The Marine Park areas were expected to have lower risk of anthropogenic disturbances than the remaining four sites.

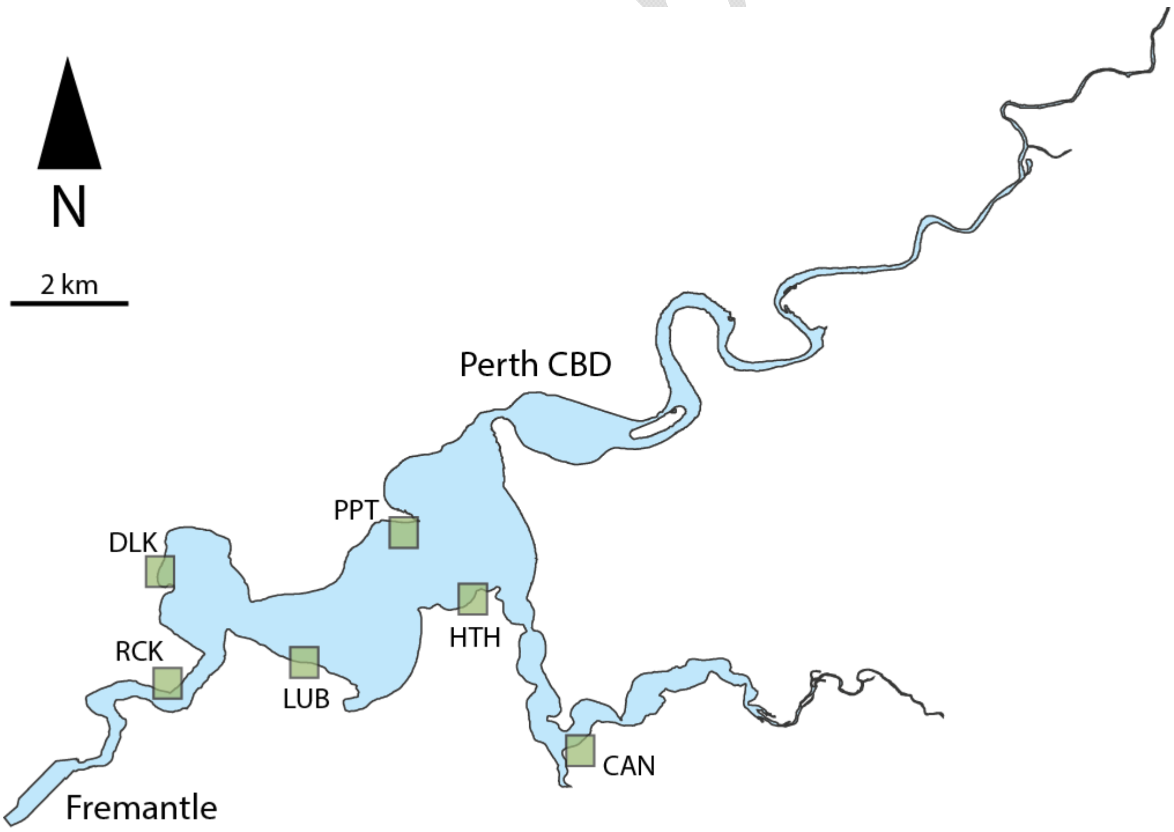

Figure 1 *Location of routine seagrass sites within the Swan-Canning estuary*

1.2 Proposed seagrass indicators

Seagrass indicators were proposed in an unpublished DWER report provided to DBCA for both **seagrass performance** (Table 2) and **key pressures** (Table 3) based on data from two years - 2011-12 and 2013-14.

It is important to consider the time period that these indicators are likely to be able to inform on, based on the sampling regime that underpins the sampling program as well as the timescale of ecological response of the plant. This is summarised in Figure 2.

It is these proposed indicators that are being assessed for their validity in this report, given that DWER has now collected ~ 5 years of data which also allows the natural variability of the seagrass and its response to be evaluated.

Table 2 *Proposed indicators of seagrass performance and classification cut-offs for scoring*

Indicators	Rationale	Measure/s contributing to indicator	Classification
Seagrass presence	Ratio of the presence and absence of seagrass observations. More observations of seagrass mean an expansion of seagrass extent within the estuary.	Transect data collected at the meadow-scale during peak seagrass abundance. Average site values were compared to classification cut-offs	Poor < 0.2 Low 0.2 to <0.5 Fair 0.5 to <0.8 Good >0.8
Seagrass cover	An increase in seagrass percentage cover is an increase in seagrass density, suggesting more favourable performance.	Transect data collected at the meadow-scale during peak seagrass abundance. Average site values were compared to classification cut-offs	Poor <5% Low 5% to <25% Fair 25% to <50% Good >50%
Seagrass productivity	Seagrass growth or productivity is a direct measure of the performance at a local scale.	Productivity was determined on up to 30 tagged rhizomes per site between January and March. Average values across the three months compared to classification cut-offs	Poor <1.5 mg apex^{-1} day^{-1} Low 1.5 to 3.0 mg apex^{-1} day^{-1} Fair 3.0 to 4.5 mg apex^{-1} day^{-1} Good >4.5 mg apex^{-1} day^{-1}

Indicators	Rationale	Measure/s contributing to indicator	Classification
Seagrass reproduction	The long-term resilience of this estuarine population (a fast-growing, colonising species) is likely to be dependent on the successful reproduction and establishment of a viable seedbank (both for genetic diversity of the population and for the capability to re-establish from seed if conditions in the estuary are temporarily unable to support seagrass).	Using both fruit and flower data observed in cores – Average values for flowering (December to March) and fruit (January to March) density were compared to classification cut-offs.	Poor <50 flowers m^{-2} and 0 fruit m^{-2} Low[1] >50 flowers m^{-2} and 0 fruit m^{-2} Fair[1] 1-20 fruit m^{-2} Good >300 flowers m^{-2} or >20 fruit m^{-2}

[1] Note originally proposed cut-offs did for low and fair categories resulted in gaps where results could not be scored. The cut-offs proposed here are in-line with the original cut-offs but now mean that all data can be scored.

Table 3 *Proposed indicators for seagrass key pressures and classification cut-offs for scoring*

Indicator	Rationale	Measure/s contributing to indicator	Classification
Macroalgal cover (light-stress)	Large accumulations of macroalgae (often 'nuisance' green macroalgae) provide a physical barrier, blocking light reaching the seagrass canopy.	10 replicate quadrats collected each month from November to March. Average cover values across site was used to compare against classification cut off.	High stress >80% Moderate-high stress 50 to <80% Moderate-low stress 20 to <50% Minimal stress <20%
δ^{13}C in leaf tissue (light-stress)	Variability in δ^{13}C occurs both due to inputs of terrestrial carbon and variation in light regimes. Interannual differences at the same site are likely to be mainly due to variation in light. The mechanism for the response to light regime is that isotopic discrimination decreases with increasing irradiance,	Leaf tissue samples collected during peak growing period (February). Average δ^{13}C values across site compared to classification cut off, which itself was site-specific.	High stress <-0.75‰ Moderate-high stress <0 to -0.75‰ Moderate-low stress 0 to +0.75‰ Minimal stress >+0.75‰

Indicator	Rationale	Measure/s contributing to indicator	Classification
	i.e. $\delta^{13}C$ values become more positive with improved light conditions		
Fsulfide in rhizomes (Sediment-stress)	The fraction of sediment-derived sulfide measured in seagrass rhizomes (by $\delta^{34}S$ analysis).	Seagrass tissue sampled in cores from January to March was measured for $\delta^{34}S$. Measurement of $\delta^{34}S$ in sediment sulfide and sulfate in overlying water also used in the calculation of Fsufide. Average values across site compared to classification cut off.	High stress >40% Moderate-high stress 30 to 40% Moderate-low stress 20 to <30% Minimal stress <20%
Carbon to nitrogen ratio (C:N) (Eutrophication)	The atomic ratio of carbon to nitrogen has been proposed as an indicator for nutrient enrichment, where increases in nitrogen relative to carbon (i.e. C:N ratio decreases) indicate eutrophication.	Leaf concentrations of carbon and nitrogen were converted to atomic ratios. Data from cores collected in February.	High stress <17 Moderate-high stress 17 to <19 Moderate-low stress 19 to 21 Minimal stress >21
$\delta^{15}N$ (Eutrophication)	The nitrogen isotope signal measured in seagrass leaves is likely to reflect the source of nitrogen. More positive $\delta^{15}N$ values measured in macrophytes are likely as a result of eutrophication (Cole et al. 2005).	Cores were collected during peak growing period (February) and leaves analysed for $\delta^{15}N$. Average values across site compared to classification cut off.	High stress >9 Moderate-high stress >7 to 9 Moderate-low stress 5 to 7 Minimal stress <5

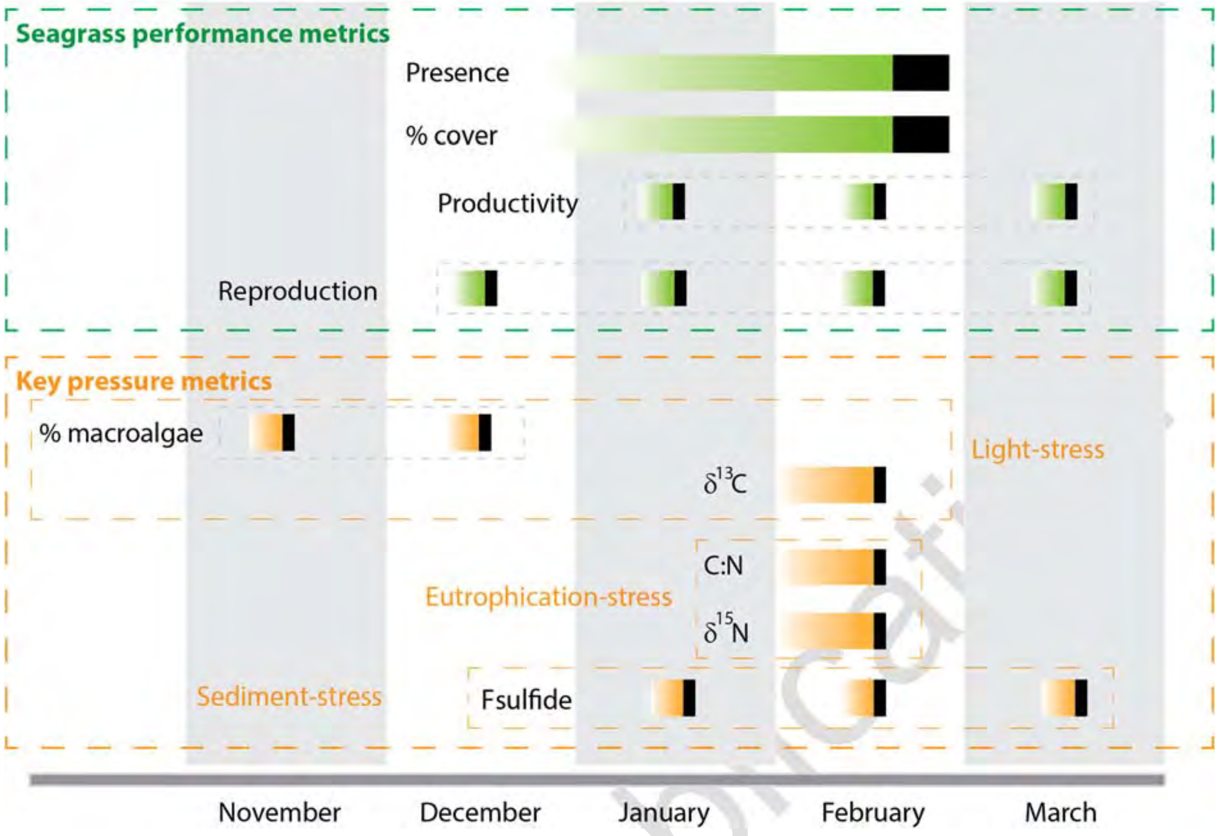

Figure 2 *Diagram showing the temporal relationship between seagrass indicators. The indicators of seagrass performance and key pressures are measured at different times during the sampling season (shown by black bars), while the period that they integrate seagrass response over also differ (green or orange shaded bar).*

1.3 Objectives of this report

This current report explicitly revisits this suite of seagrass indicators, assessing their soundness over 5 years of study (summers of 2011-12, 2013-14, 2014-15, 2015-16 and 2016-17). The aims of this report are to:

1) describe the natural variability of seagrass cover, species composition and other seagrass meadow characteristics as related to changes in key climatic conditions,
2) understand the sensitivity of seagrass metrics in response to anthropogenic stressors such as water quality and sediment stress,
3) refine and validate the seagrass indicators proposed, and
4) provide protocols for the use of these indicators.

Detailed data is provided in Appendix C. Additionally appendices are provided to report complementary information and data collected during the validation period but not directly part of the seagrass indicator suite of measures.

2 Climatic and environmental conditions 2011-2017

Seagrass measures (and derived indicators) may show significant variation over time. Observed changes may be attributed to seasonal growth cycles or other natural inter-annual variations such as climate. Additionally, aspects more directly related to human-induced pressures, such as water quality, may influence the seagrass response in a given year.

A number of climatic and environmental attributes are considered to have a direct or indirect influence on seagrass performance, including tide height, temperature, hours of sunshine, rainfall, water clarity.

In this section, we aim to address the following questions:

- How representative was the climate of the 5 years[3] in which we sampled seagrass when compared to the last ~25 years?
- What was the typical annual variation of climatic and environmental data within the validation years when seagrass was studied? Was the climate of some years of study more conducive to seagrass performance than others?
- Does the routine water quality monitoring for the Swan-Canning estuary show differences in water quality between years of seagrass study that might affect annual seagrass performance?

2.1 How does the climate of the seagrass validation years compare to historical records?

Seagrass indicator responses may exhibit significant variability inter-annually, even when ecological condition remains fairly stable. These changes may be attributable to the within-year variations in environmental condition which result in natural growth cycles of the seagrass, or the variations in environmental condition across multiple years, driven by differences in climate. As the seagrass studied, *Halophila ovalis*, is a colonising seagrass, species the magnitude of these variations are expected to be greater than for other opportunistic or persistent seagrasses (Kilminster et al. 2015).

The following plots (Figure 3) show climate (rainfall, temperature and sunshine) from two periods thought to influence annual seagrass performance for 2011-12 to 2016-17, as well as the average conditions in the past ~25 years (variable depending on available data record). Data within this section has been obtained from the Bureau of Meteorology http://www.bom.gov.au/climate/data/stations/ for station Perth Metro 009225 and the Department of Transport for Fremantle and Barrack Street.

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[3] Throughout this document, the validation period will be referred to as 5 years, although for some measures of seagrass attributes, such as species composition and meadow-scale transect data, 6 years of data are available.

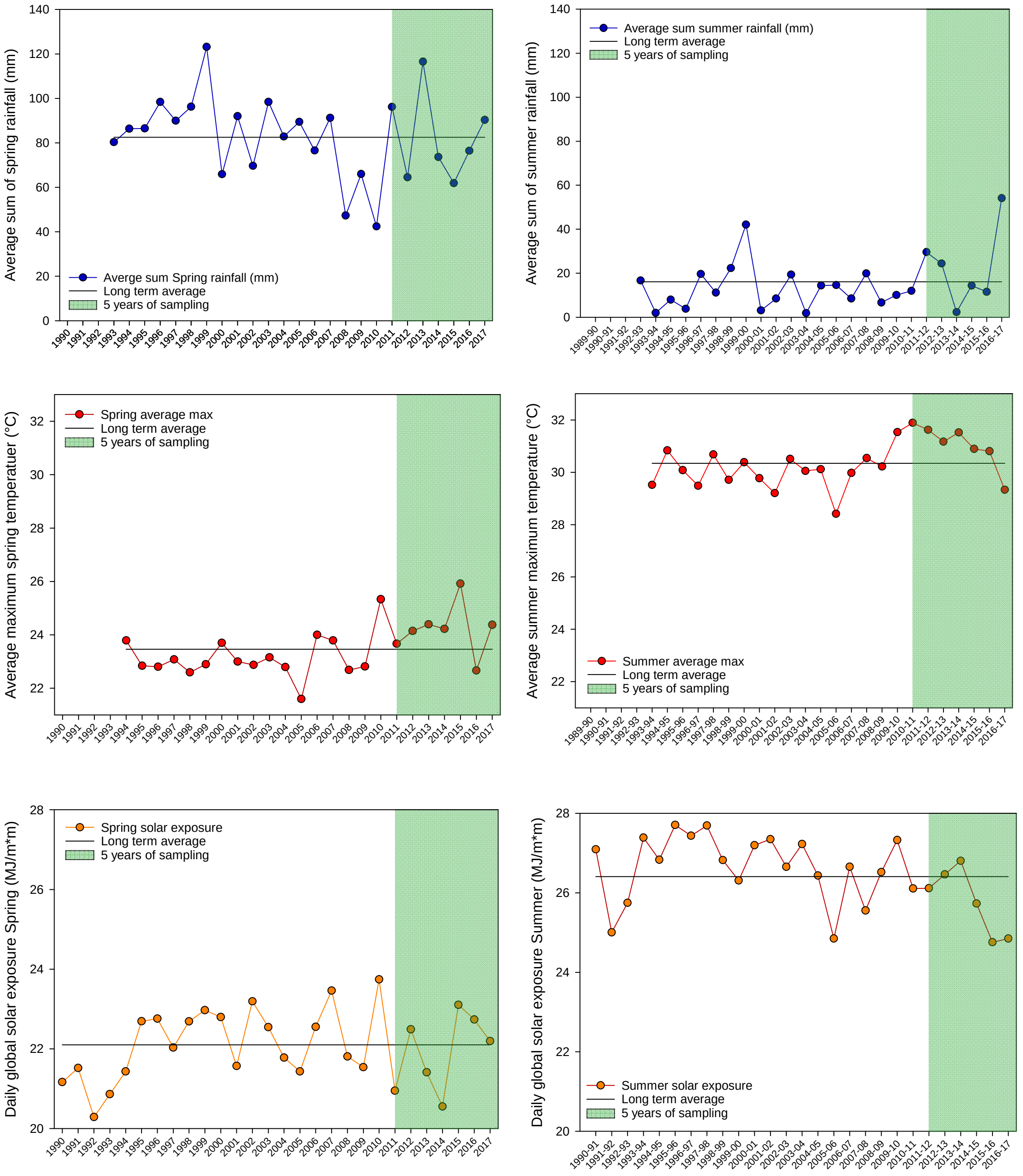

Figure 3 *Plots showing annual climatic conditions across the validation period (shown with green background), compared to the recent historical record: A) sum of rainfall per month in Spring period, B) sum of rainfall per month in Summer sampling period, C) average temperature in Spring, D) average temperature in Summer period, E) average solar exposure in Spring, and F) average solar exposure in Summer. Long-term averages shown with horizontal line.*

Both of these periods were considered ecologically relevant time periods for seagrass in the Swan-Canning estuary – 'Spring' which is data pooled for September, October and November; and 'Summer sampling period' which covers December, January, February and March – in line with the period of most sampling effort. By considering the data in this fashion, we are looking to see that the validation period encompassed the climatic conditions that seagrass are likely to be exposed to in the future (i.e. were these years a good test of the indicators?).

In general, Spring conditions appeared within range previous experienced (except for temperature, which appeared somewhat elevated in the validation period). For the Summer sampling period, when seagrass were expected to be most productive, the validation period was generally warmer. These hotter conditions were reflected in the Bureau of Meteorology reporting of significant climatic conditions in this period (Appendix C). 2016-17 had unusually high summer rainfall resulting in major river flows across much of southern Western Australia. Two weather events (29/01/2017 to 1/02/2017 and 10/02/2017 to 12/02/2017) delivered approximately 270 gigalitres of water from the Avon River into the Swan Estuary. This resulted in conditions in Summer of 2017 which were not typical of the preceding years.

2.2 Was climate of some years more conducive to good seagrass performance than others?

Halophila ovalis, as a colonising species, is highly responsive to the environmental conditions that it is exposed. In a management sense, we need to acknowledge the natural variability that is inherent from year to year in seagrass condition, due to factors that we cannot manage for. Therefore, recognising factors that lead to good or sub-optimal performance, particularly related to climate, are critical to understanding how to interpret the seagrass indicators for management. Where seagrass performance deviates from what might have been expected due to the climatic conditions experienced in any given year, anthropogenic factors may be responsible, and therefore management actions may be possible.

The external climatic factors that we believe may influence seagrasses in the Swan-Canning estuary (for which we can obtain data) are shown in Table 4. For each factor, in each year of validation data, we have provided an expert opinion of how conditions that year may have influenced seagrasses in the Swan-Canning estuary in a relative sense compared to the other years of study where green indicates conditions were good, and orange that conditions were sub-optimal. We suggest as a summary of climate in the validation years based on the information in Table 4:

- 2011-12 – poor climatic conditions for seagrass performance
- 2012-13 – good climatic conditions for seagrass performance
- 2013-14 - good climatic conditions for seagrass performance
- 2014-15 –average climatic conditions for seagrass performance
- 2015-16 – average climatic conditions for seagrass performance
- 2016-17 - poor climatic conditions for seagrass performance

Table 4 *Overview of differences between study years of climate variables thought to influence seagrasses, where dark green is more favourable, to dark orange indicating less favourable conditions for seagrasses.*

Climate variable	Potential influence on seagrass performance	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017
Tide[a]	Higher average tides result in a greater depth of overlying water above seagrasses, which reduces light availability. Too low tides may promote desiccation of very shallow seagrasses in some instances.	+15.1	+4.8	-3.3	-3.8	-9.9	-2.8
Water temperature[b]	Higher temperatures are likely to promote greater growth of seagrass and algae, unless beyond physiological tolerances.	-0.09	n.d.	+0.51	+0.27	+0.05	-0.75
Solar exposure	More hours of sunshine across summer period are likely to promote better conditions for photosynthesis.	0.33	0.68	1.02	-0.06	-1.03	-0.94
Benthic light[d]	Greater benthic light available (measured in situ as photosynthetically active radiation) may result in greater productivity. Note although light loggers deployed in 2011-12, data is not directly comparable to subsequent years.	n.d.	n.d.	+9.2	-0.07	-1.16	-7.9
Hours of saturating light[e]	Maximum rates of photosynthesis occur when light levels are saturating. Greater seagrass growth may occur with more hours of light above saturation.	n.d.	n.d.	+1.41	+0.68	+0.38	-2.47
Spring Rainfall (Sept-Nov)	Rainfall brings nutrients from the catchment, so may promote algal blooms in competition with seagrass. Changes in salinity and sunshine (due to cloud cover) are also possible. The effect on seagrass is likely to be related to the timing and duration of events.	14.6	-17.0	35.0	-7.9	-19.7	-5.1

[a] Values shown are deviation from the average tide height (of 0.83 cm) measured at both Fremantle and Barrack Street tide gauges in the period 2011-2017, December to March. Positive values mean tide was higher, negative values mean tide was lower than the average condition in this six year period.

[b] Average water temperature observed between December and March for all sites pooled as a deviation from the average condition measured in the period (24.75 °C).

[c] Average summer solar exposure calculated annual for the December to March period using data from Bureau of Meteorology's daily global solar exposure model. Deviation from the average value of 25.78 MJ m^{-2}.

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[d] Benthic light as measured insitu with Odyssey PAR loggers. Described as the deviation from the average daily moles of photosynthetically active radiation across the January to March period pooled for all sites and years (average = 20.07 moles m^{-2} d^{-1}). Note, benthic light will integrate the influences of changing water clarity and water depth due to tide.

[e] Described as the deviation from average Hsat (hours above saturation of 200 μmol m^{-2} s^{-1}; average = 7.49 hrs) as determined from insitu Odyssey PAR loggers between December and March, pooled for all sites.

[f] Spring rainfall (September to November) values shown are the deviation away from the average monthly rainfall received in the spring period of 81.5 mm.

Tidal variation

For seagrasses within estuaries, tide height may strongly influence the depth of the water overlying shallow benthic seagrass communites, thus resulting in dramatic differences in light climate, as light is absorbed as it passed through water. Although the Swan-Canning estuary experiences a microtidal regime, there can be interannual variations in mean tide height related to frequency of storm-cells or high pressure systems. Across the six years of seagrass data collection, there was up to a 25 cm difference in the average tide observed across the December to March period, which is substantial for these shallow seagrass meadows. Plots of average monthly tide data for each year are shown in Appendix B (Figure A 2).

2011-12 had significantly higher tide heights, and 2015-16 lower tide heights than other years of study (Figure A 2). In fact, 2011-12 had the highest average tide recorded across the December to March period between 1990-91 and 2016-17 for Barrack Street.

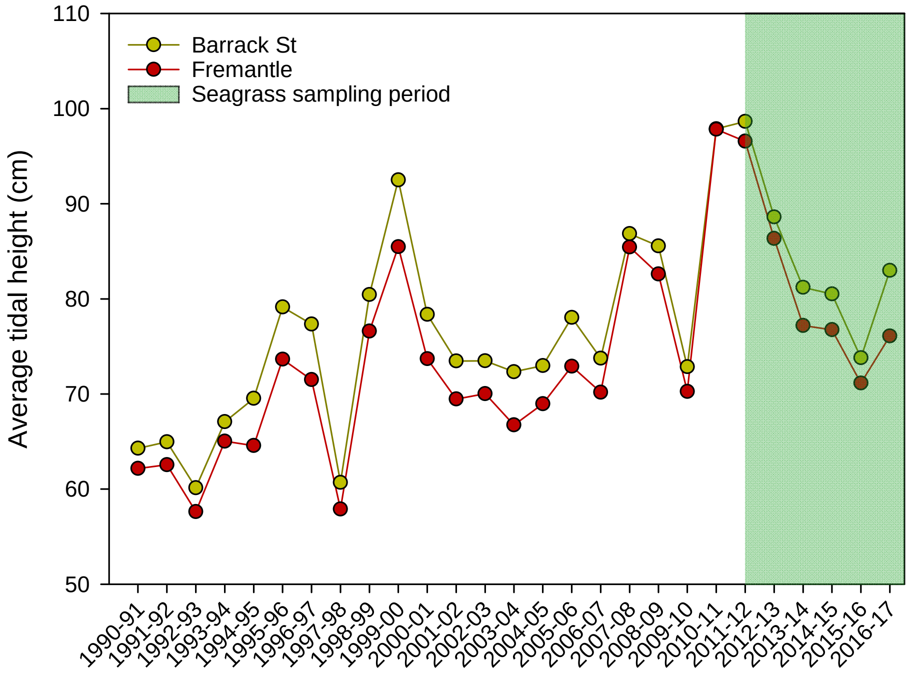

Figure 4 *Average tidal height during December to March at Barrack Street and Fremantle for December to March periods from 1990-91 to 2016-17.*

Water temperature

Water temperature is likely to have a complex relationship with seagrass growth under natural conditions in the estuary given interactions with other biotic components in the estuary (such as macroalgae, phytoplankton and sediment microbial communities). However, temperature accounted for 38-70% of the variation in seagrass productivity at sites in the Swan-Canning estuary investigated by Hillman *et al.* (1995). Culture experiments of *Halophila ovalis* also showed that no growth occurred <10 °C, was severely limited between 10-15 °C, and the highest growth rates investigated occurred at 25 °C (Hillman et al. 1995).

Of the five years studied, 2016-17 was notably cooler (Figure A 3).

Solar exposure

Seagrasses require light to photosynthesise, and differences inter-annually in available sunshine during the growing season may influence seagrasses performance. Information on solar exposure is taken from Bureau of Meteorology.

2013-14 had on average more than 2 hour more of sunshine than 2015-16 and 2016-17 across the sampling period (December to March).

Benthic light[4]

Light reaching the benthos is a combination of the climate (e.g. hours and intensity of sunshine) and also the clarity of the water overlying the seagrass (e.g. turbidity, phytoplankton and/or macroalgal accumulations). Benthic light has been reported here as moles m^{-2} d^{-1}, and therefore are a cumulative measure of the total light received during a given day. Benthic light was measured as photosynthetically active radiation with insitu loggers.

On average, light received by seagrasses was 20.07 moles m^{-2} d^{-1} over the January to March period. LUB, the shallowest location, received the most light – up to 42.55 moles m^{-2} d^{-1}. Light conditions were best in 2013-14 and worst in 2016-17 where turbid flood waters dramatically reduced the light available (Figure A 4 and Figure A 5).

Hours of saturating irradiance (Hsat)

Saturating light for photosynthesis was taken as 200 μmoles photons $m^{-1}s^{-1}$ based on Hillman et al. (1995), although recent work by Said (2017) reports that the saturating irradiance for photosynthesis of *Halophila ovalis* can vary substantially depending on location and time of year. This Hsat metric reports the number of hours per day where the benthic light exceeds the saturating light threshold.

On average, seagrass received 8-9 hours of light greater than the saturating threshold in years 2013-14, 2014-15, and 2015-16, while this was lower at only 5 hours in 2016-17

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[4] Note benthic light and Hsat information is only available for 2013-14 to 2016-17, whilst measured in 2011-12, it is not comparable to the later datasets due to differences in calibration. The publication Shaffer, JM & Beaulieu, JJ 2012, 'Calibration of the Odyssey™ Photosynthetic Irradiance Recorder™ for absolute irradiance measures', *Journal of Freshwater Ecology,* vol. 27**,** pp. 599-605. allowed for the conversion of Odyssey PAR logger data to absolute measures of irradiance.

(Figure A 4). Note, hours of compensating irradiance – the irradiance where photosynthesis and respiration are equal is also shown in Figure A 4., and the value for compensating irradiance used was 40 μmoles photons $m^{-1}s^{-1}$ again derived from Hillman et al 1995.

Spring rainfall

Spring rainfall can deliver nutrients from the catchment to the estuary, just prior to increasing temperature and sunshine in the estuary. Supply of nutrients may favour algal blooms which compete with seagrass for available light. Average monthly spring rainfall was calculated for the BOM station Perth Metro.

2011-12 and 2013-14 were the years with the highest spring rainfall, while 2012-13 and 2015-16 were dry in comparison.

2.3 Validation years – evidence from routine water quality sampling

Water quality from the routine monitoring program (Swan Canning Environmental Monitoring and Reporting project which has operated since 1994) was explored for the years 2011-2017 which made up the validation period for the seagrass monitoring program. We aimed to see if significant inter-annual variations in water quality existed which might affect the condition of the seagrass during the period where seagrass were being monitored (December to March)

For seagrass sites in the Lower Swan, data was pooled for routine water quality sites NAR, HEA, ARM, BLA, and SAL was expected to reflect conditions for the Canning estuary (i.e. seagrass site CAN) – see Figure A 1 for site map of routine water quality monitoring sites in relation to seagrass monitoring sites.

Overall, there appeared to be little difference between years 2011-12 to 2015-16, while water quality was clearly different in 2016-17 due to the extreme summer rainfall event previously discussed in section 2.1.

Chlorophyll a

Chlorophyll a is a measure of the amount of photosynthetic pigment present within the water sampled, and may be interpreted as a proxy for generalised phytoplankton activity.

Chlorophyll a was low across all validation years except for 2017, where peaks of both surface and integrated chlorophyll were present in February and March (Figure 5). Surface Chlorophyll was likely elevated due to the presence of *Procentrum minimum* in March in the Lower Swan, a larger dinoflagellate species that could also take advantage of the extra nutrients available at this time (see Figure A 6). *Procentrum minimum* was also present at SAL at this time in the Canning Estuary.

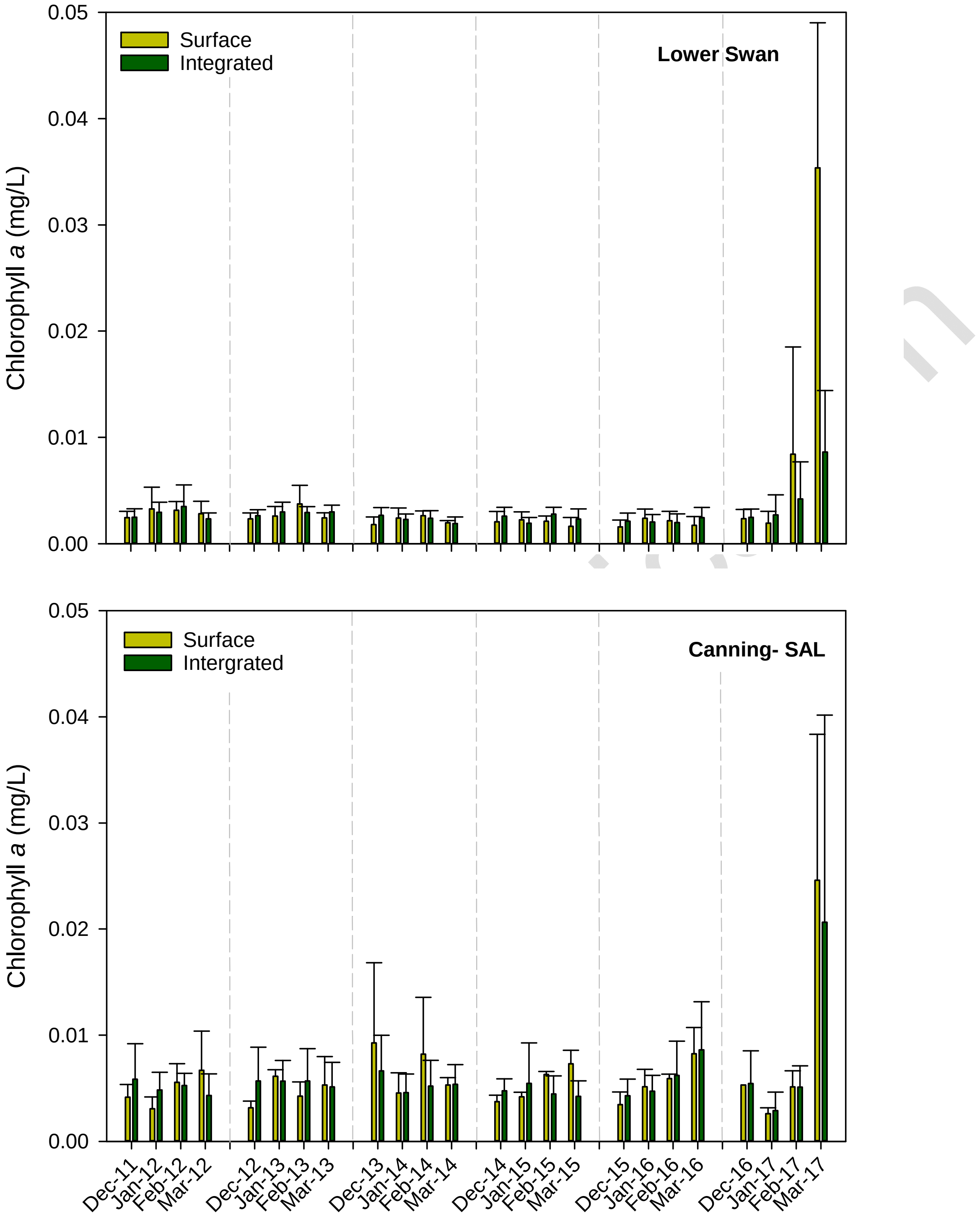

Figure 5 *Chlorophyll a (surface and integrated) measured at sites in the Lower Swan (pooled for NAR, HEA, ARM, BLA) and Canning (SAL).*

Water clarity

Light extinction co-efficients have been measured as ‘In-Channel PAR’ fortnightly with LICOR instruments. Data was obtained fortnightly across sites in the Swan-Canning estuary, and the profiles used to determine spatial and temporal variation in light attenuation in the estuary as well as the depth to which sufficient surface irradiance would likely penetrate the water allowing seagrass growth. This is known as euphotic depth.

It must be acknowledged that there are large gaps in data due to occasions where equipment was not working. For example, there is no data during the summer flood event of 2017 and during the 2015-16 there were times where only two sampling periods produced usable data. Table 5 shows median euphotic depth 10% for each site using data from December to March of each study year.

Euphotic depth (10% of surface irradiance) varied spatially and temporally in the Swan estuary, ranging between 1.9 to 5.3 m. Spatially, euphotic depth decline from the marine extent of the estuary (BLA, ARM and HEA) to Perth Waters (NIL). Temporally, euphotic depth was more variable at the marine extent of the estuary and was more stable at NAR. Euphotic depth in the Swan River was much deeper than in the Canning River (1.9 to 2.3 m).

Deeper euphotic depths towards the marine extent of the estuary are reflected by seagrass observations at depths of 5-6 m at RCK and 3-4 m at DLK, showing deeper penetration of surface irradiance, compared to CAN where seagrass is typically limited to depths of 1-2 m.

Table 5 *Average euphotic depth (10% surface irradiance) for sites in the Lower Swan and Canning*

Estuary site (region)	2013-14[1]	2014-15	2015-16[2]	2016-17[3]
BLA (Marine channel)	4.5	4.3	4.5	5.3
ARM (Melville Waters)	4.1	4.2	3.7	4.0
HEA (Melville Waters)	3.6	3.1	3.8	3.0
NAR (Perth Waters)	2.6	2.3	1.9	2.2
SAL (Canning estuary)	1.9	1.6	2.3	2.3

[1] Data for 2013-14 season only began in February 2014, and there are only four sampling occasions

[2] BLA and ARM only has one usable reading, HTH had two while NAR had three readings

[3] There is only data available for December 2016. Due to issues with the LICOR there is no data available during the 2017 summer flood. Hence values should be interpreted with caution

Other measures of water quality

Seasonal nutrient concentrations for Lower Swan and Canning sites are shown in Figure A 6 and Figure A 7 for TN, TP, DON, NOx, NH4 and FRP for bottom and surface water samples. Nutrients were generally higher for the Canning site (SAL) compared to values for the Lower Swan (pooled for sites NAR, HEA, ARM, BLA). Nutrient concentrations in the December to March period were elevated in 2016-17 compared to other years of study.

Seasonal physical measurements of water quality (salinity, dissolved oxygen, and temperature) were again most variable in 2016-17, likely a result of the summer flood event Figure A 8.

2.4 Summary of climatic and environmental conditions 2011-2017

The climate within the 5 years we sampled had some extreme events relative to historical data. Most notably, summer temperatures were much warmer than previous periods, with a number of records exceeded during this period (see Appendix C).

Tide heights were unusually high in the first year of sampling (2011-12) over the seagrass growing period (December to March) compared to the other years of monitoring, and compared to the preceding ~20 years. These higher tides will reduce the light reaching the seagrass canopy due to a greater depth of water overlaying the seagrass through which light needs to penetrate.

A large summer flood event delivered 270 gigalitres of water from the Avon River into the Swan Estuary in February 2017. This event significantly influenced conditions in the estuary with substantial decreases in light available, as well as higher nutrient concentrations, lower salinity and lower temperature.

Overall we suggest that:

- 2011-12 and 2016-17 had the poorest climate for seagrass performance
- 2014-15 and 2015-16 had average climate conditions for seagrass performance
- 2012-13 and 2013-14 were considered good climates for seagrass performance

3 Seagrass metrics – variability across validation years

Metrics (or measures)[5] of seagrass performance were measured across the 2011-17 period. These have been reported in the following section split into categories relating to 1) meadow characteristics, 2) seagrass productivity, 3) seagrass reproduction, 4) and broader site surveys with transect observations.

3.1 Meadow characteristics

We have assumed that greater leaf density, apex density, branching and biomass are indications of better seagrass performance as these characteristics directly influence the ability of the plant to photosynthesise, and/or expand vegetatively. Characteristics, such as leaf mass and biomass allocation to above and belowground plant parts, may be altered by the plant in response to environmental conditions and were not assigned a directional influence on seagrass performance.

The comparative difference between study years is shown in Table 6. 2014-15 had the greatest apex density, branch density, leaf density and biomass, suggesting that seagrass performance was very good. Conversely, 2013-14 had the lowest apex density, branch density and leaf density and moderately low biomass – suggesting poor seagrass performance.

Detailed data on meadow characteristics is shown in Figure A 9 and Figure A 10 for apex density, branch density, leaf density and total biomass for each site in each year of monitoring. Generally apex and branch density showed variability both between sites and years. For leaf density and total biomass, most sites had peaks in 2014-15. Site LUB however, consistently reduced for both leaf density and total biomass in each subsequent year. Site RCK had the densest meadows in all years, reflected in both leaf density and total biomass.

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[5] Throughout this report we adopt the terminology of metric to the actual parameter measured (usually a continuous variable), to distinguish from the term indicator (generally classified into 4 categories in this report)

Table 6 *Overview of relative differences between study years for meadow characteristics. Dark green is considered a more favourable seagrass response, while dark orange indicates less favourable seagrass responses.*

Meadow characteristic Measurement	Potential influence on eagrass health	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017
Apex density[a]	More growing tips per square metre suggest greater vegetative seagrass growth potential	-164.9	n.d	-267.5	+195.7	+261.9	-32.1
Branch density[b]	More branches per square metre suggest greater vegetative seagrass growth potential	-17.7	n.d	-63.8	+73	+30.2	-22.4
Leaf density[c]	Greater leaf density indicates greater resistance potential of the seagrass to disturbance	-385.4	n.d	-728.6	+1373.7	+265.8	-541.6
Leaf mass[d]	Average leaf mass varies between sites, however it is not clear whether greater leaf mass is positive or negative (or neither) attribute of seagrass health	+0.21	n.d	+0.77	+0.31	-0.95	-0.33
Above below ground biomass ratio[e]	Changes in allocation of resources between above-ground (photosynthetic) tissues and belowground may be informative, but not clear the direction of effect on seagrass health	-0.12	n.d	-0.04	-0.01	-0.11	+0.26
Total Biomass[f]	Greater biomass indicates a more productive seagrass meadow	+14.5	n.d	-0.08	+41	-6.3	-48.5

[a] Values shown are deviation from the overall mean apex density (1481.5 apex per m^{-2}) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

[b] Values shown are deviation from the overall mean branch density (543.3 branches per m^{-2}) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

[c] Values shown are deviation from the overall mean leaf density (7503 leaves per m^{-2}) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

[d] Values shown are deviation from the overall mean leaf mass (6.6 mg) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

[e] Values shown are deviation from the overall mean above to below-ground biomass ratio (0.66) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

[f] Values shown are deviation from the overall mean total biomass (149.4 g DW m^{-2}) measured at all sites between December to March in the period 2011-2017 (excluding 2012-13).

3.2 Seagrass productivity

For all of the measures of seagrass productivity listed in Table 7, in all cases, faster rates were considered to be indicative of better seagrass health. In terms of general seagrass productivity, 2013-14 was the best year, followed by 2011-12, with 2016-17 clearly the worst year. It should be noted that there appears to be a trade-off as to whether the seagrass invests in vegetative growth or in sexual reproduction.

Productivity measures for rhizome extension rate (mm apex^{-1}day^{-1}) and total growth rate (mg apex^{-1}day^{-1}) are shown in Figure A 11. Generally vegetative growth was greater in 2011-12 and 2013-14 than in 2014-15 and 2015-16 (when sexual reproduction was at its peak). Productivity was lowest in 2016-17 as a consequence of the summer flood waters.

Growth rates were highest for PPT in 2013-14, potentially a consequence of low starting density in November and December (as shown in Figure A 23), with the plant investing greater effort in vegetative expansion.

Table 7 *Overview of relative differences between study years for seagrass productivity. Dark green is considered a more favourable seagrass response, while dark orange indicates less favourable seagrass responses.*

Productivity measurement	Potential influence on seagrass health	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017
Leaf formation rate[a]	Higher leaf formation rates would indicate faster growth	+0.07	n.d	+0.03	+0.03	-0.01	-0.12
Rhizome extension rate[b]	Faster rhizome extension rates would indicate greater capacity to vegetatively expand over an area	+0.85	n.d	+1.09	-0.3	-0.19	-1.4
Above ground growth rate[c]	Faster above-ground growth indicates greater investment in photosynthetic tissues	+0.11	n.d	+0.41	+0.14	-0.03	-0.63
Below ground growth rate[d]	Faster below-ground growth indicates greater investment in below-ground storage tissues	+0.32	n.d	+0.62	-0.01	-0.18	-0.73
Growth rate[e]	Faster growth in general means a greater potential for vegetative expansion	+0.38	n.d	+1.06	+0.12	-0.19	-1.35

[a] Values shown are the deviation from the overall leaf formation rate (0.42 new leaves per apex per day) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[b] Values shown are the deviation from the overall rhizome extension rate (3.67 mm apex^{-1} d^{-1}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[c] Values shown are the deviation from the overall above-ground growth rate (1.64 mg DW apex^{-1} d^{-1}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[d] Values shown are the deviation from the overall above-ground growth rate (1.69 mg DW apex^{-1} d^{-1}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[e] Values shown are the deviation from the overall above-ground growth rate (3.28 mg DW apex^{-1} d^{-1}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

3.3 Seagrass reproduction

For all of the measures of seagrass reproduction listed in Table 8, in all cases, greater investment in sexual reproduction was considered to be indicative of better seagrass health. Investment in flowering has also been suggested to be a stress response of seagrasses (Cabaço & Santos 2012), however our observations of *Halophila ovalis* in the Swan-Canning suggest that the seagrass invests in reproduction more profusely when environmental conditions are good.

Table 8 *Overview of the relative differences between study years for seagrass reproduction metrics. Dark green is considered a more favourable seagrass response, while dark orange indicates less favourable seagrass responses.*

Reproduction characteristic	**Potential influence on seagrass health**	**2011-2012**	**2012-2013**	**2013-2014**	**2014-2015**	**2015-2016**	**2016-2017**
Flower density[a]	More flowers are likely to result in a more resilient seagrass population, with greater genetic diversity	-253.1	n.d	-246.4	+365.1	+259	-135.2
Male flowers density[b]	Male flowers are less energetically costly to produce than females. It is likely to be a negative influence on seagrass health if relatively more male flowers are produced than females.	-222	n.d	-150	+156.1	+210.4	-3.7
Female flower density[c]	Female flowers are more energetically costly to produce than males, so it is likely to be a positive influence on seagrass health if relatively more female flowers are produced than males.	-113.5	n.d	-90.9	+215.4	+84.7	-100.4
Fruit density[e]	More fruit is likely to result in a more resilient seagrass population, with greater genetic diversity – however fruits can only be observed in female plants.	-219	n.d	-225.9	+285.3	+236.9	-77.4
Reproductive effort[f]	Reproductive effort is a measure that standardizes the flowering effort with the leaf density (can also be described other ways). Higher values mean that relatively more flowering is occurring, and suggests plants are growing in conditions that favour reproduction	-0.03	n.d	-0.03	+0.06	+0.05	-0.04

[a] Values shown are the deviation from the overall mean flower density (603.3 flowers m^{-2}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[b] Values shown are the deviation from the overall mean male flower density (372.8 male flowers m^{-2}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[c] Values shown are the deviation from the overall mean female flower density (192.8 female flowers m^{-2}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[d] Values shown are the deviation from the overall mean fruit density (303.4 fruit m^{-2}) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

[e] Values shown are the deviation from the overall mean reproductive effort (standardized flowering effort to leaf density) (0.14 flowers per leaf pair) measured at all sites for January to March in the period 2011-2017 (excluding 2012-13).

The best years for *Halophila ovalis* reproduction were 2014-15 and 2015-16 (Figure 6) and 2011-12 was clearly the least successful year for reproduction. Generally flowering density is greatest in December and January, while fruit density reaches its maximum in January in most years, with high numbers also observed in February. For 2016-17, much of the flowering and even fruit production would have been complete before the floodwaters adversely influenced environmental conditions.

There was large variation in sexual reproduction across sites, with LUB having poor reproduction (with no fruit observed in most years) and RCK and HTH showing the most successful production in fruit across most years (Figure A 12). *Halophila ovalis* is dioecious, and variability in the proportion of male and female flowers observed also differed across sites and years of study (Figure A 13).

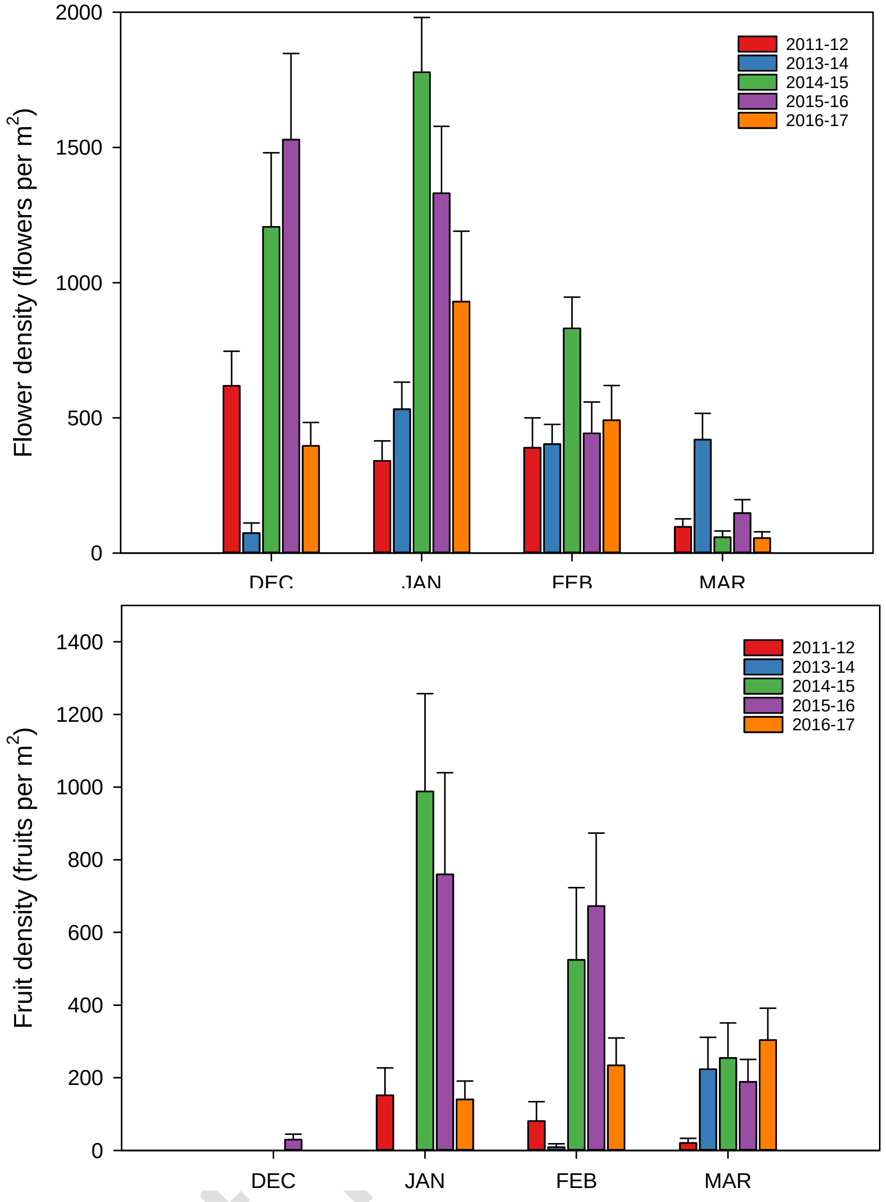

Figure 6 *Monthly flower and fruit densities for 2011-12 to 2016-17 (excluding 2012-13) pooled for all sites in the Swan-Canning estuary.*

3.4 Seagrass transect surveys

Species composition

Generally pattern of seagrass species composition was similar across sites in each year of study. *Halophila ovalis* by far most dominant seagrass present and *Zostera muelleri* is only found towards the marine end of the estuary. CAN showed steady increase in %cover (only *H. ovalis* present) across the study period and *Ruppia megacarpa* became more abundant in 2017.

Of interest, *Posidonia australis* was observed in small patches at the RCK site in 2015-16. This persistent-type seagrass is not commonly observed in estuaries, and historically was not present in the Swan-Canning (e.g. surveys by Hillman in the 1980s). Its presence in the lower Swan is likely to reflect the prevalence of more marine and stable conditions associated with reduced rainfall. However, the *Posidonia australis* patches did not appear to survive the summer flood event in 2017.

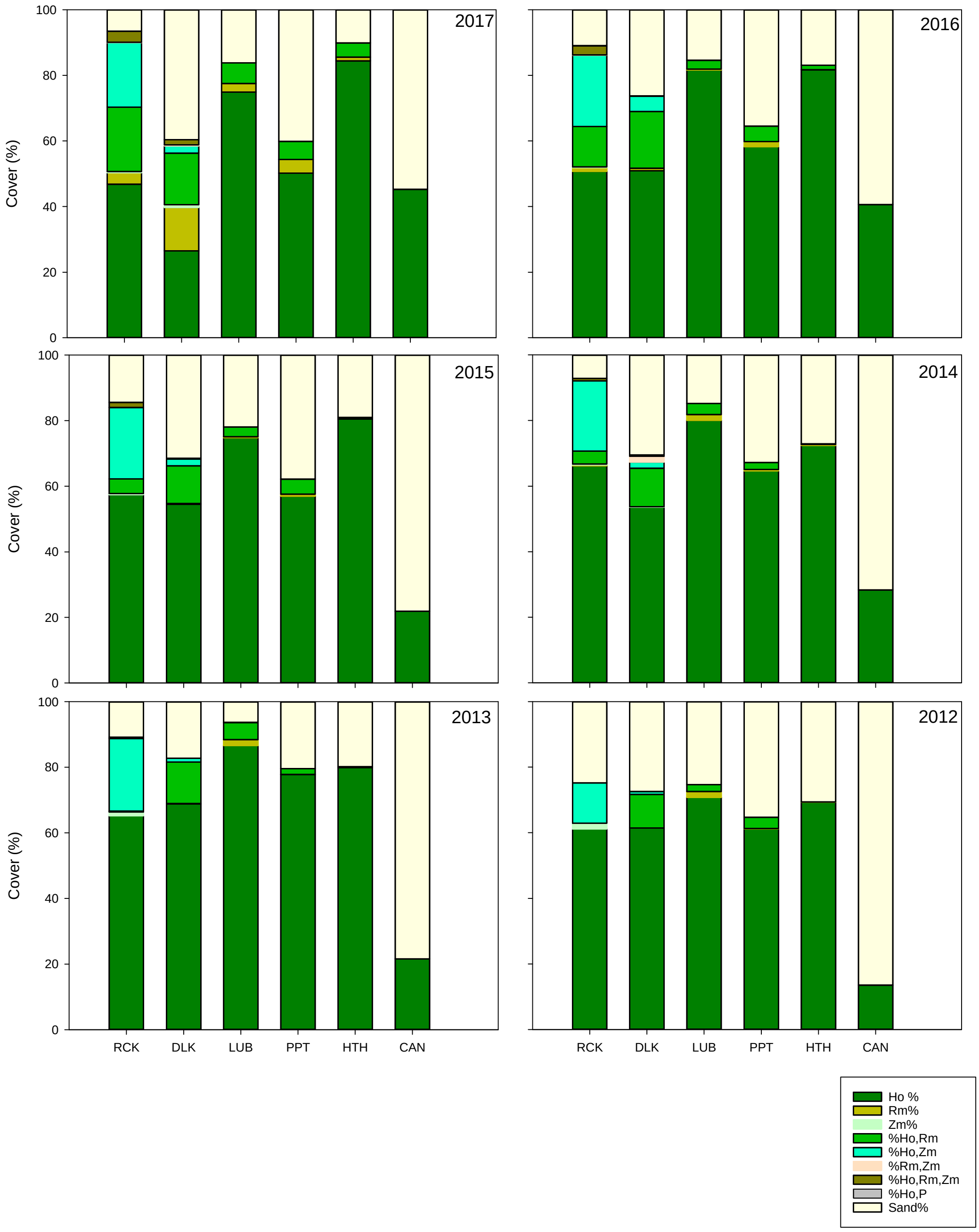

Figure 7 *Percentage cover of seagrass assemblages at each site, where Ho = Halophila ovalis, Rm = Ruppia megacarpa, Zm = Zostera muelleri and P = Posidonia australis.for six years of seagrass assessments.*

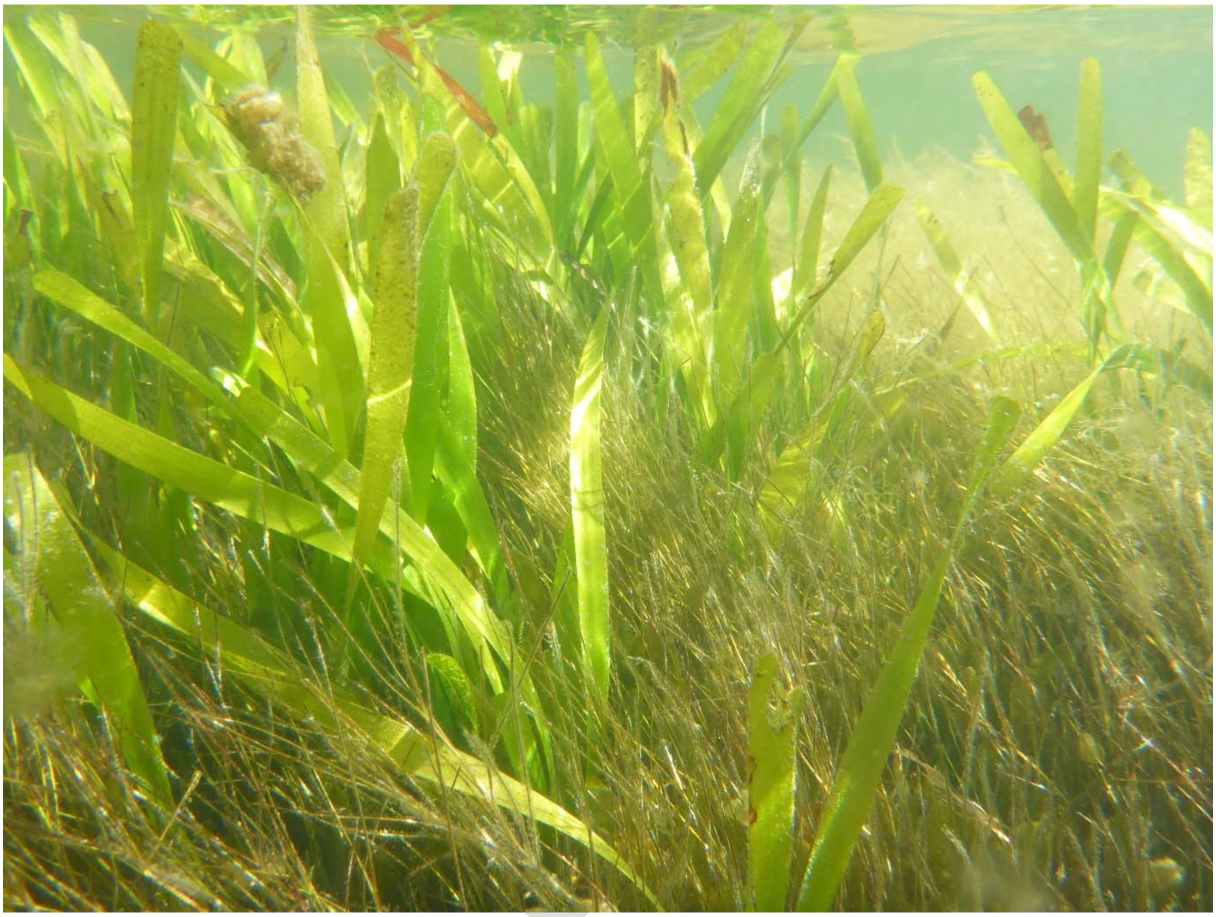

Figure 8 *Posidonia australis in a mixed seagrass bed at site RCK, photo taken in January 2017.*

Seagrass presence and cover

Across the whole estuary, at the meadow-scale, seagrass presence was fairly stable across the validation period, averaging 69.2% of observations. Seagrass presence[6] was highest in 2013 (74%) and lowest in 2012 (61.2%) and all subsequent years fell between these values. Note, due to the timing of the survey in 2017, half of the sites were monitored before impacts from the floodwaters were evident, while the remaining half of sites were clearly affected.

There were larger annual fluctuations in seagrass cover[7] across the years (data from all sites pooled) with the lowest mean cover observed in 2012 (24.2%) and the highest in 2013 (47.5%). Detailed data from each site for percent seagrass cover in each year is shown in Figure A 16, Figure A 17 and Figure A 18, and for most sites the lowest cover was observed in 2012 across the full depth transect. Statistics demonstrating significant changes in meadow presence and percent cover are shown in Appendix E.

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[6] Annual seagrass presence calculated using the sum of seagrass presence (presence = 1, absence = 0) for each transect interval (10 m).

[7] Average seagrass cover was calculated using the midpoint of the percentage range of the cover classes for each of the 10 m transect intervals.

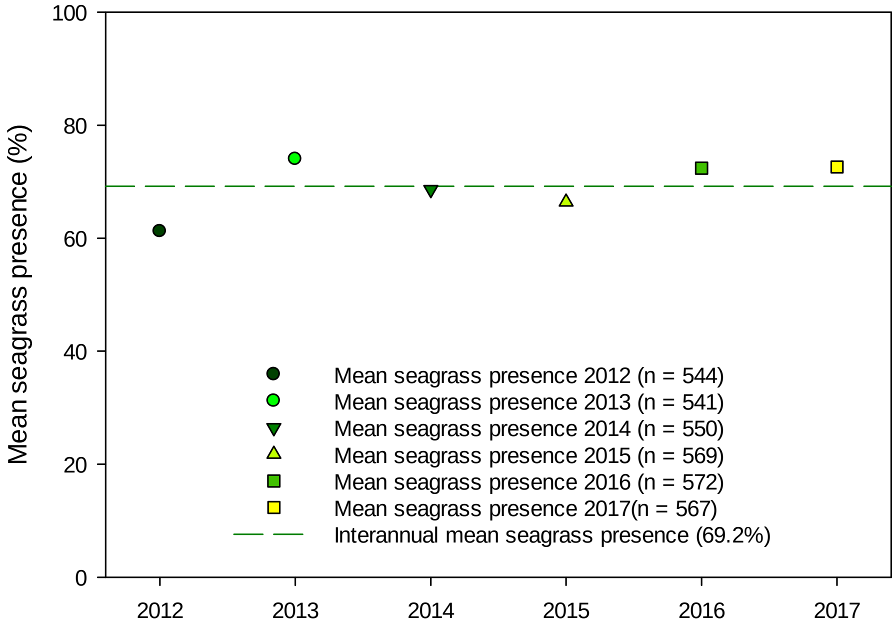

Figure 9 *Average seagrass presence across all sites, for surveys at the meadow-scale from 2012 to 2017.*

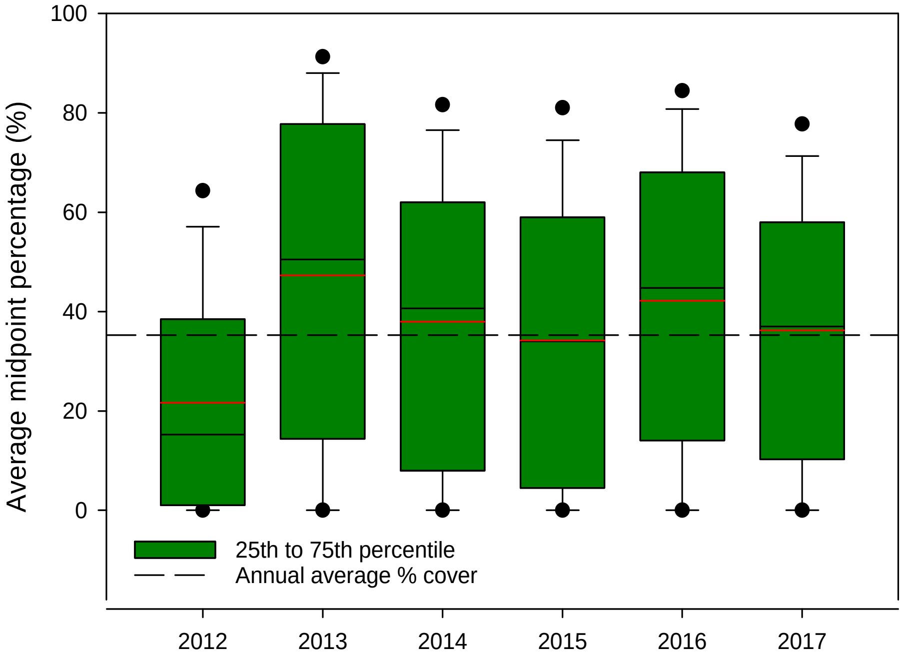

Figure 10 *Boxplots of seagrass cover (pooled for all sites) across the 6 years of observations (mean of mid-points in each year shown in red line, median as black line).*

4 Seagrass key pressures – variability across validation years

Three key pressures are described as increasing the vulnerability of the seagrass population in the Swan-Canning estuary: *light-stress, sediment-stress* and *eutrophication*. While these are environmental pressures that can be linked to climate conditions, there are also clear links with both immediate and historical anthropogenic activities. Other non-anthropogenic pressures; for example, salinity and temperature, also influence seagrass condition, however little can be done to manage non-anthropogenic pressures and so these are not the focus of the current work.

There may be some disconnect between the pressure the seagrass is exposed to and its response – due to either a time lag or the seagrass only responding once a particular threshold is reached. The indicators proposed to describe these key pressures are listed in Table 3 and are considered functional indicators since they aim to link environmental stress and biotic response to allow targeted management actions.

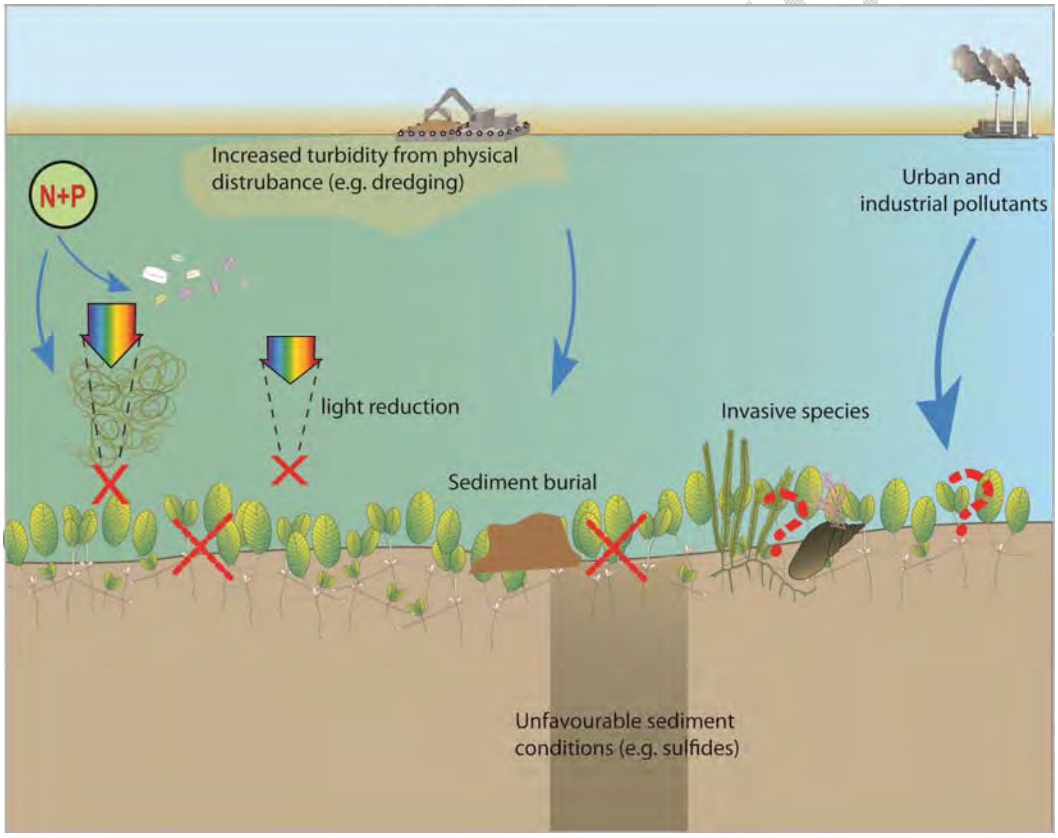

Figure 11 *Key anthropogenic pressures thought to influence seagrass performance in the Swan-Canning.*

4.1 Key pressures metrics – variation by year

Some of the key pressures may have substantial inter-annual variation. An overview of the relative differences between study years for the underlying metrics that were measured is shown in Table 9.

Using 2013-14 as an example to explore the relative differences in these key pressure metrics, we see that macroalgae cover was very high in 2013-14 and while δ^{13}C measures suggested light conditions were good for this year. Considering environmental variability between years explored in Table 4, these findings make sense as 2013-14 had the most hours of solar exposure across the summer season (~2 hours more than 2015-16). Additionally, spring rainfall was greatest in 2013-14 – likely delivering a pulse of nutrients to fuel macroaglal growth in the November-December period.

Comparing δ^{13}C measured in leaf tissues to solar exposure in December to March of each year, results in a Pearson correlation of R=0.87, suggesting at the estuary-wide scale δ^{13}C in seagrass tissues provides a reasonable estimate of relative light climate.

Table 9 *Overview of relative differences between study years for metrics of key pressures. Dark green is considered a more favourable for seagrass, while dark orange indicates less favourable for seagrass.*

Key pressure metric	Potential influence on seagrass performance	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017
Macroalgal cover[a]	High quantities of macroalgae compete with seagrass for light and nutrients. Nov-Dec cover has also been correlated with low sexual reproductive effort of *Halophila ovalis* in the Swan-Canning	+4.5	n.d.	+20	-10.9	-15.5	+1.8
δ^{13}C in leaves[b]	Variability in δ^{13}C occurs both due to inputs of terrestrial carbon and variation in light regimes. If light is primary source of variation, then δ^{13}C become more positive with improved light conditions.	+1.17	n.d.	+2.17	-0.17	-3.01	-0.16[8]

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[8] Note – Sampling of seagrass for tissue analysis occurred on the 15th and 16th of February in 2017 so would only have been expected to assimilate part of the influence of the floodwaters.

Key pressure metric	Potential influence on seagrass performance	2011-2012	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017
Fsulfide in rhizome[c][9]	The fraction of sediment-derived sulfur measured in sediments is a useful indicator of sediment stress (higher values indicate additional stress)	-4.9	n.d.	-6.82	+3.33	+4.81	+3.58
C:N[d]	The atomic ratio of carbon to nitrogen within plant tissues may be an indicator of nutrient enrichment, where reduced ratios indicate eutrophication.	-0.62	n.d.	+0.11	+2.62	+0.65	-2.76
δ^{15}N[e]	The nitrogen isotope signal measured in seagrass leaves is likely to reflect the source of nitrogen. More positive δ^{15}N likely a result of eutrophication	+0.55	n.d.	-0.15	+0.1	+0.27	-0.77

[a] Values shown are the deviation from the overall mean macroalgal cover (29.9%) measured at all sites for November and December in the period 2011-2017 (excluding 2012-13).

[b] Values shown are the deviation from the overall mean δ^{13}C in leaves (-12.49 per mil) measured at all sites in February in the period 2011-2017 (excluding 2012-13).

[c] Values shown are the deviation from the overall mean Fsulfide in rhizomes (32.47) measured at all sites in January, February and March in the period 2011-2017 (excluding 2012-13). Note – Fsulfide was calculated using site-specific values of mean δ^{34}S in sulfate (overlying water) and sulfide (measured on the chromium reducible fraction from sediments) collected in March of each year.

[d] Values shown are the deviation from the overall mean of the atomic C:N ratio in leaves (19.25) measured at all sites in February in the period 2011-2017 (excluding 2012-13).

[e] Values shown are the deviation from the overall mean δ^{15}N in leaves (7.6 per mil) measured at all sites in February in the period 2011-2017 (excluding 2012-13).

4.2 Key pressures metrics – variation by site

Some of the key pressures may differ more substantially between sites than between years, and consequently an overview of the key pressure metrics by site is provided in Table **10.**

Using LUB as an example for interpreting these relative differences between sites in the underlying metric used to calculate the indicators of key pressures. Macroalgae cover was worst at LUB, and LUB also had values of C:N and δ^{15}N which suggested eutrophication (relative to the other sites), however δ^{13}C was more positive at this site than any other – likely due to it being the shallowest site monitored Table **10 Table 10** Overview of relative

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[9] The exposure of plant tissues to sediment sulfides can be inferred from sulfur isotope data, measured in each plant part and expressed as Fsulfide as per Frederiksen, MS, Holmer, M, Borum, J & Kennedy, H 2006, 'Temporal and spatial variation of sulfide invasion in eelgrass (*Zostera marina*) as reflected by its sulfur isotope composition', *Limnology and Oceanography,* vol. 51**,** pp. 2308-2318.

differences between sites for key pressures metrics. Dark blue is considered more favourable for seagrass while dark purple indicates less favourable for seagrass.

Key pressure metric[a]	**Potential influence on seagrass performance**	**RCK**	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
Macroalgal cover[a]	High quantities of macroalgae compete with seagrass for light and nutrients. Nov-Dec cover has also been correlated with low sexual reproductive effort of *Halophila ovalis* in the Swan-Canning	-17.8	-18.9	+24.5	+14.4	+15.9	-18.3
$\delta^{13}C$	Variability in $\delta^{13}C$ occurs both due to inputs of terrestrial carbon and variation in light regimes. If light is primary source of variation, then $\delta^{13}C$ become more positive with improved light conditions.	+0.13	-0.87	+1.81	+0.82	-0.65	-1.24
Fsulfide in rhizome	The fraction of sediment-derived sulfur measured in sediments is a useful indicator of sediment stress (higher values indicate additional stress)	+4.63	+1.56	-3.08	-0.28	+10.27	-13.10
C:N	The atomic ratio of carbon to nitrogen within plant tissues may be an indicator of nutrient enrichment, where reduced ratios indicate eutrophication.	+1.13	+1.80	-3.82	+0.2	+2.1	-1.4
$\delta^{15}N$	The nitrogen isotope signal measured in seagrass leaves is likely to reflect the source of nitrogen. More positive $\delta^{15}N$ likely a result of eutrophication	+0.04	-0.44	+1.43	-0.51	-0.16	-0.38

[s] Values shown are the deviation from the overall mean values for each metric – as described in the footnotes of Table 9.

Comparing $\delta^{13}C$ measured in leaf tissues and the site light conditions measured by in-situ loggers. Pearson correlations were strongest for the total light seagrass were exposed (daily moles m^{-2}) in the previous month i.e. January – understandable since the tissue sampled in February analysed for $\delta^{13}C$, most likely was up to a month old.

- R=0.71 ($p<0.0001$) for $\delta^{13}C$ versus daily moles m^{-2} measured in January
- R=0.52 ($p<0.001$) for $\delta^{13}C$ versus daily moles m^{-2} measured in January, February and March.
- R=0.59 ($p<0.0001$) for $\delta^{13}C$ versus hours above saturating irradiance in January
- R=0.45 ($p<0.0001$) for $\delta^{13}C$ versus hours above compensating irradiance.

A moderate correlation (R=0.48, $p<0.0001$) was observed for the deviation of $\delta^{13}C$ at each site from its long-term value (as used in the indicator described in Table 3) when compared to the total daily moles received in January.

4.3 Macroalgae observations

Macroalgae are a natural part of the estuary. However proliferation, particularly of nuisance green algae (such as *Chaetomorpha* or *Cladophora*), may directly compete with seagrasses - blocking light reaching the seagrass (and utilizing available nutrients). Spring rainfall delivers a nutrient pulse to the estuary from catchment runoff, just as temperatures and light increase. This can result in proliferation of macroalgae and subsequently reproductive effort of *Halophila ovalis* reduces (Figure 12).

Macroalgae presence has been observed in two ways across the validation period:

- monthly between November and March with quadrat observations (10) at each site, matched to monthly seagrass cover (Figure A 22, Figure A 23 and Figure A 24)
- during the transect survey of seagrass cover at the meadow-scale during late summer (expected peak biomass of seagrass) from 2014-17 (Figure A 19, Figure A 20 and Figure A 21).

The focus of these observations has been to identify the dominant species present and estimate the relative density (as percent cover). There was no attempt to identify all species present, or measure biomass of the macroalgae.

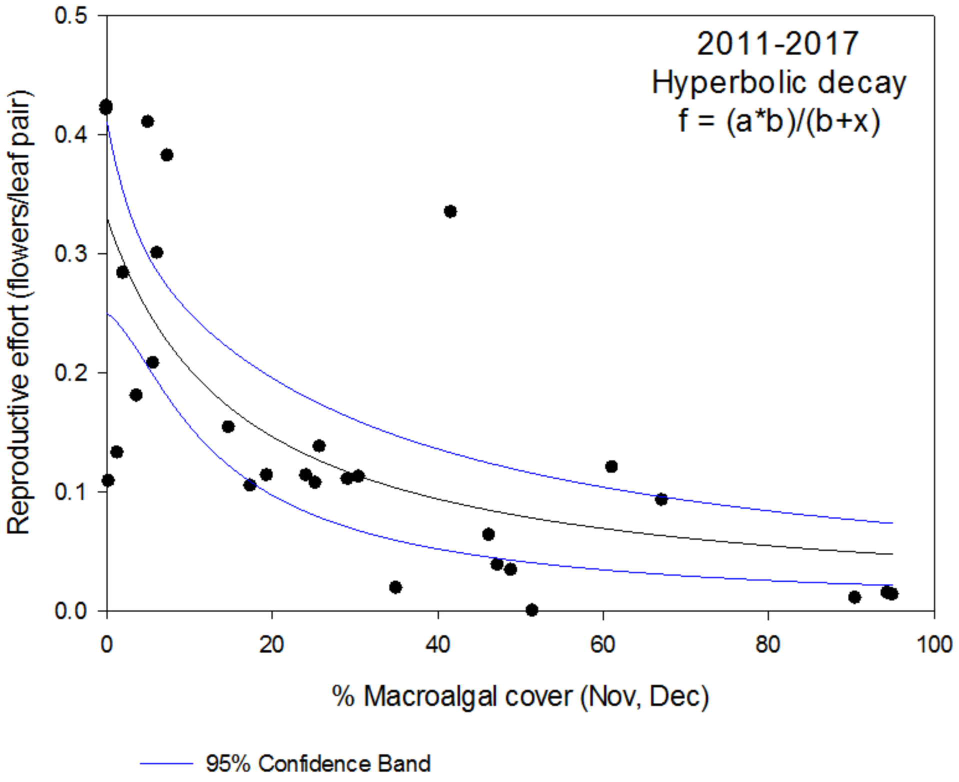

Figure 12 *Relationship between reproductive effort of Halophila ovalis and macroalgae present in November and December*

Chaetomorpha linum was the dominant macroalgae observed, however at some sites *Gracilaria* spp. and *Cladophora* spp. were abundant. Less abundant, but commonly observed were *Cystoseria* spp., *Colpomenia* spp. and *Laurencia* spp. Diversity of macroalgae appears to increase towards the marine end of the estuary (for example, 9 types of macroalgae were identified at RCK in 2017, while only three were present at CAN in the same year). This pattern in macroalgal diversity was also observed by Astill and Lavery (2004).

For RCK and DLK, macroalgal cover was higher closer to shore (Figure A 19), while LUB[10], PPT and HTH macroalgal cover was more consistent across the depth transect (Figure A 20 and Figure A 21). Macroalgal cover increased dramatically at CAN in 2017 (seen in both Figure A 21 and Figure A 24). The highest macroalgal cover across the full sampling period (November to March) was generally at sites LUB and PPT in most years (Figure A 23)

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[10] Note LUB transects do not actually encompass the area where plant-scale measurements are taken (an idiosyncrasy introduced in the first year of sampling)

4.4 Chemical and isotopic analysis of seagrass tissue

Some of the indicators described in Table 3 rely on the chemical and/or isotopic analysis of seagrass tissue. Seagrass leaves were analysed for % C, N, P and δ^{13}C, δ^{15}N in February of each year. Additionally seagrass leaves, rhizomes and roots were analysed for %S and δ^{34}S in January, February and March of each year. Plots of these data can be found in Appendix C.

Carbon concentration within the leaf tissues were fairly stable between sites and across years (ranging from 27.4 to 33.3%). Nitrogen and phosphorus concentrations were more variable, particularly between sites ranging from 1.35 to 3.05% for nitrogen and 0.22 to 0.49% for phosphorus. There appears to be an increasing trend over time in %P within the leaf tissues of *Halophila ovalis* with the highest percentage occurring in 2017 (Figure A 25). Of the atomic ratios shown in Figure A 26, site differences seemed to dominate for N:P ratios, while a decreasing trend for C:P ratio appeared evident with time across the validation period.

For sulfur within seagrass tissues, generally %S was higher in leaf tissues than roots or rhizomes, although occasionally spikes in sulfur were observed in roots (Figure A 27 and Figure A 28). When these spikes co-occurred with lower δ^{34}S in tissues (Figure A 29 and Figure A 30), it is strongly indicative of sulfide intrusion from sediments.

The typical patterns for sulfur isotope dynamics were generally observed where leaves have the most positive δ^{34}S values (reflecting the positive δ^{34}S of sulfate in seawater), roots the most negative (reflecting negative δ^{34}S of sediment sulfides), with the δ^{34}S signature of rhizomes intermediate (Figure A 29 and Figure A 30).

A notable change occurred for sulfur dynamics within seagrass tissues at RCK in 2017. In January, leaves, roots and rhizomes were all positive and relatively low %S (suggesting that sediment-stress of sulfide intrusion into the plant was not occurring to any significant degree), however by March the roots and rhizomes both had negative δ^{34}S and concentrations of sulfur in the roots had more than quadrupled (~2x higher in leaves and rhizomes). This data is best interpreted as increased sulfide intrusion (and ‘sediment-stress’) related to the poor light climate related to the unusual summer flood, and similar trends in the data can also be seen at the other sites excluding CAN (although the change is most dramatic at RCK).

For δ^{13}C there was significant variability both between sites and years of study, and the most negative δ^{13}C were observed in 2016. Values of δ^{15}N showed far more site-fidelity, and were relatively consistent between years. Site LUB had the most positive δ^{15}N in most years, with a similar site pattern observed until the final year of sampling in 2017.

5 Proposed indicator scores (site x year)

5.1 Seagrass performance –scored as per Table 2

Seagrass presence (P/N° observation ratio)						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	0.75	0.73	0.75	0.65	0.69	0.14
2012-13	0.89	0.83	0.94	0.80	0.80	0.22
2013-14	0.93	0.70	0.85	0.67	0.73	0.28
2014-15	0.86	0.69	0.78	0.62	0.81	0.22
2015-16	0.89	0.74	0.85	0.65	0.83	0.41
2016-17	0.93	0.60	0.84	0.60	0.90	0.45

Seagrass %cover						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	26.5	27.1	28.2	37.5	23.8	1.4
2012-13	38.8	41.2	68.4	55.2	64.8	9.2
2013-14	57.0	36.7	48.0	39.1	37.0	13.6
2014-15	44.2	35.3	30.1	43.3	47.9	3.2
2015-16	56.7	44.3	45.1	30.3	56.4	21.8
2016-17	58.8	27.7	33.3	30.5	54.1	14.9

Seagrass productivity						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-2012	3.3	3.4	3.3	4.3	4.2	3.5
2012-2013						
2013-2014	3.6	3.6	4.1	6.7	3.8	4.3
2014-2015	3.7	3.4	3.4	3.8	2.9	3.1
2015-2016	3.9	2.3	2.6	3.7	2.9	3.2
2016-2017	2.8	2.3	1.6	1.7	1.4	1.8

Seagrass reproduction (annual flowers | fruits m^{-2})						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-2012	400 | 184	342 | 101	394 | 143	176 | 9	31 | 9	893 | 60
2012-2013						
2013-2014	542 | 18	452 | 350	48 | 0	290 | 92	166 | 0	642 | 5
2014-2015	1378 | 1335	1703 | 37	35 | 0	915 | 241	722 | 1768	843 | 111
2015-2016	1392 | 1257	1951 | 83	0 | 0	266 | 60	432 | 1566	1133 | 276
2016-2017	1099 | 560	811 | 135	144 | 0	44 |53	362 | 655	45 | 13

5.2 Key pressures – scored as per Table 3

Macroalgal cover (%)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	30.5	14.8	24.1	46.3	90.5	0.0
2012-2013						
2013-2014	18.8	29.1	94.4	61.1	95.0	1.3
2014-2015	5.6	5.0	35.0	41.6	25.8	0.0
2015-2016	2.0	0.0	51.5	25.3	0.3	7.4
2016-2017	3.6	6.1	67.1	47.3	17.4	48.9

δ^{13}C in leaves

	RCK	DLK	LUB	PPT	HTH	CAN
Site-specific value	-12.36	-13.36	-10.68	-11.67	-13.14	-13.73
2011-2012	1.74	1.85	1.35	1.19	1.40	-0.50
2012-2013						
2013-2014	1.96	1.10	2.97	2.60	3.23	1.18
2014-2015	-0.30	-0.12	0.19	-0.69	-0.51	0.39
2015-2016	-3.54	-2.76	-3.59	-2.62	-3.58	-1.98
2016-2017	0.14	-0.07	-0.93	-0.48	-0.54	0.91

Fsulfide in rhizome

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	37.04	26.98	27.08	27.65	29.85	16.83
2012-2013						
2013-2014	28.73	27.55	32.27	17.18	27.78	20.41
2014-2015	38.10	39.60	28.55	34.59	56.01	17.97
2015-2016	40.87	48.35	25.71	37.01	50.91	20.89
2016-2017	40.79	27.70	33.35	44.53	49.16	20.81

C:N atomic ratio in leaves

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	19.9	18.9	16.9	18.3	17.9	19.9
2012-2013						
2013-2014	21.4	22.4	15.1	18.0	19.8	19.5
2014-2015	22.3	24.8	16.5	25.8	25.5	16.1
2015-2016	19.3	21.0	15.8	20.8	24.6	17.9
2016-2017	19.0	18.2	12.7	14.3	18.9	15.8

δ^{15}N in leaves

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	8.10	7.20	10.40	8.32	7.16	7.68
2012-2013						
2013-2014	7.38	6.74	9.44	6.30	6.85	7.84
2014-2015	7.29	6.97	9.63	7.24	7.58	7.48
2015-2016	7.35	7.33	9.10	7.21	8.26	7.94
2016-2017	8.07	7.47	6.59	6.40	7.33	5.13

6 Validation of metrics and indicators

We have chosen to evaluate our choice of metrics and indicators of seagrass performance and key pressures by three criteria:

1. Is there evidence of the metric or indicator providing ecologically relevant information?
2. Are the classification categories used for the indicator reasonable and informative? Are the cut-offs adequately placed to allow for change in grade between good years and bad years?
3. Are there interactions or considerations that need to be considered for interpretation or application of the indicator?

In some cases, evidence will be taken from year to year comparisons, while for others evidence between sites or at an individual site may be used. This section will discuss each proposed indicator in turn.

6.1 Seagrass presence

Ecologically relevance: Seagrass presence indicator generally suggested that conditions were good or fair at all sites at all times for all except CAN, where seagrass was usually quite patchy. This suggests a stability in response of the seagrass to the environmental conditions at the meadow-scale. Given that the meadows monitored are rather shallow, it is not too surprising that this measure is relatively stable across time.

Classification categories: The classification categories are chosen to be easy to explain, and directly associated with the prevalence of seagrass habitat where denser is better. This choice however does mean that meadows near the upstream extent (e.g. CAN) are penalised when they are unlikely to ever have meadows as dense as further downstream. Other programs have used deviation from historic baseline for individual sites to score their seagrass condition – however this means that scores are not directly comparable between sites. For communication simplicity, we recommend keeping the current ecologically relevant categories.

Interactions and/or considerations: Our current methodology typically can pick up changes in meadow presence of ~5 per cent (see Appendix E). These measures can be used to show trajectory of change, even if the indicator itself is not changing.

Significant retraction at the deeper-edge of seagrasses within the estuary could be occurring and not be demonstrated by this indicator metric, due to site choice. This measure could be supplemented by sampling across the deeper edge, and or whole of estuary monitoring on a regular basis.

We also trialled scoring seagrass presence data from 6 transects rather than 10 transects per site, and believe there is sufficient agreement between these comparisons to recommend reducing the effort at each site (Table A 13).

6.2 Seagrass cover

Ecologically relevance: The seagrass cover indicator is more sensitive than seagrass presence indicator to environmental conditions. Seagrass cover was fair to good at most sites in most years, however percent cover at CAN, although it increased in most years was still low.

Classification categories: The proposed classification categories (Table 2) appear slightly too harsh when considering the requirement for the full site (across the depth profile) to meet 50% cover to be scored as having good cover. We recommend adjusting these categories to be <5% poor, 5-20% low, 20-40% fair and >40% good. The adjusted scores are for all sites and years are shown in Table A 12.

Interactions and/or considerations: Our current methodology typically can pick up changes in meadow cover of ~5 per cent (see Appendix E). These measures can be used to show trajectory of change, even if the indicator itself is not changing.

The depth profile of the site is likely to influence this score. It is much harder for a site like DLK (which reached 4-5 m depth) to score good cover across the whole site, than a more consistently shallow site.

We also trialled scoring seagrass cover data from 6 transects rather than 10 transects per site, and believe there is sufficient agreement between these comparisons to recommend reducing the effort at each site (Table A 13).

6.3 Seagrass productivity

Ecologically relevance: Seagrass productivity is a direct measure of growth of seagrass – directly relatable to the seagrasses ability to vegetatively expand and recover from disturbances.

Classification categories: Overall the categorization cut-offs seem ok, although it is possible that the growth signal is being dampened by averaging over the three months of measurement. This results in only rarely good condition being recorded.

Interactions and/or considerations: We trialled scoring seagrass productivity from individual months and also January and February together to assess if effort could be reduced for this indicator (Table A 17). From this exercise, we can see that following the flood event in 2016-17, the cut-off categories suggest poor or low growth at all sites in March, as we would expect of the indicator. Our recommendation is to reduce the effort of measuring seagrass productivity to only January and February (and not in March) as much of the same information is captured, results in a similar pattern of scoring, although slightly more generous with good being recorded a few more times.

6.4 Seagrass reproduction

Ecologically relevant: Colonising seagrasses have little ability to resist disturbances, rather relying on recovery mechanisms – which may be asexual or sexual. The establishment and persistence of a seedbank is considered important for ongoing survival of *Halophila ovalis* in the estuary. While research is underway to understand the links with seedbanks and viability of the seed stores within it (see Appendix A) – a detailed understanding of how much flowering and fruiting is needed each year to maintain viable seedbanks for recovery is not yet known.

Classification categories: Sexual reproduction of *Halophila ovalis* at the majority of Swan-Canning sites is prolific relative to marine observations. It makes sense then that most sites are scored as good. Classification categories were chosen to such that evidence of successful fruiting was given a fair condition, while prolific flowering and fruiting given good condition.

Interactions and/or considerations: *Halophila ovalis* is dioecious, meaning that there are separate male and female plants. This measure should not overly discriminate if sampling only in a male patch (as will never have fruits presence). This is why both flowers and fruit are contributing to the indicator.

We explored whether the sampling effort could be reduced from four months of core collection and laboratory analysis. Originally the flowers were scored on data from December to March, and the fruit for data from January to March. Satisfactorily similar scores were obtained for using flowering and fruiting from January to March (Table A 15), but could not be reduced further to either one or two months due to the variability between years of the peak in reproduction (Table A 16). From year to year, fruit and flower peaks occurred in different months (see Figure A 14 and Figure A 15) which means that sampling in January to March is necessary to capture the reproductive success of *Halophila ovalis.*

6.5 Macroalgal cover

Macroalgal cover measured in late-spring (November and December) was proposed an indicator of light stress for seagrass, based on the an initial relationship observed between macroalgal cover at this time and the seagrass sexual reproduction as well as the observation that dense quantities of macroalgae would limit light reaching the benthos.

Ecologically relevance: There is a clear ecological relationship between late-spring macroalgae and *Halophila ovalis* sexual reproduction (Figure 12), where greater macroalgae results in less reproduction. Spring macroalgae was most abundant in 2013-14 (Table 9) when higher than average spring rainfall (likely delivering catchment-derived nutrients to the estuary), warm waters and higher than average solar exposure (Table 4) created conditions that favoured macroalgae. Conversely, spring macroalgae was lowest in 2014-15 and 2015-16, when spring rainfall was relatively low.

Classification categories: Category cut-offs were chosen based on evaluation of Figure 12, and their performance over multiple years appear to be meaningful considering the relationship between the annual summed score and spring rainfall (Figure A 1).

Interactions and/or considerations: Consideration of this indicator as a 'light-stress' indicator may be not be the best way to interpret this measure. The pressure is catchment-influenced eutrophication, related to rainfall and the effect is on seagrass reproduction. We recommend keeping this indicator but reporting it separately to both 'light-stress' and 'eutrophication-stress'.

6.6 Stable carbon isotope ratio

The carbon stable isotope ratio within seagrass tissues was assumed to be influenced by both the source of carbon available to seagrass and light availability (as discussed in detail within Kilminster and Forbes (2014)). For the δ^{13}C indicator to inform only on light-stress, we aimed to account for the differences in source of carbon by examining the deviation of the δ^{13}C in leaves from a site-average value. Our first attempt at this used data from 2011-12 and 2012-13, however this resulted in 'poor' condition unrealistically too often (Table A 18). The plausibility of the indicator was improved by using the long-term site averages as shown in section 5.2.

Ecologically relevance: The data collected supports a strong relationship between light climate the seagrasses are exposed to and the value of δ^{13}C measured in the leaves, as at the estuary-wide scale, a strong correlation was observed for solar exposure from December to March each year and the average annual δ^{13}C in seagrass leaves.

At the site-level, the correlations between absolute δ^{13}C values and the benthic light environment measured in the previous month were stronger than the correlations between the deviation from the long-term average δ^{13}C values and the benthic light environment (as proposed for the indicator).

These analyses suggest that the absolute δ^{13}C value is more ecologically meaningful to use as an indicator. Therefore, despite the natural estuarine-catchment gradients which may affect the δ^{13}C value of the source carbon, light availability is the dominant factor that influences δ^{13}C measured in seagrass tissues within the Swan-Canning estuary.

Classification categories: New classification values are proposed based on the absolute δ^{13}C value where > - 10 per mil = low stress, -10 to -12 per mil = moderately-low stress, -12 to -15 per mil = moderately-high stress and < -15 per mil = high stress. Site and year classifications according to this new indicator using absolute δ^{13}C value is shown in Table A 19. These classification cut-offs appear to score the system appropriately when you consider the annual condition as shown in Figure A 42.

Interactions and/or considerations: δ^{13}C values in leaves appear to inform well on total light conditions in the previous month. Currently this is sampled only once across the season, but in terms of effort versus cost it seems significantly better value than in-situ light loggers which have a significant data-processing requirement, and data collection issues (e.g. sensor disturbance and/or bio-fouling).

While differences in source of carbon (e.g. terrestrial inputs along the estuarine-catchment gradient) did not appear to significantly influence the observed $\delta^{13}C$ values, it is possible that under some conditions this could still be a significant effect. Seagrass was not analysed for $\delta^{13}C$ in March 2017, when catchment-derived carbon may have been a significant influence following the unusual summer flood. This indicator should be interpreted with caution following significant rainfall.

6.7 Fsulfide in rhizomes

The concept of a sediment-stress indicator was first explored in Kilminster and Forbes (2014) and further developed in Kilminster et al. (2014). The idea is that seagrass growth and survival may be constrained by sediment conditions, such as anoxic processes that produce sulfide (which is toxic to plants). Examining the seagrass tissues themselves (using sulfur isotopes) provides an indication of sulfide intrusion into the plant from sediment, integrated of the plant matter's lifespan.

Ecologically relevance: Seagrass growth was found to be negatively correlated with a higher degree of sulfide intrusion (Fsulfide) in seagrass rhizomes for data from 2011-12 and 2013-14 (Kilminster et al. 2014). Considering 5 years of data, the relationship appears at first to break down. It is likely that the trade-off between sexual reproduction and vegetative growth is driving this outcome, as sexual reproduction was relatively low in the first two years. However if we excluding January (the month with highest reproductive effort) from the analysis, the negative relationship of Fsulfide and growth is observable.

Additionally as previously discussed, following the unusual summer flood in 2017, increased sulfide intrusion (and 'sediment-stress') related to the poor light climate was observable in the sulfur stable isotope data, most notably at RCK, but to a lesser degree at other sites (except CAN – both low in sediment organic matter but also least influenced by the floodwaters).

Classification categories: The pattern of scoring makes intuitive sense. We would expect good light conditions to mean that Fsulfide is reduced, and sites with higher organic matter (e.g. RCK compared to CAN) should be more susceptible to sulfide intrusion.

Interactions and/or considerations: Higher temperatures may accelerate microbial rates, although as this is likely to affect both sulfate reduction and sulfide oxidation, the direction of effect is not clear.

We explored whether the sampling effort and analytical cost could be reduced by examining the effect of scoring Fsulfide if we only measured it in February only (as per the other chemical measures), or in January and February. The January and Feburary combination produced the most satisfactory concordance with the Fsulfide categorization for three months of data. Therefore we recommend Fsulfide to be measured for January and February rather than the three months of January, February and March.

6.8 C:N ratio in leaves

Lower C:N atomic ratios indicate relatively more nitrogen within seagrass tissues and have been proposed as a possible indicator of nutrient availability (Fourqurean et al. 1997), although other authors suggest this ratio may also inform on light availability (McMahon et al. 2013) .

Ecologically relevance: Our data shows a strong correlation between %N and C:N (R=0.91). The cut-off values were previously chosen informed by quartiles and means for data in the Swan-Canning for 2011-12 and 2013-14. This means interpretation of this indicator should be as relative differences in nutrient availability between sites within this system, rather than as categories that necessarily indicate ecological effect on seagrass condition.

Classification categories: The proposed classification values appear to provide a good range of classifications, and site LUB (which we believe is influenced by nutrient enrichment) had poor performance each year. Site CAN also showed poor performance in the most recent years, which is also consistent with the elevated nutrient concentrations observed in the routine water quality sampling.

Interactions and/or considerations: This indicator appears to be performing well, our recommendation is not to alter it.

6.9 Stable nitrogen isotope ratio in leaves

Anthropogenic influences (e.g. wastewater, sewage and/or fertiliser) usually result in an enrichment of the δ^{15}N values within macrophytes. We expect that more positive δ^{15}N is related to localised eutrophication.

Ecologically relevance: Within our data there appears to be evidence of negative ecological outcomes with more positive δ^{15}N. Site LUB has displayed high δ^{15}N (ranked poor) for years 2011-12 to 20115-16, and during this time, LUB showed signs of poor performance, such as low to no reproduction, reduced leaf mass and biomass, and generally elevated amounts of macroalgae compared to other sites.

Classification categories: Similar patterns are observed between C:N and δ^{15}N, although no good performance is shown for this indicator. Compared to the much lower δ^{15}N in leaves of seagrass from other systems this is probably fair. For example, across all years, the Swan-Canning averages δ^{15}N = 7.6, whereas the mean for the Leschenault δ^{15}N = 1.2.

Interactions and/or considerations: This indicator appears to be performing well, we recommend to not alter it.

7 Annual seagrass condition

7.1 Seagrass performance

Each of the indicators for seagrass performance (with the revised cut-offs or sampling periods as recommended in the previous section) was given a score of 1 to 4, where an indicator that scored most poorly (i.e. red) was given a low score of 1, and good (i.e. green) was given a score of 4. These scores were averaged across all indicators and then the sites were classified for seagrass performance as follows:

- poor (red) where average score is <2
- low (orange) where average score is 2 to 2.5
- fair (yellow) where average score is >2.5 to 3
- good (green) where average score is >3.

ANNUAL SEAGRASS PERFORMANCE

	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	3.5	3.25	3.25	3.5	3.25	2.25
2012-13[11]	4	4	4	4	4	2
2013-14	3.5	3.5	3.25	3.75	2.75	2.75
2014-15	3.75	3.5	2.75	3.75	3.75	2.5
2015-16	3.75	3.5	3	3.25	3.75	3
2016-17	3.5	3	2.5	3	3.5	2.25

Generally seagrass performance is good across the Swan-Canning sites. Condition at site CAN (at the upper extent of the distribution), shows poorer performance than other sites and site LUB has shown significant evidence of stress and reduced performance over time (likely related to eutrophication pressure). 2016-17 had lower performance generally than all other sites and if the flood had occurred a few weeks earlier (e.g. before sites were surveyed), the effect on the overall performance would have been more notable.

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[11] Scored for seagrass presence and cover only

7.2 Overall seagrass key pressures

Summary statistics for the key pressures were obtained by giving each of the indicators a score of 1 to 4 and treating each of the five functional-level indicators independently, where an indicator that scored most poorly (i.e. red) was given the low score, and good (i.e. green) was given a high score. These scores were averaged across all indicators and then the sites were classified as follows:

- high stress where average score was <2
- moderate stress where average score was between 2 and 3
- minimal stress where average score was > 3.

	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	2.6	2.8	2.4	2.6	2	3
2012-13						
2013-14	3.2	3	1.8	3	2.8	2.8
2014-15	2.8	3.25	2.2	2.8	2.5	2.6
2015-16	2.2	2.75	1.8	2.6	2.4	3
2016-17	2.6	2.75	2.2	2	2.2	2.75

Combining these key pressures indicates overall seagrasses in the Swan-Canning are under moderate stress. Site LUB was generally the most stressed, followed by PPT and HTH, then CAN, while RCK and DLK were the least stressed. This seems consistent with our observations and intuitive understanding of the system across the five years of study.

8 Recommendations for ongoing seagrass monitoring

8.1 Management objectives

Under DBCA's River Protection Strategy, Objective 4 highlights the desire to protect, manage and enhance biodiversity, while Objective 3 seeks to ensure management decisions are based on appropriate knowledge. Information on seagrass is required annually for reporting. Ensuring viable and resilient seagrass communities is an important component of effective estuary management.

From these statements, it is not clear what the degree of acceptable change might be, whether this is a change in absolute area or density, or if the intent is more about maintaining a resilient seagrass community into the future (accepting that there will be significant changes following disturbances). If seagrass is lost from a broadscale disturbance, how quickly does it need to be able to recover to fulfil the requirement of providing a viable habitat?

It is clear, however, that annual information on seagrass condition is required for reporting purposes, so that is the main criteria that our following recommendations are based around, as well as seeking to provide the best value-for-effort from the program.

8.2 Hierarchical monitoring

We recommend that the principles of hierarchical monitoring are maintained within the program. Specifically, this means including the broad-scale mapping of the whole estuary at 3-5 year intervals and measuring seagrass performance at multiple scales.

The long-term tide plot (Figure 4) shows that the estuary-wide map last produced by DWER in 2010 was carried out at close to the highest tide for the last ~25 years. Understandably, a reduction in area of seagrass was noted compared to previous maps. It is recommended that estuary-wide mapping of seagrass is carried out again (preferably during a year when tide heights are more average).

8.3 Performance monitoring

We recommend that performance monitoring is carried out annually at the six sites previously monitored. Following the recommendations of section 6, this would include:

- Seagrass surveys using six transects rather than 10 to determine seagrass presence and cover – undertaken in February
- Tagging of seagrass for growth in January and February
- Collection and processing of cores in January, February and March for reproduction assessment

The suite of indicators seems to work well and covers aspects of resistance and recovery (important for resilience). Additional meadow characteristics will be collected with analysis of the cores which provide supporting evidence.

This recommendation is a reduction of sampling effort from what was previously undertaken as described below and we expect it will still be possible to adequately score seagrass performance.

What was used for the 5 years of sampling		
Task	**Days**	**Number of people**
P/N and % cover (mapping x 10 transects for 6 sites	2 weeks	6
Production (tagging)	9 days (1 day to tag 1, day to retrieve, 1 day to process x 3 months)	2
Reproduction (cores)	21 days (2 days per month in field 3 days per month in lab for 4 months)	3-4

With changes- in good conditions		
Task	**Days**	**Number of people**
P/N and % cover (mapping x 6 transects for 6 sites	1 weeks	3
Production (tagging)	6 days (1 day to tag, 1 day to retrieve, 1 day to process x 2 months)	2
Reproduction (cores)	15 days (2 days per month in field 3 days per month in lab for 3 months)	3

Seagrass presence/absence and percent cover could be scored using 6 transects at each site rather than 10. This would be hugely time saving, as it could be achieved by three experienced staff in approximately one week, reducing the time and number of people by half. Productivity may be able to be scored by just sampling over two months in January and February. This reduced necessity to collect data in March which would reduce the number of working days by a third, while still adequately describing changes in seagrass growth. Adequate scores for reproduction could be obtained by collecting samples in January to March rather than December to March, reducing effort by approximately 30%.

8.4 Key stresses

As with seagrass performance metrics, we recommend an rationalised program for assessing key stresses in the future, based on the evaluation discussion of key stress indicators in section 6.

We recommend annual sampling for:

- Monthly assessment of macroalgal cover by 10 random quadrat observations per month from November to March, for all sites. These assessments are quick (~10 minutes per site) and have no cost beyond staff-time. The relationships between macroalgal density and seagrass reproduction suggest this is a useful indicator of pressure on an individual seagrass site.
- Sample seagrass leaf tissue in February for $\delta^{13}C$ as a surrogate for light measurements – 5 replicates per site. There are substantial time, effort and cost-savings related to not deploying light-loggers. Each logger costs approximately $900 (including wiper unit) and we deploy 2 loggers per site to compensate for public-disturbance and equipment failure. Even still, data for light climate has been very patchy across the years of study, and we have lost quite a few logger units. The staff-time required for calibration, deployment, retrieval, equipment maintenance and cleaning, data cleaning and analysis is in the order of a week per site. In contrast, analytical costs of $\delta^{13}C$ would be less than $200 per site.

We recommend less frequent than annual sampling (perhaps ~3 yearly):

- For indicators of eutrophication stress – e.g. C:N and $\delta^{15}N$, these measures vary more between sites than between years, so sampling frequency could be reduced. However, similar to $\delta^{13}C$, analytical costs are relatively cheap, and field and laboratory staff time not particularly onerous. Therefore, If budget allowed, these measures could be implemented annually, or expanded over a greater number of sites to identify potential hot-spots of eutrophication within the estuary.
- Measures of sediment-stress appear to give good site specific information and can assist in the interpretation of seagrass responses, particularly following disturbance events. These measures also vary related to annual light conditions and potentially reproduction effort. While some information will be lost by a reduced sampling effort, there is also little direct management actions that can be applied with knowing that the sediment is a stressor to the seagrass. Measuring this indicator less frequently is recommended, again perhaps investing in expanding assessment to other sites in the estuary not routinely monitored to assess their vulnerability to sediment stress.

Appendices

Appendix A — Collaborative projects

A number of informal collaborative student and ad-hoc research investigations have been supported though data provision, knowledge transfer and scientific discussions by the DWER seagrass project team since the inception of the project. These investigations and research projects have filled knowledge gaps and sought to value-add to the data collected routinely during the 5 year validation period. This section is not intended to be a comprehensive list of all associated projects, nor provide full details of the work and outcomes – rather highlight the existence of this additional information (which was generated during the same period of study) for the interested reader to follow up independently.

- PhD: Biodiversity, biosecurity and management of sessile invertebrate assemblages in Western Australia. – *University of Western Australia*

A part of **Tiffany Simpson's** PhD research investigated the colonial ascidian *Didemnum perlucidum* which was present in the Swan River estuary not only on artificial substrates but fouling seagrasses. The ascidian's presence had a measurable effect on seagrass biomass and interaction with *Batillaria australis*. Seasonal temperature changes appeared to control the patchy distribution and spread of the invasive ascidian.
Supervised by Thomas Wernberg, Justin McDonald and Dan Smale

- PhD: Investigation of microbial relationships in seagrass rhizospheres – *University of Western Australia*

Belinda Martin aims to develop 'microbial indicators' for seagrass health in the Swan-Canning and Leschenault estuaries. Her work sought to find correlations with seagrass health indicators (especially sulfide intrusion) and specific microbial taxa and/or change in community composition, then assess the use of these taxa as early 'indicators' for when seagrass are under stress. DWER data used is from 2016-2017.
Supervised by Gary Kendrick, Pauline Grierson, Deirdre Gleeson, Jeremy Bougoure, and Megan Ryan.

- Honours: Freshwater future: the influence of exposure to extreme summer rainfall events on the resistance and recovery patterns of an estuarine seagrass - *Edith Cowan University*

Chanelle Webster developed metrics to assess the severity of the February 2017 flood and response of *Halophila ovalis* in the Swan-Canning estuary. She used DWER data from six routine seagrass monitoring sites (and assisted with data collection) plus additional sites she monitored exclusively using consistent methods. The influence of meadow form (transitory and enduring meadows *sensu* Kilminster et al. (2015) was explored in relation to resistance and recovery an unusual summer flood event.
Supervised by Dr Kathryn McMahon.

- Honours: *Halophila ovalis* germination mediated by seasonal temperature gradients within the Swan-Canning estuary

Rob Sellers investigated the germination response of *H. ovalis* to simulated in situ temperatures (over 17 weeks) and sampled seedbanks within the estuary to validate germination observations. His study suggested role of temperature fluctuations in breaking dormancy and cueing germination, where spring/summer temperatures resulted in 16% germination compared to less than 1% in winter temperatures. Supervised by: *Gary Kendrick, Dr John Statton, David Merritt, and Kieryn Kilminster*

- Investigation by DWER regarding remote sensing of seagrass

Ben Marillier and **Vanessa Forbes** trialled a number of approaches for using remote sensing to inform on seagrass in the Swan-Canning could complement seagrass assessments at the meadow and plant scale. This study produced a successful classification of seagrass cover on the shallow sandy banks of the Swan Estuary using 2 m resolution WorldView2 satellite images collected in 2014 and 2016. A supervised classification procedure was adopted, supported by quadrat survey data collected at field sites throughout the estuary. Pre-processing steps including masking of deep water and water column correction helped to improve the accuracy of subsequent maximum likelihood classification. Key limitations associated with the techniques include, 1) water clarity limits the effective depth at which seagrass can be mapped (maximum water depth of ~1.5m) and accurate habitat classification depends on the water clarity on the time of image capture, 2) high resolution images are required to identify individual patches of seagrass and environmental conditions on the time of image capture significantly influence the usability of the image, 3) species classification is not possible, and 4) potential for pixels to be erroneously classified as seagrass where macroalgae dominate. Remote sensing is not a complete solution to producing an estuary-wide map, as it is unable to obtain information on the deeper seagrasses, however could form part of a hybrid solution to a whole-of-estuary survey.

- Investigation by DWER regarding sulfide concentrations within sediment

Kieryn Kilminster in collaboration with David Welsh and Will Bennett (Griffin University) and Marianne Holmer (Southern Denmark University), measured concentrations of sulfide using DGT (Diffusive gradient thin-films) in the sediment at seagrass sites during March. Results from this work showed that concentrations of sulfide were highest in the most productive seagrass meadows. Concentration of sulfide in the sediment was not a good predictor of the amount of sulfide intrusion in the seagrasses.

- Investigation by DWER and Kings Park regarding seed dormancy triggers

DWER seagrass team in collaboration with scientists at Kings Park – Dave Merritt and Kingsley Dixon, undertook preliminary investigations into the seedbank persistence (using a seed burial experiment at site CAN) and triggers for breaking dormancy and germination. From the seed burial experiment, most of the seeds appeared to disappear (germinate, rot or be consumed) within a year of burial, leading to the conclusion from this limited study that annual production of seeds at this location was important for continued seagrass habitat.

Appendix B — Additional detail about sampling

Table A 1 *Schematic showing schedule of seagrass sampling undertaken in each seagrass sampling period from 2011-12 to 2016-17.*

2011–2012	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)		🟩			🟩				🟩				🟩				🟩			
H. ovalis productivity (collection)			🟩			🟩				🟩				🟩				🟩		
Chaetomorpha survey			🟩			🟩				🟩				🟩				🟩		
Seagrass reproduction			🟩			🟩				🟩				🟩				🟩		
PAR surveys in-estuary																				
Nutrient tissue content (C,N,P) and δ^{15}N and δ^{13}C in leaves			🟩			🟩				🟩				🟩				🟩		
Sediment stress indicator			🟩			🟩				🟩				🟩				🟩		
Seagrass extent and distribution																		🟩	🟩	
2012–2013	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)																				
H. ovalis productivity (collection)																				
Chaetomorpha survey																				
Seagrass reproduction																				
PAR surveys in-estuary																				
Nutrient tissue content (C, N, P) and δ^{15}N and δ^{13}C in leaves																				
Sediment stress indicator																				
Seagrass extent and distribution														🟩	🟩			🟩		
2013–2014	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)										🟩				🟩				🟩		
H. ovalis productivity (collection)											🟩				🟩				🟩	
Chaetomorpha survey			🟩				🟩				🟩				🟩				🟩	
Seagrass reproduction							🟩				🟩				🟩				🟩	
PAR surveys in-estuary	🟩		🟩		🟩		🟩		🟩		🟩		🟩		🟩		🟩		🟩	
Nutrient tissue content (C, N, P) and δ^{15}N and δ^{13}C in leaves															🟩					
Sediment stress indicator											🟩				🟩				🟩	
Seagrass extent and distribution															🟩	🟩	🟩			

2014–2015	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)										🟩				🟩				🟩		
H. ovalis productivity (collection)											🟩				🟩				🟩	
Chaetomorpha survey		🟩					🟩				🟩				🟩				🟩	
Seagrass reproduction							🟩				🟩				🟩				🟩	
PAR surveys in-estuary				🟩		🟩			🟩		🟩		🟩		🟩		🟩		🟩	
Nutrient tissue content (C, N ,P) and δ^{15}N and δ^{13}C in leaves															🟩					
Sediment stress indicator											🟩				🟩				🟩	
Seagrass extent and distribution													🟩	🟩	*					

2015–2016	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)										🟩				🟩			🟩			
H. ovalis productivity (collection)											🟩				🟩				🟩	
Chaetomorpha survey				🟩			🟩				🟩				🟩				🟩	
Seagrass reproduction							🟩				🟩				🟩				🟩	
PAR surveys in-estuary		🟩		🟩		🟩		🟩	🟩		🟩		🟩		🟩		🟩		🟩	
Nutrient tissue content (C ,N, P) and δ^{15}N and δ^{13}C in leaves															🟩					
Sediment stress indicator											🟩				🟩				🟩	
Seagrass extent and distribution													🟩	🟩	🟩					

2016–2017	November				December				January				February				March			
week	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
H. ovalis productivity (tagging)										🟩				🟩				🟩		
H. ovalis productivity (collection)											🟩				🟩				🟩	
Chaetomorpha survey				🟩		🟩					🟩				🟩				🟩	
Seagrass reproduction						🟩					🟩				🟩				🟩	
PAR surveys in-estuary		🟩		🟩		🟩		🟩												
Nutrient tissue content (C, N, P) and δ^{15}N and δ^{13}C in leaves															🟩					
Sediment stress indicator											🟩				🟩				🟩	
Seagrass extent and distribution													🟩	🟩						

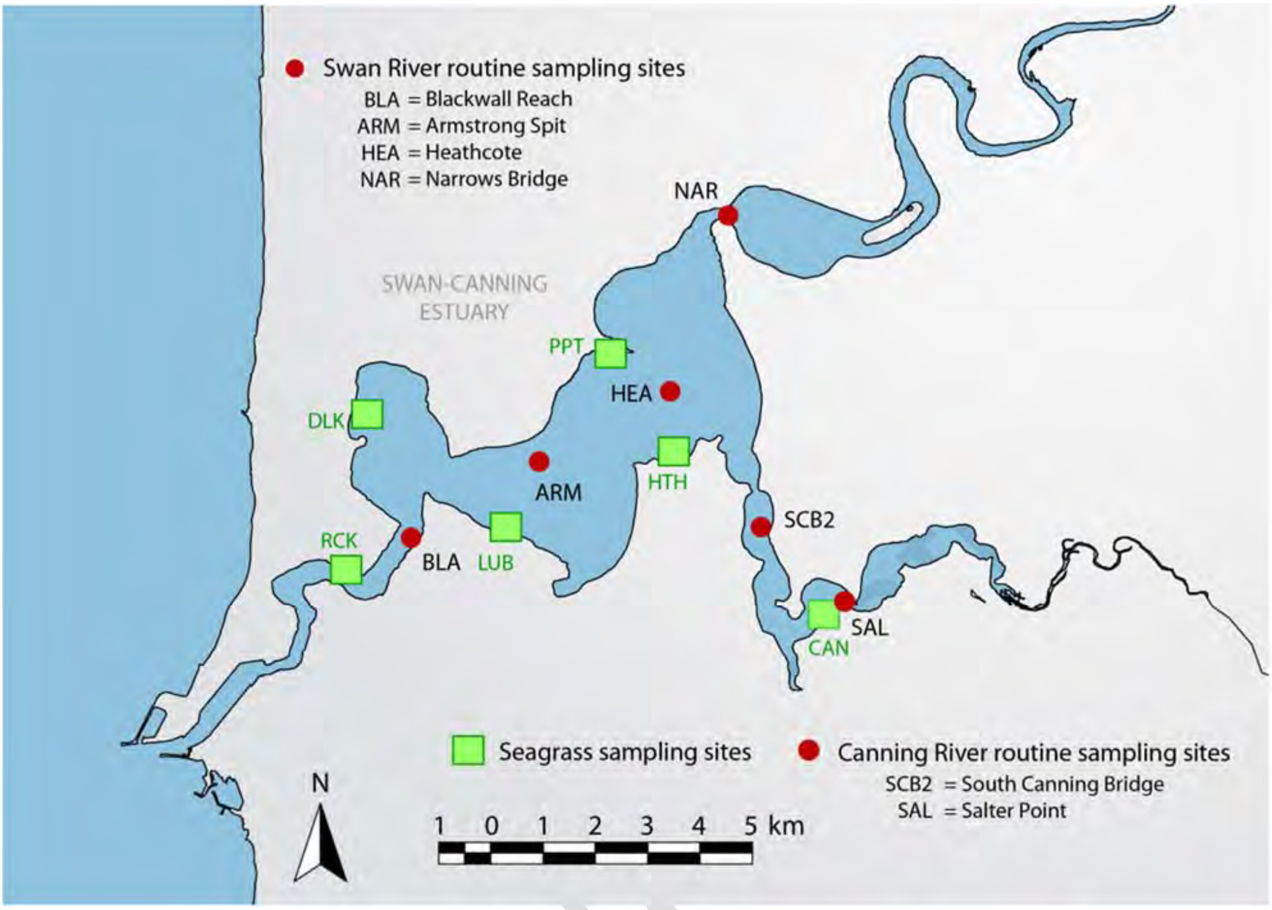


Figure A 1 *Locations of routine Swan and Canning water quality monitoring and seagrass sampling sites.*

Appendix C — Climatic conditions of note

Climatic events of note that occurred between 2011 and 2017, during the seagrass monitoring program

2011

- Wettest year on record, averaged across the whole state
- Most of the rainfall was received during the first four months of the year; due to a very active monsoon and one of the strongest La Niñas on record as well as exceptionally warm Indian Ocean temperatures
- Maximum and minimum temperatures were above the long term average

2012

- Temperature was well above average across the whole state, with the south-west of the state experiencing its 2nd warmest year in terms of average mean maximum temperatures
- The south-west of the state received below average rainfall

2013

- Was the warmest year on record, averaged across the whole state
- In terms of average mean minimum temperatures, it was the 2nd highest across the state
- High spring rainfall, it was the wettest September for 40years in Perth Metropolitan area

2014

- Another warm and dry year, in term of average mean maximum temperatures it was the fourth-highest in the Perth Metropolitan area and warmest year across the state and 9th highest in terms of average mean minimum temperature
- Long dry spell from late spring of 2013 through to early summer of 2014

2015

- Second warmest year on record for the whole state and equal warmest year on record in the Perth Metropolitan area
- Average mean maximum and minimum temperatures where record highest in the South-west land division
- Spring was the warmest on record for both the south-west and across the whole state (September 8th warmest of record, October warmest and November 4th warmest on record)
- Below average rainfall, particularly in the south-west

2016

- Coolest year in Perth in over a decade in terms of average mean maximum temperatures, and coolest years since 2010 in terms of average mean minimum temperature
- Highest rainfall for three years, yet still below long term average
- Prolonged heatwave across most of Australia from late February to mid-March, Perth experienced a four day spell of 40°C or higher in early February

2017

- Across the whole state rainfall was above average and was the 9th wettest year on record (42% above average), and wettest year in six years for the Perth Metropolitan area
- 8th warmest year on record for the state, in terms of average mean maximums
- Perth received exceptional rain fall in February and March; 114.4mm of rain was received in the Perth Metropolitan area over 24 hours, the second highest amount of rainfall received in one day in 142 years

Appendix D — Detailed data during validation period

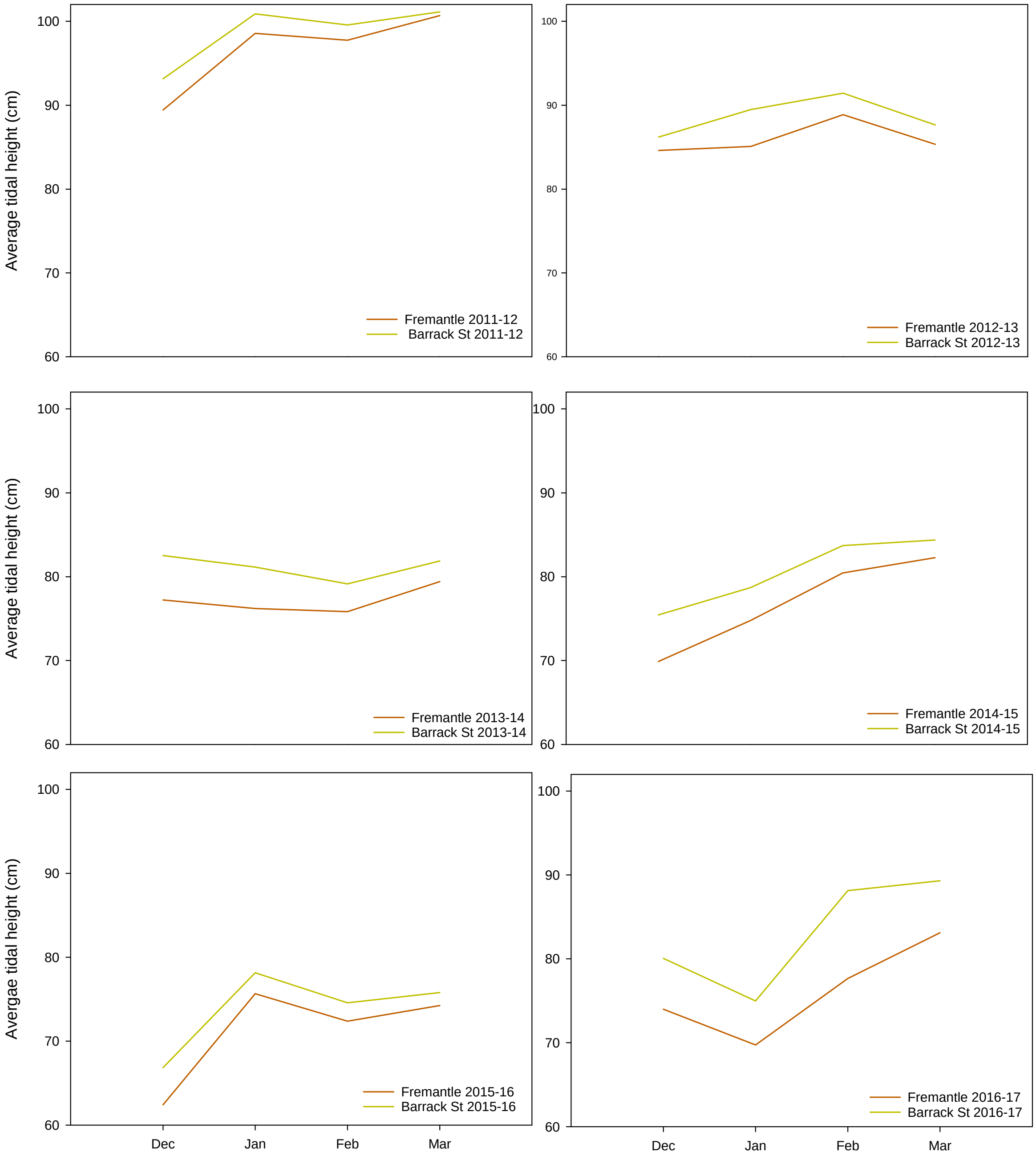


Figure A 2 *Monthly average tide height (Dec-Mar) for the six years from 2011-12 to 2016-17) for Fremantle and Barrack Street tide gauges (data provided by Department of Transport).*

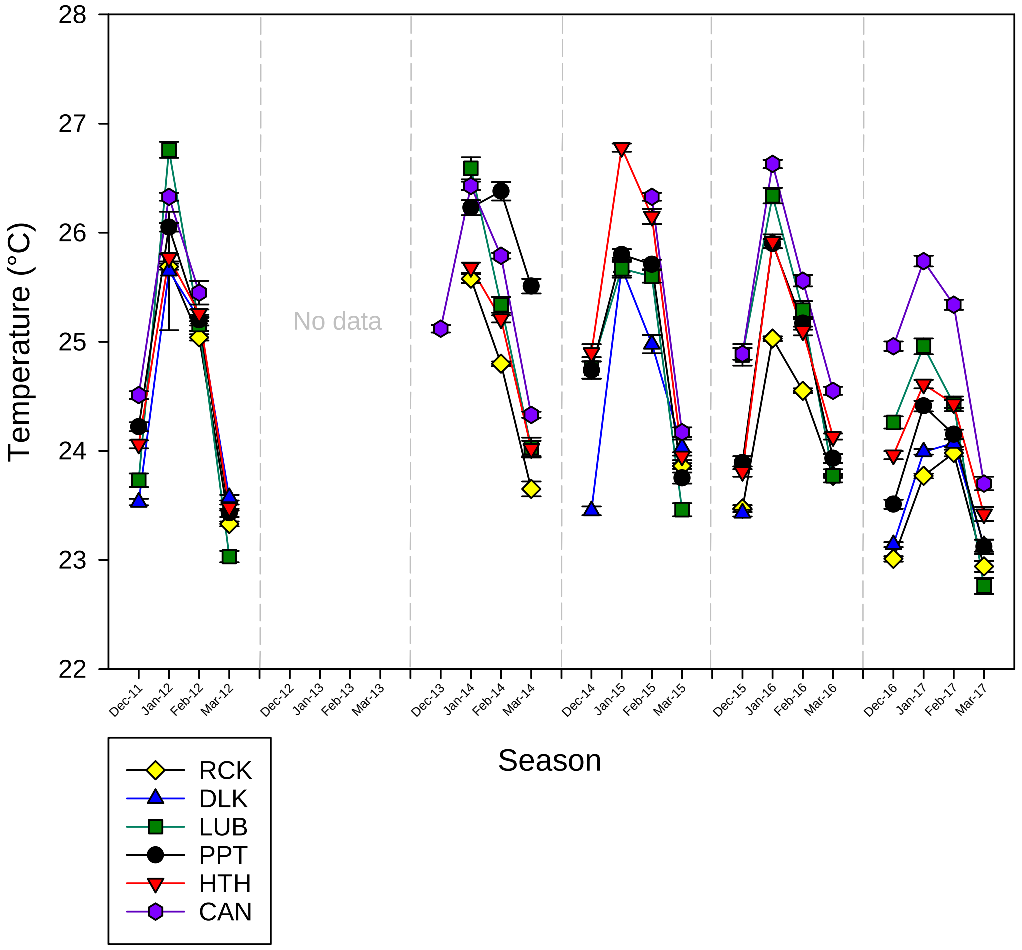


Figure A 3 *Average monthly maximum insitu temperature for Swan Canning sites – measured with insitu HOBO temperature loggers*

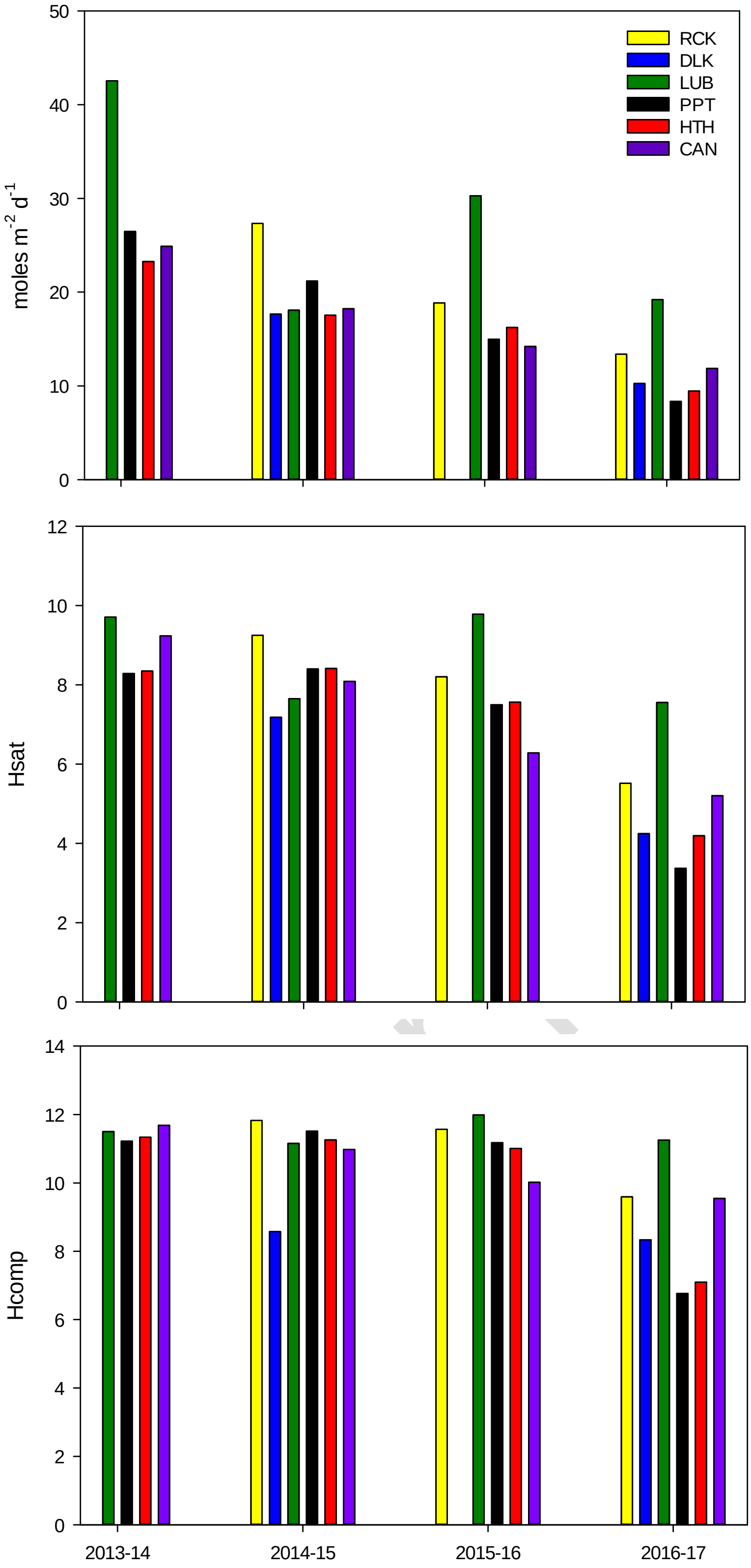


Figure A 4 *Light metrics by site x year-season for total moles m^2 d^{-1} and hours above saturating irradiance and compensation irradiance for 2013-14 to 2016-17.*

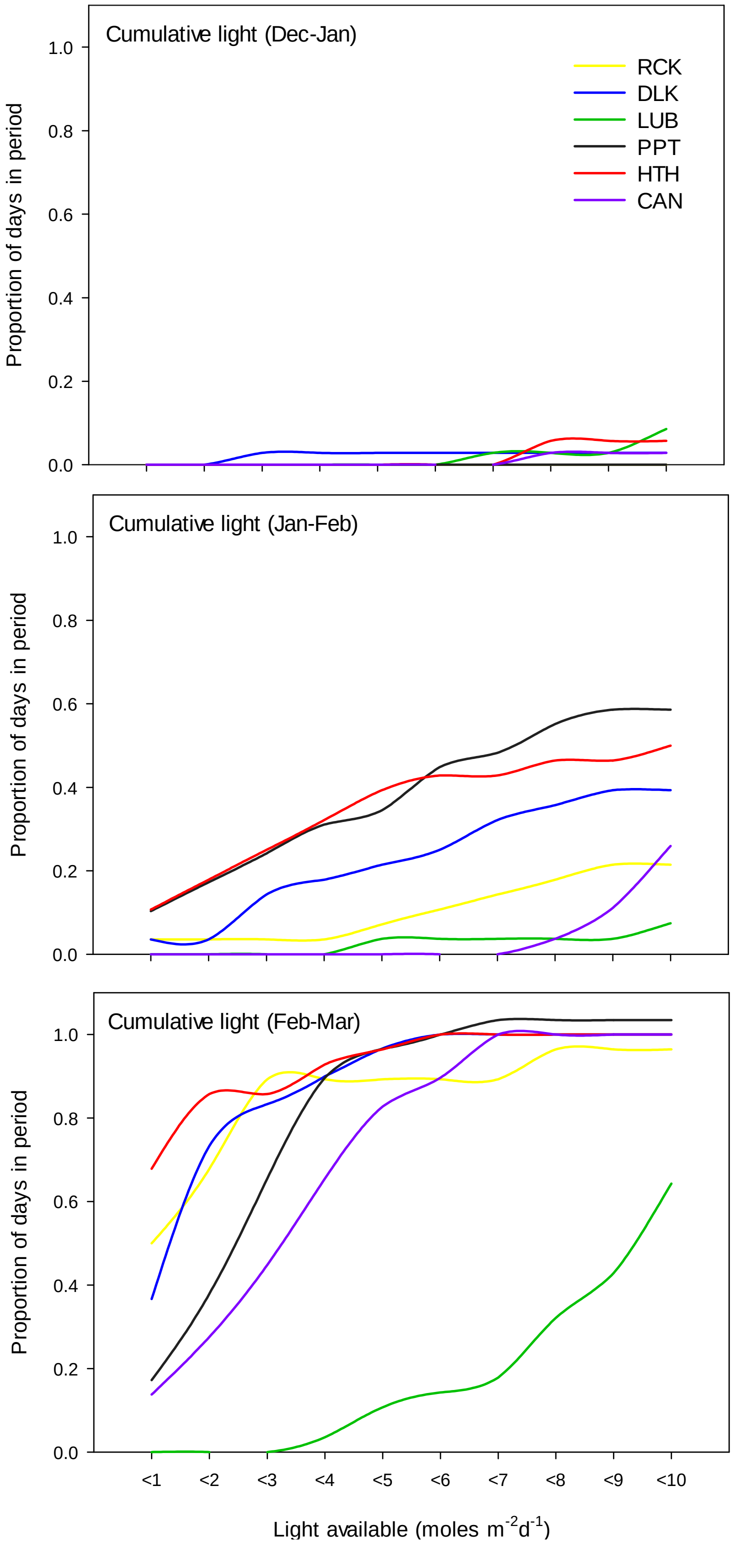


Figure A 5 *Cumulative light per site for December 2016 to March 2017 showing significant influence of flood waters on light climate. Low values in December indicate the majority of days had >10 moles m^{-2}d^{-1} available.*

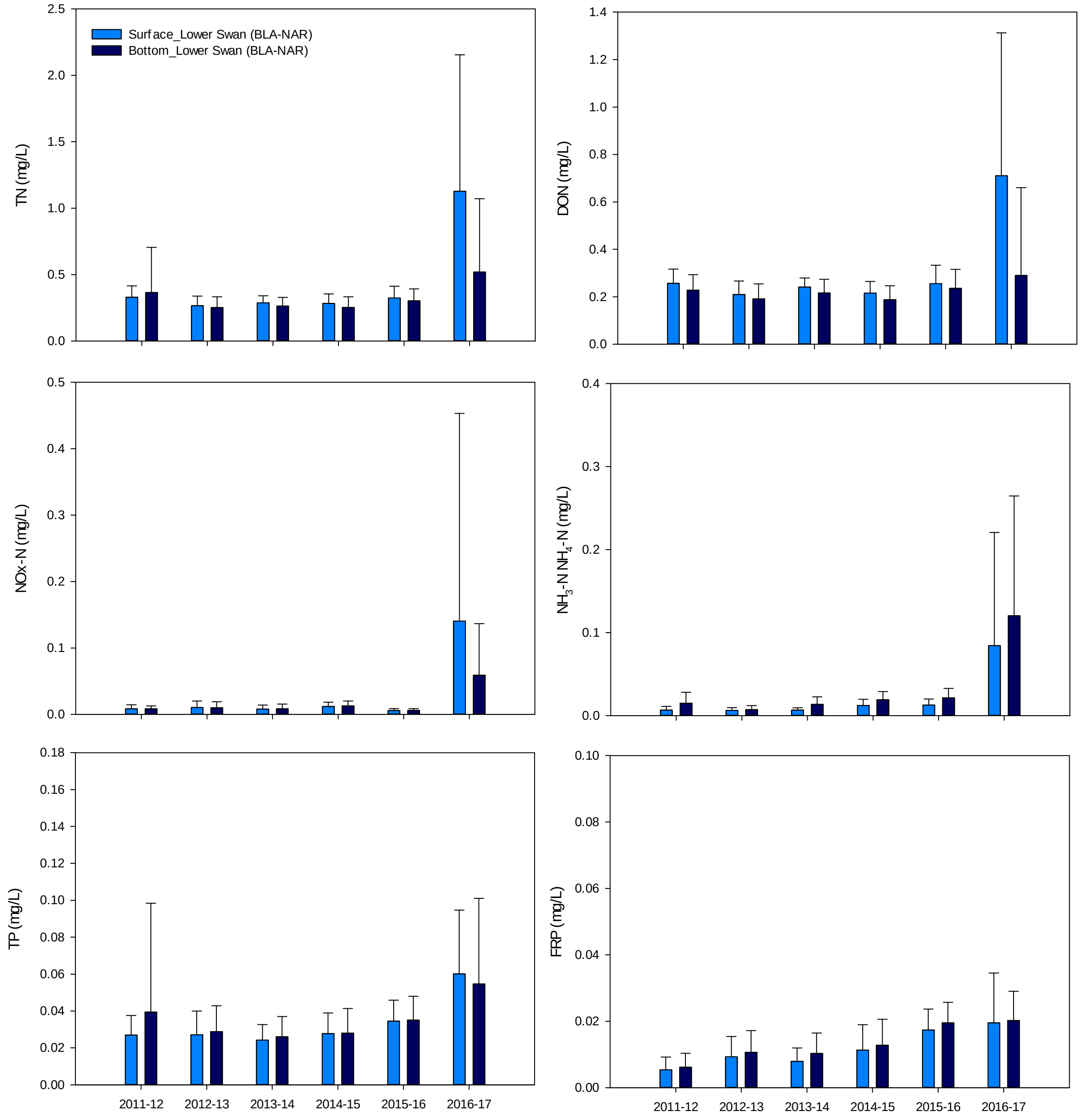


Figure A 6 *Seasonal nutrient concentrations in Lower Swan (NAR, HEA, ARM, BLA) for surface and bottom water samples*

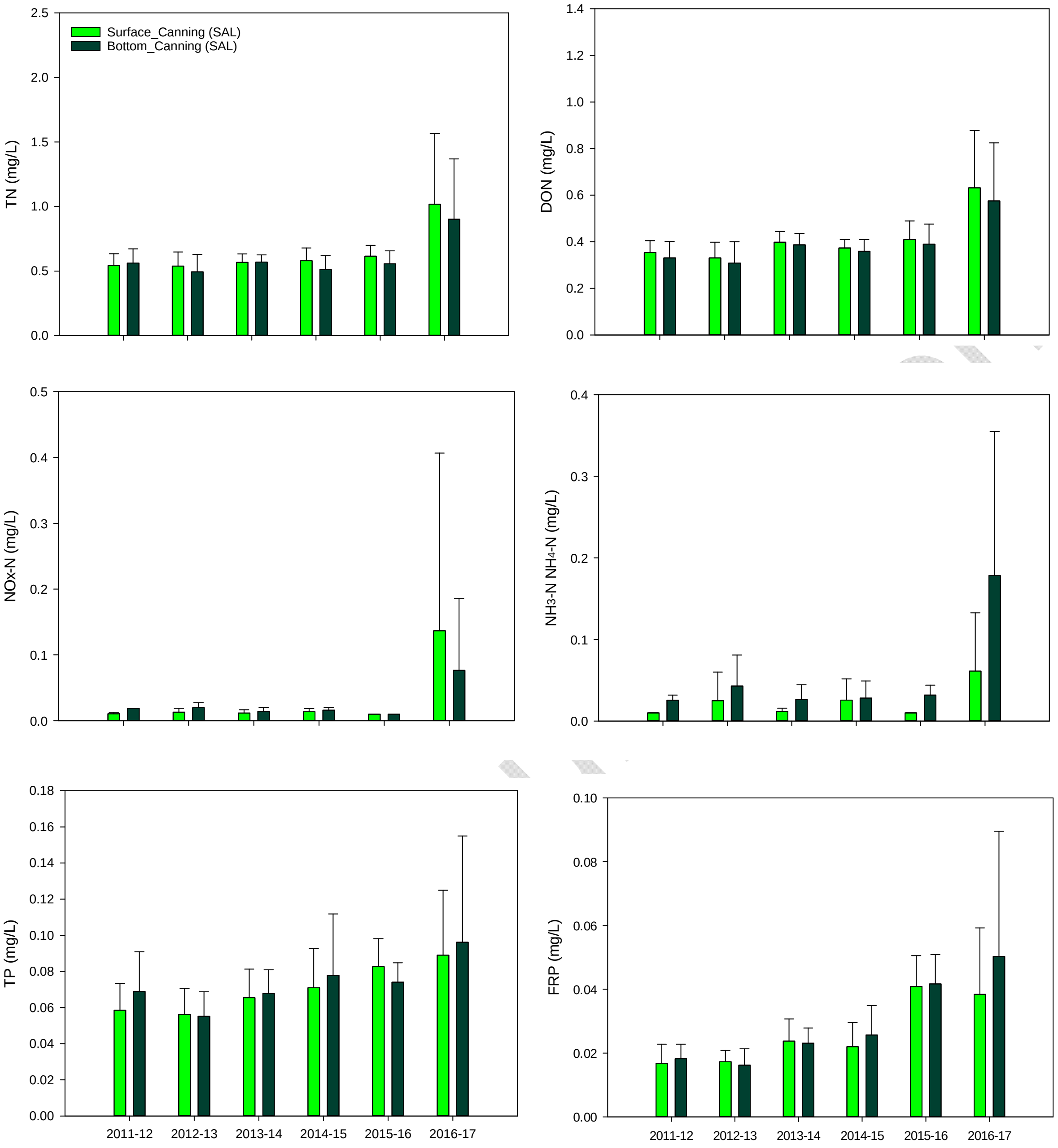


Figure A 7 *Seasonal nutrient concentrations in Canning (SAL) for surface and bottom water samples averaged for December to March each year-season*

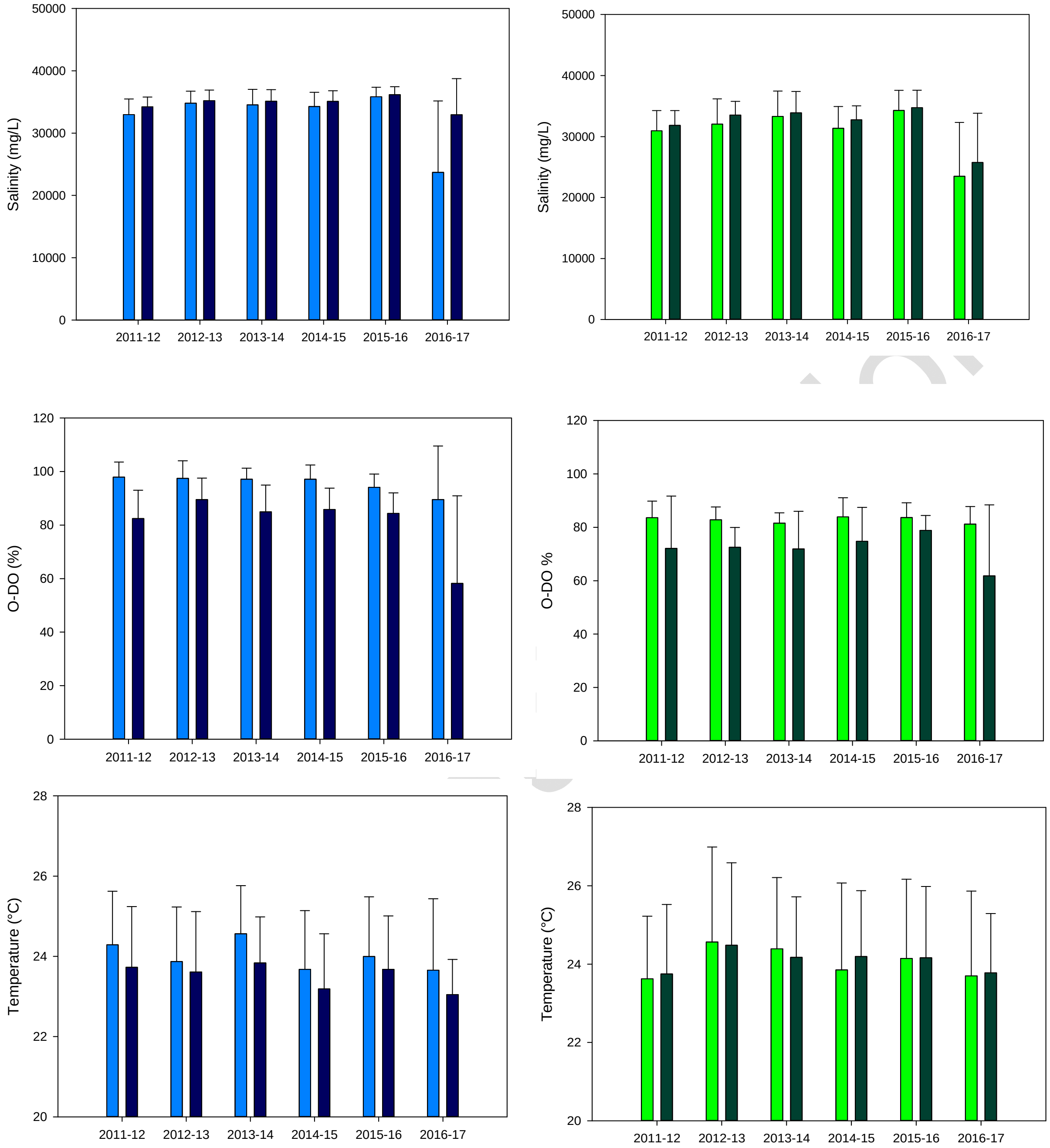
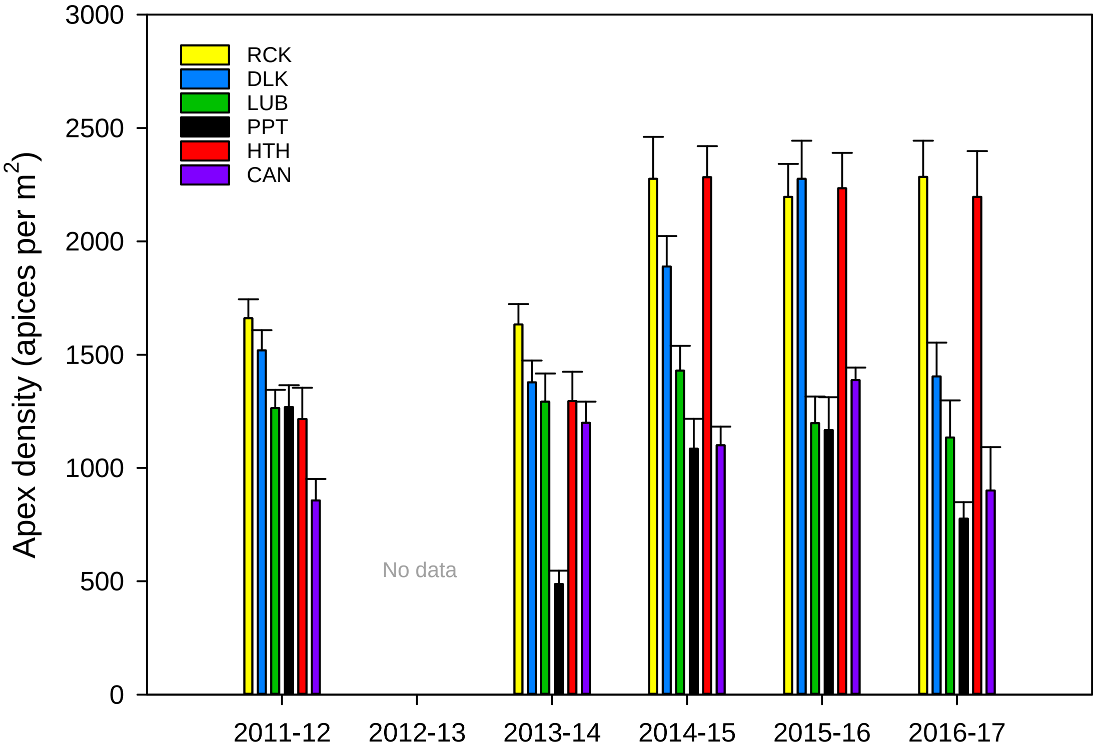


Figure A 8 *Seasonal physical water quality conditions (salinity, dissolved oxygen and temperature for Lower Swan sites (blue bars) and Canning site (green bars) averaged for December to March each year-season.*



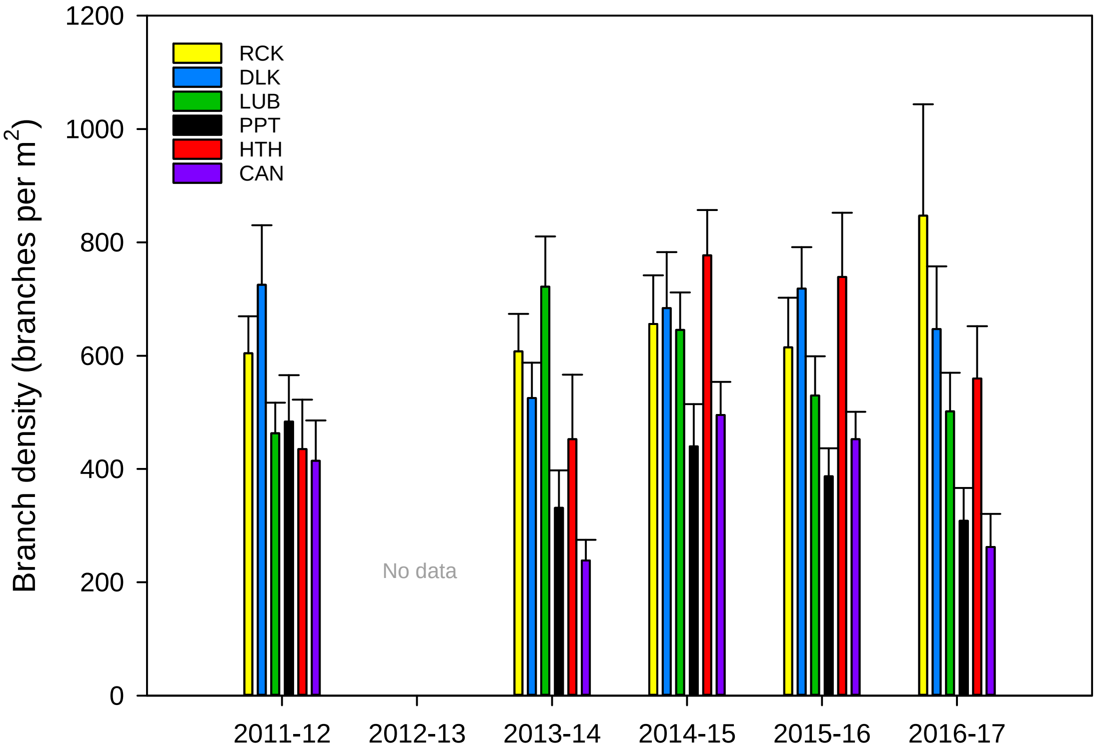
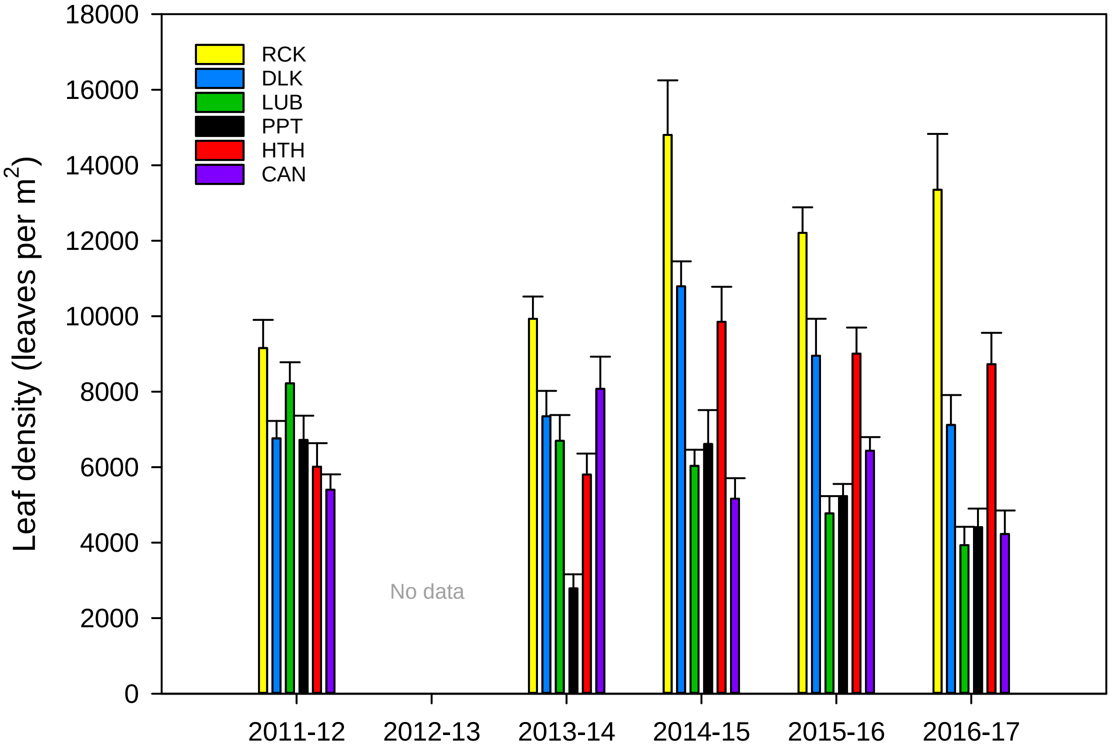


Figure A 9 *Average apex and branch density per site, across the validation period (mean + st. err, n=5).*



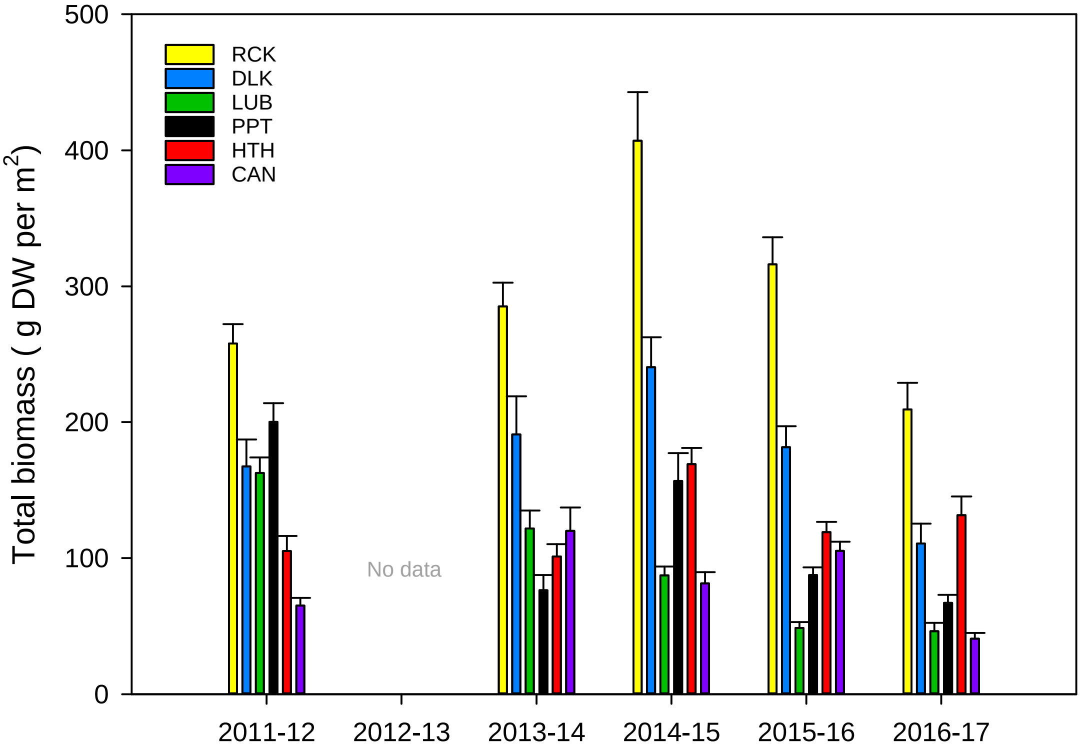


Figure A 10 *Average leaf density and total biomass per site, across the validation period (mean + st. err, n=5).*

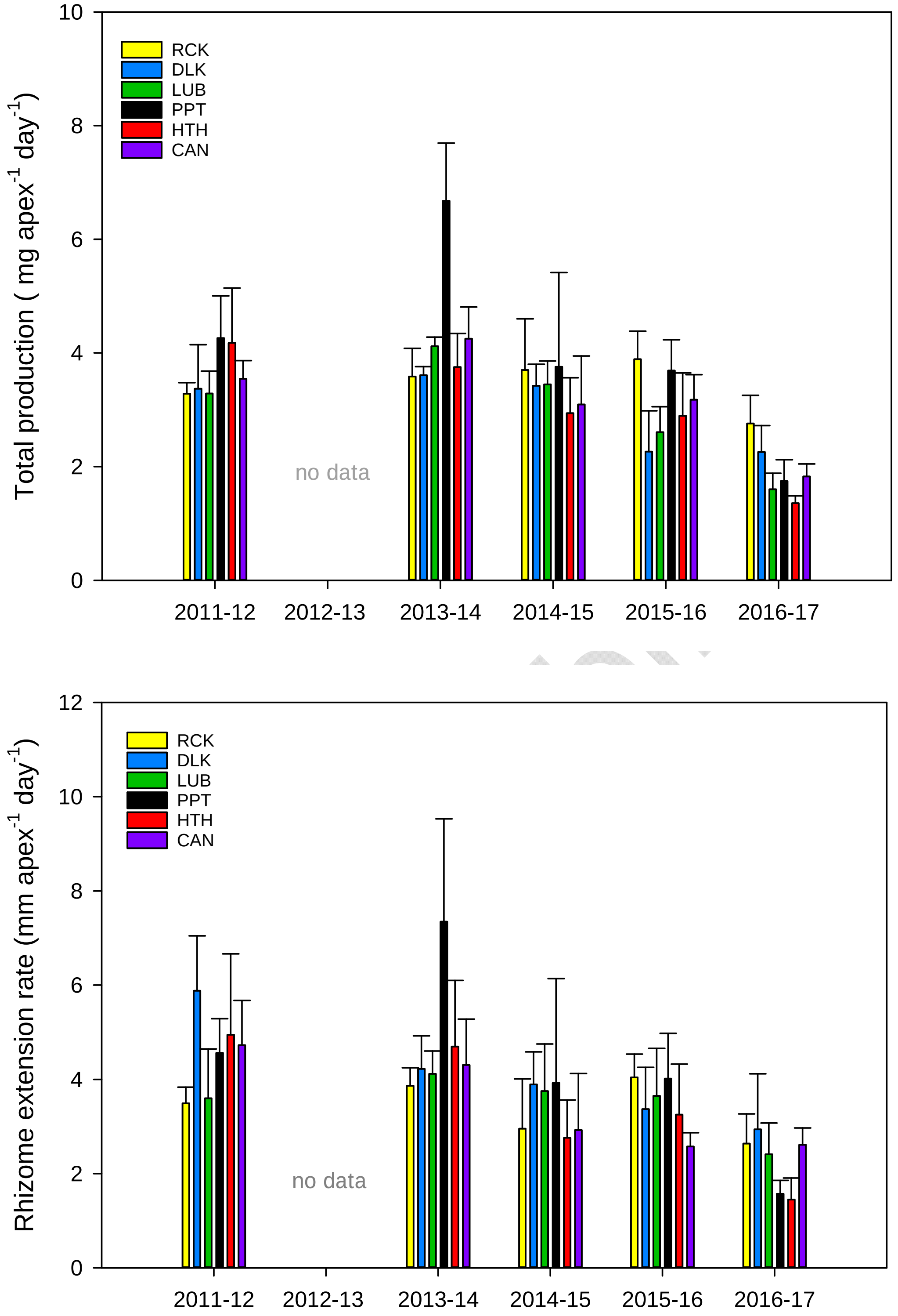


Figure A 11 *Productivity measures (total production and rhizome extension per apex per day) for six sites in the Swan-Canning from 2011-12 to 2016-17.*

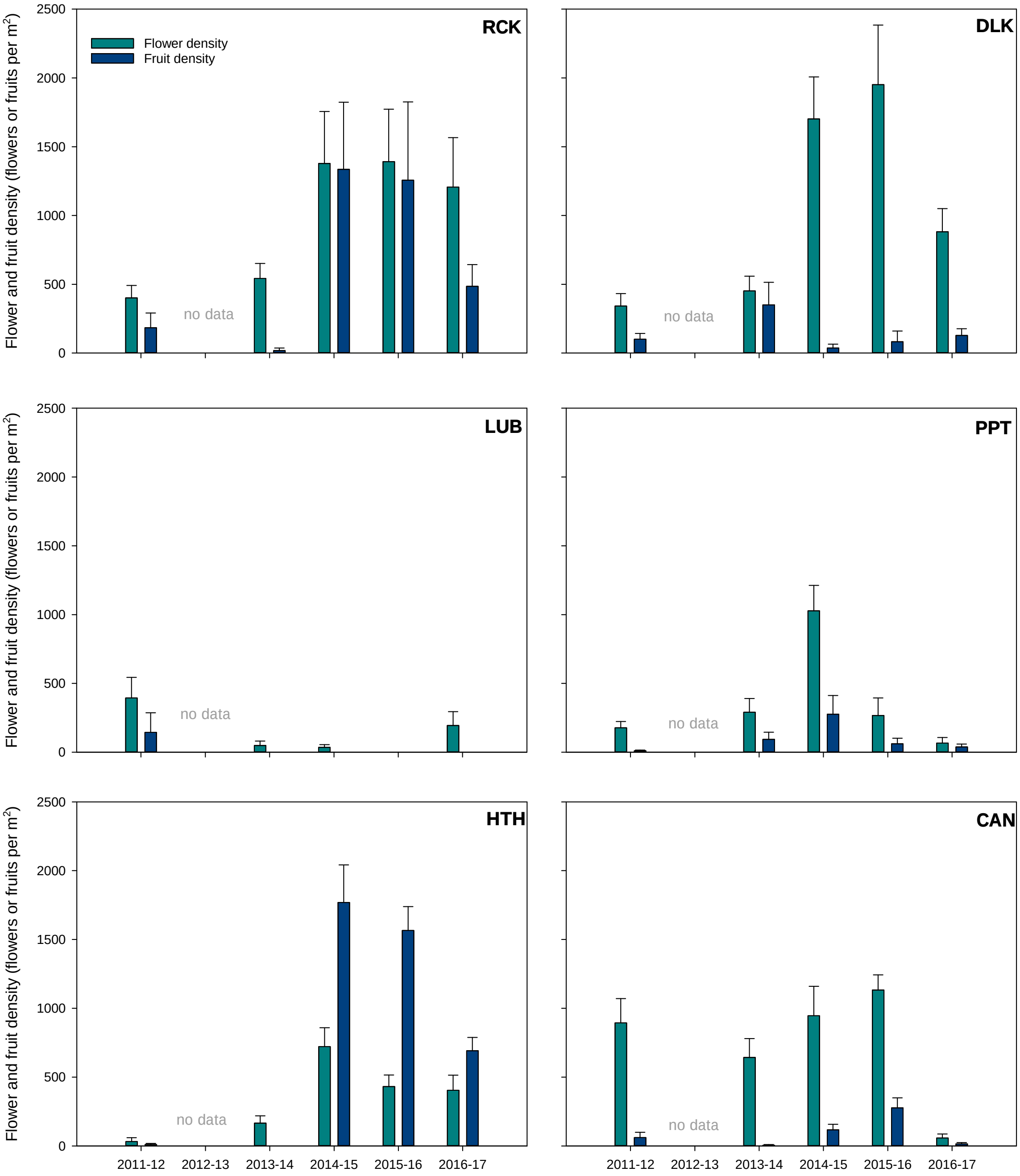


Figure A 12 *Annual flower and fruit production measured at sites in the Swan-Canning across the validation period.*

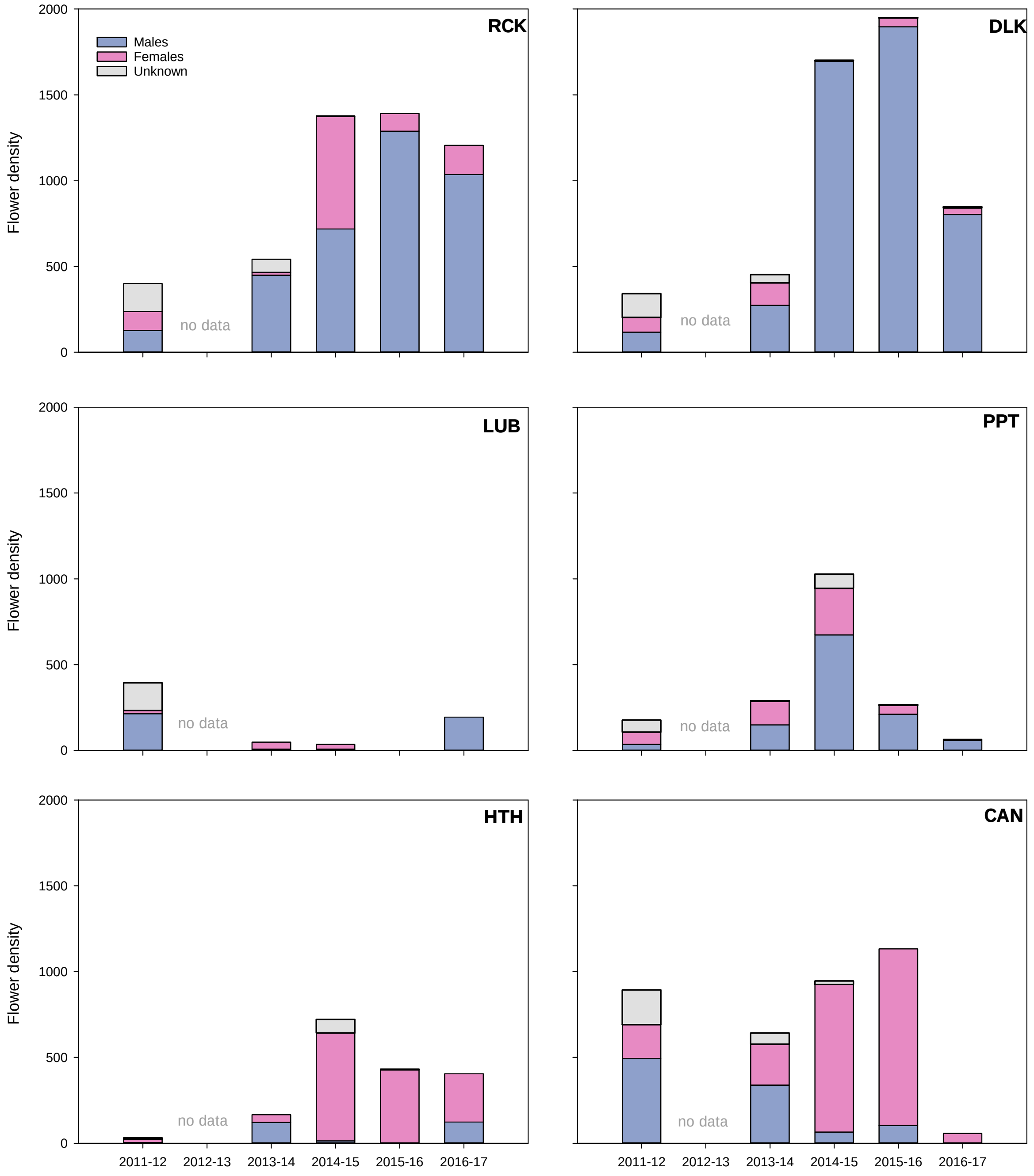


Figure A 13 *Average flower density (flowers m*$^{-2}$*, also showing proportion of male and female flowers, where known) for all sites across the validation period.*

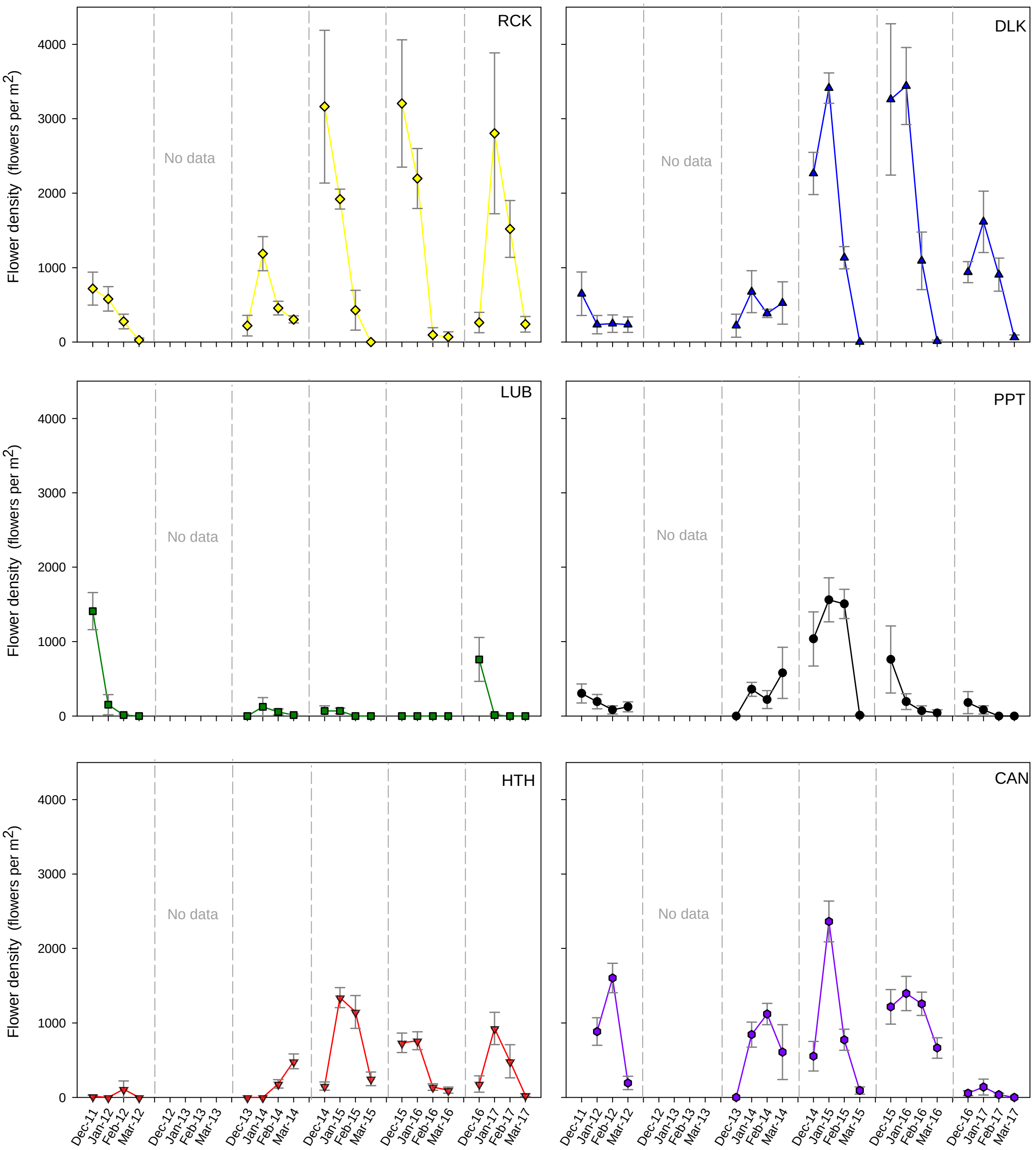


Figure A 14 *Monthly average flower density (± st err, n=5) for all sites from 2011-17.*

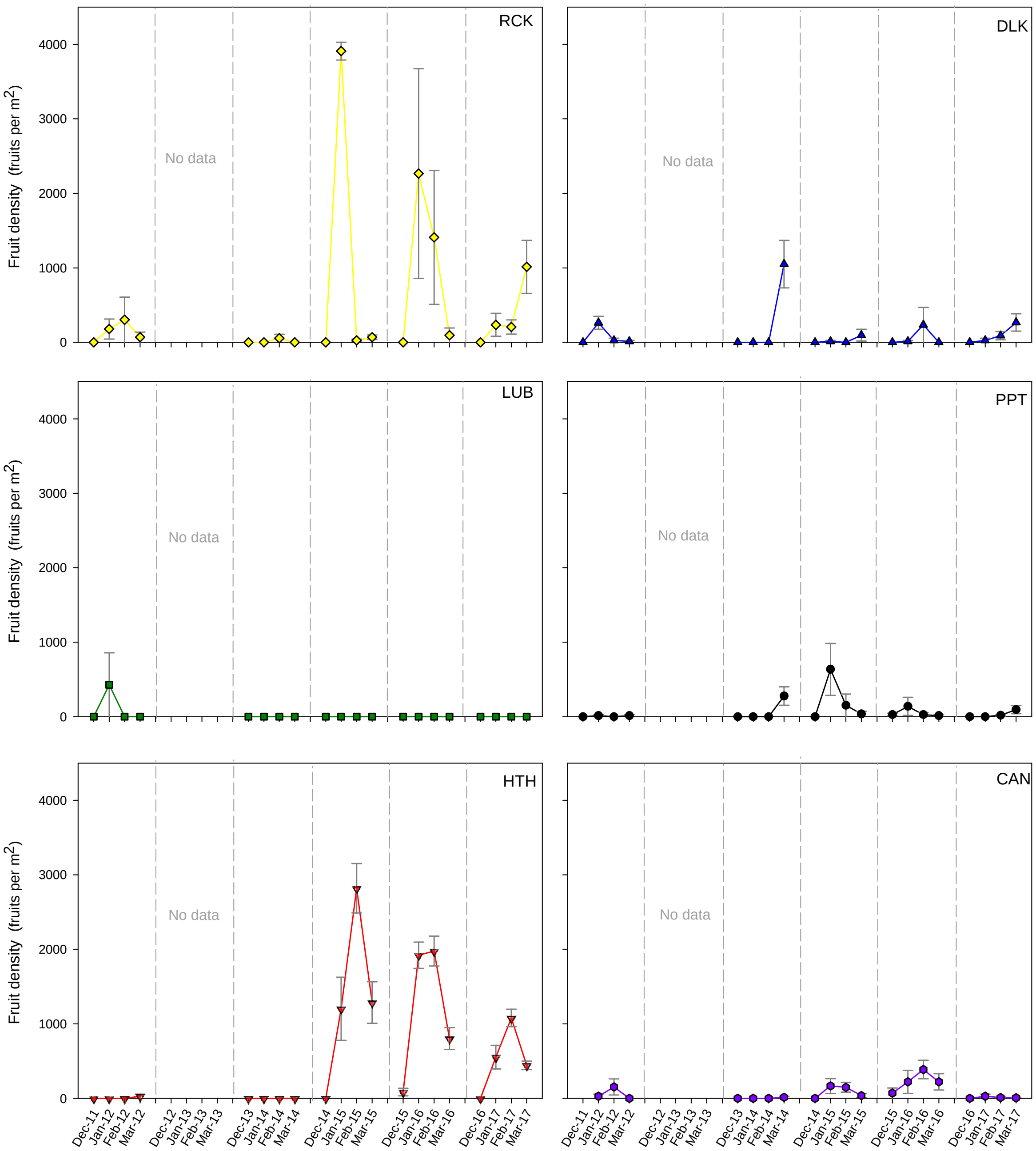
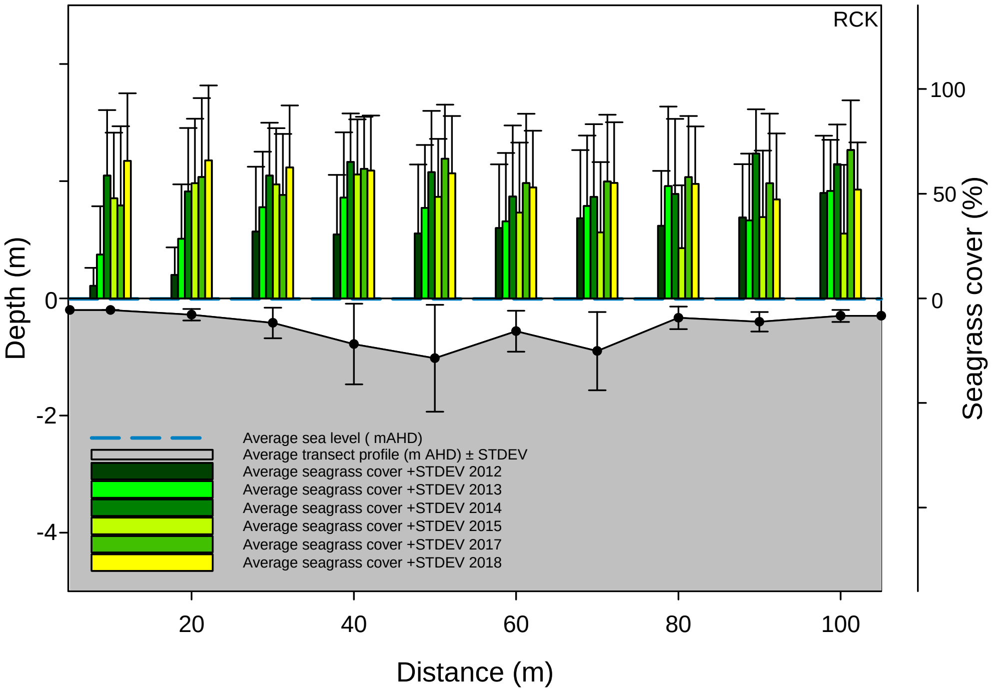


Figure A 15 *Monthly average fruit density (± st err, n=5) for all sites from 2011-17.*



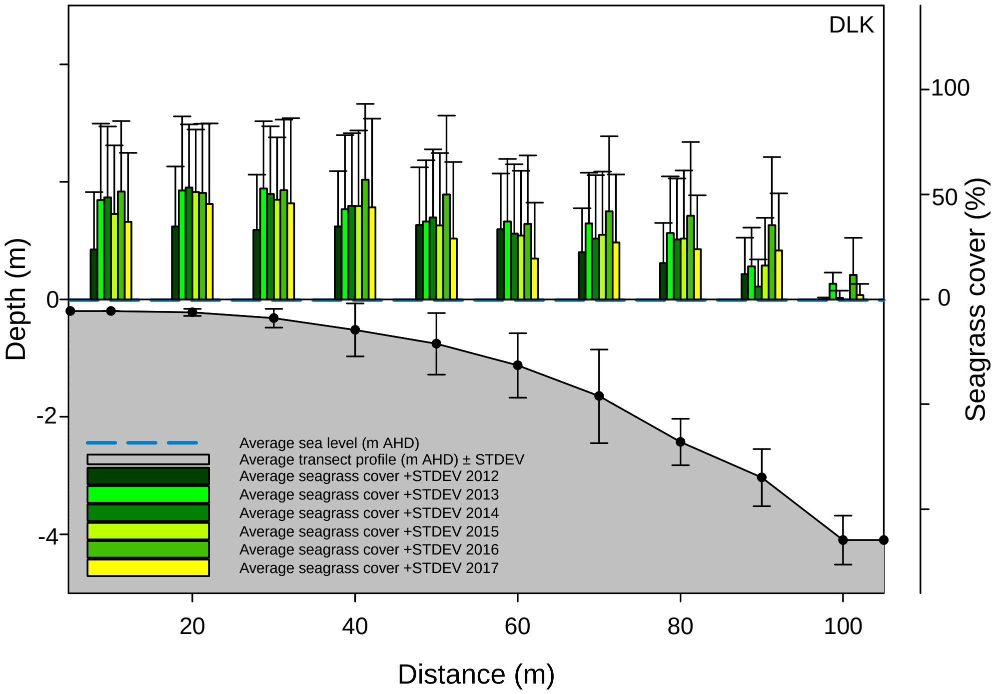
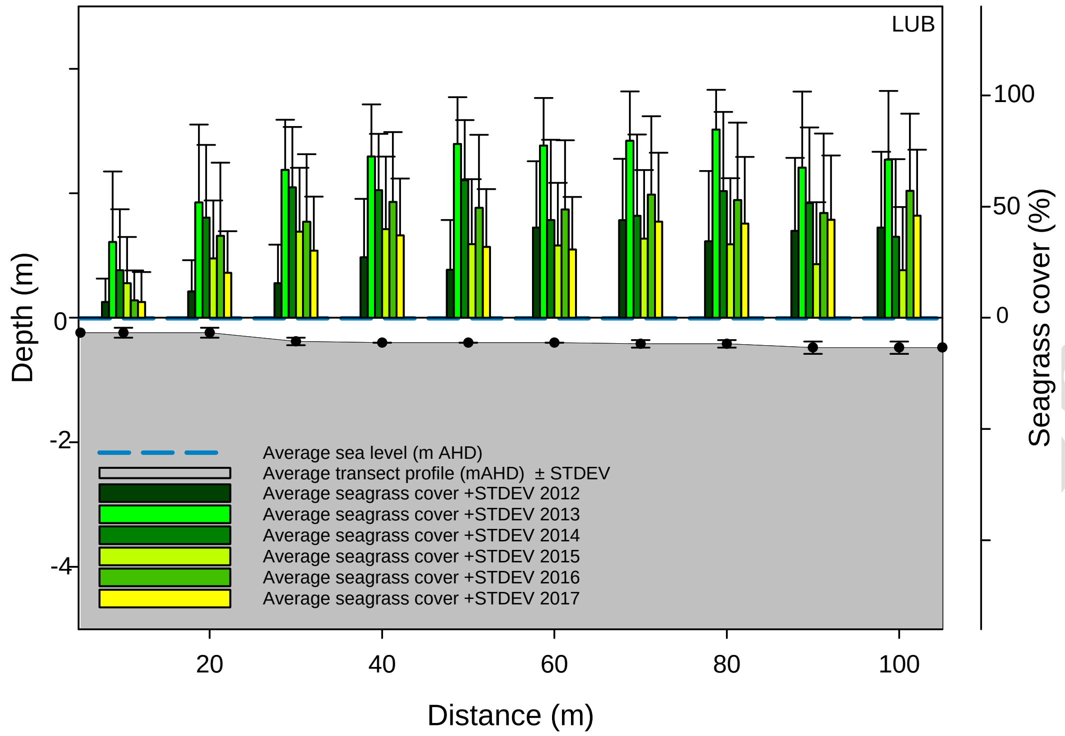


Figure A 16 *Average seagrass cover (%) over for RCK and DLK in years 2012-17. Data shown is mean, + standard deviation, pooled for each 10 m interval and summarizing 10 transects per site. Site topography also shown beneath in grey.*



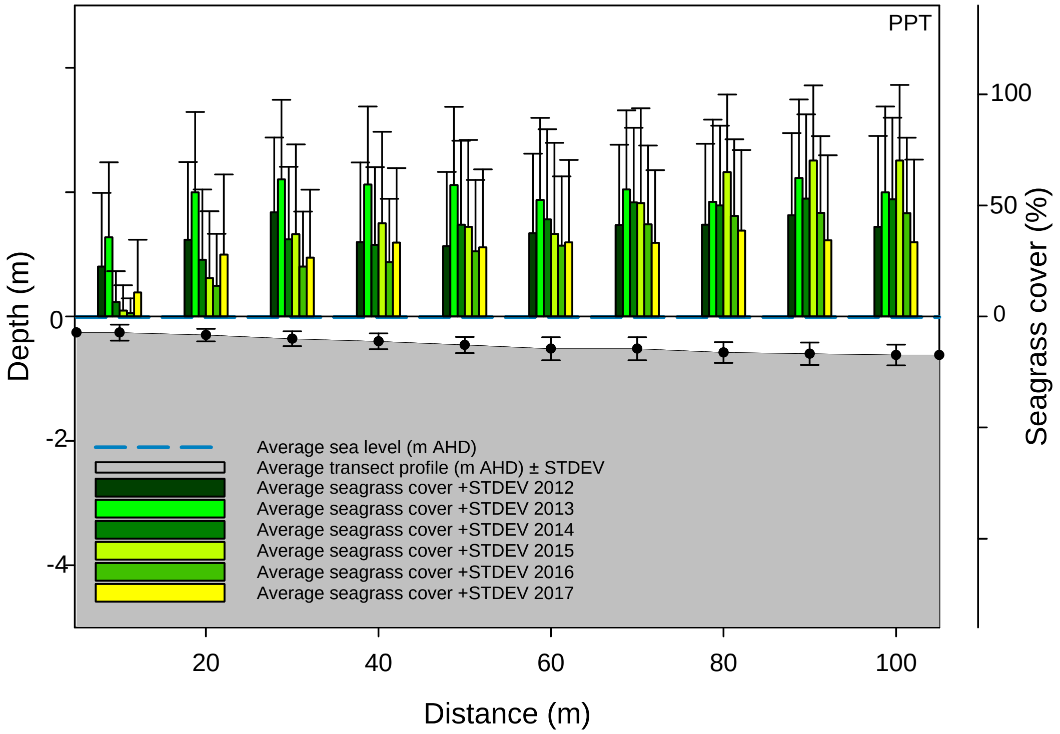


Figure A 17 *Average seagrass cover (%) over for LUB and PPT in years 2012-17. Data shown is mean, + standard deviation, pooled for each 10 m interval and summarizing 10 transects per site. Site topography also shown beneath in grey.*

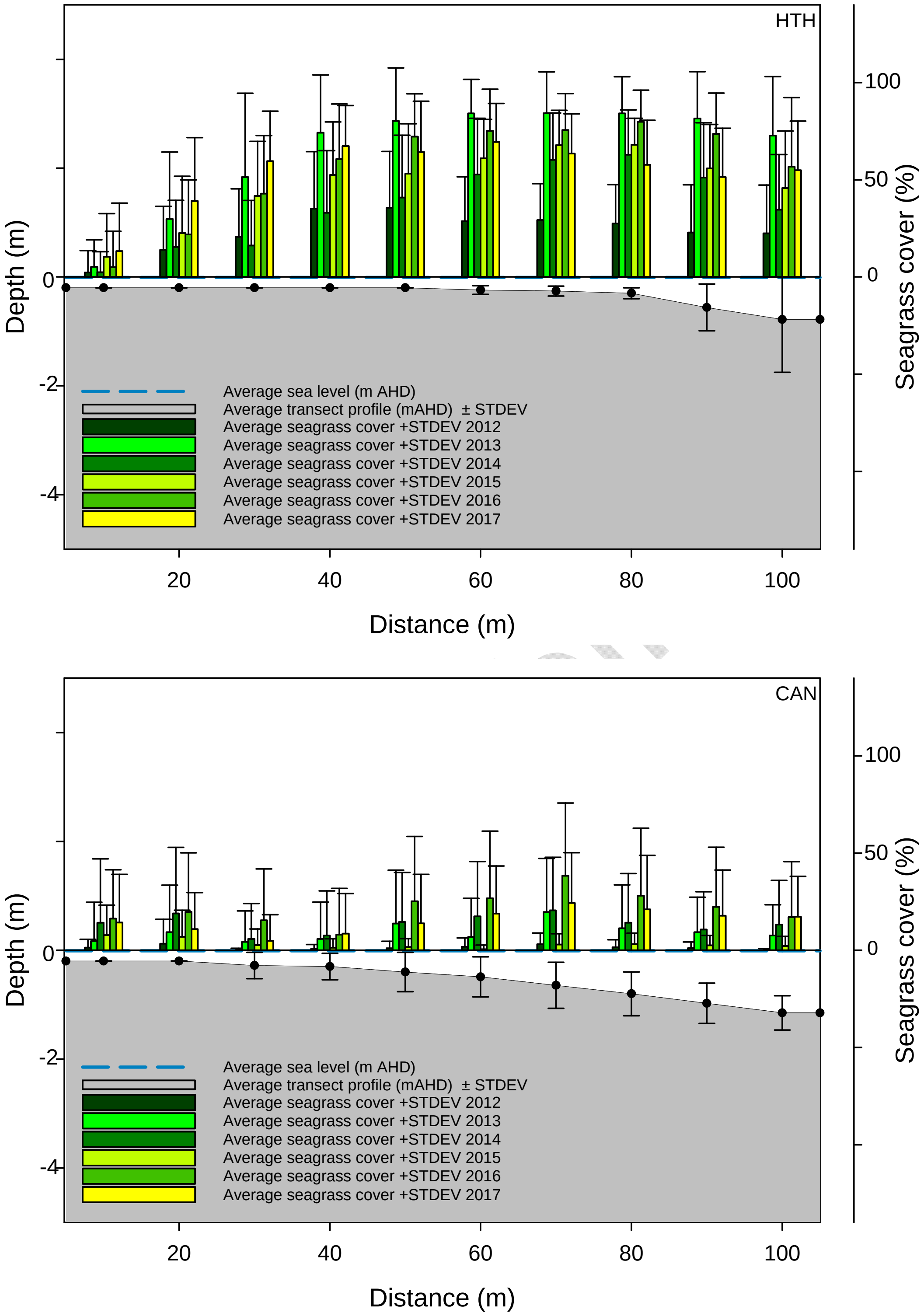


Figure A 18 *Average seagrass cover (%) over for HTH and CAN in years 2012-17. Data shown is mean, + standard deviation, pooled for each 10 m interval and summarizing 10 transects per site. Site topography also shown beneath in grey.*

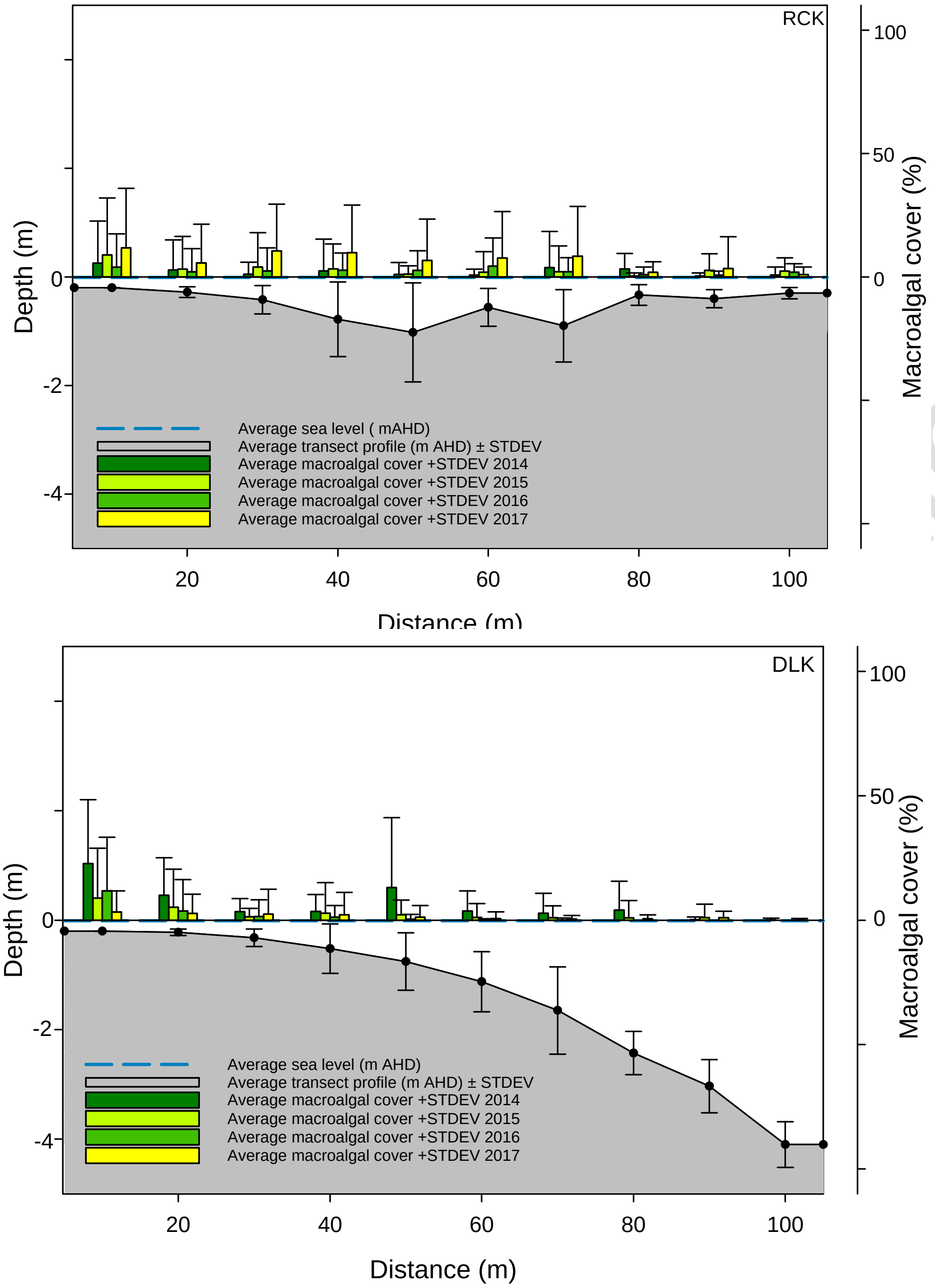
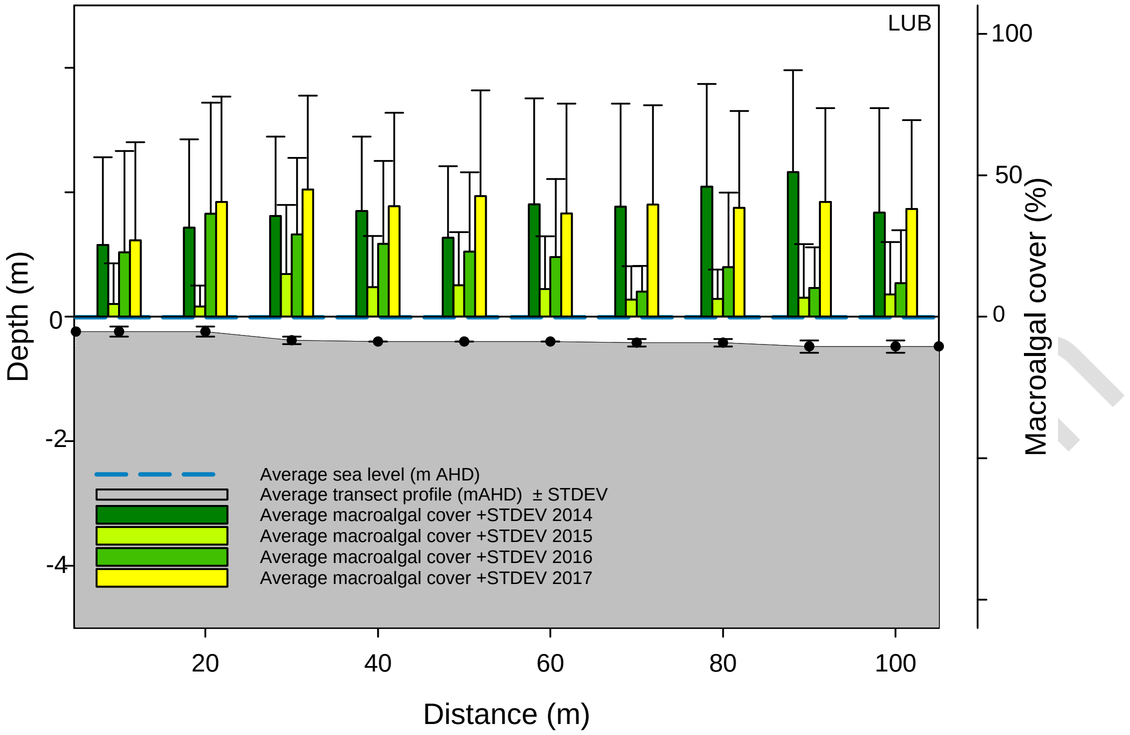


Figure A 19 *Average macroalgal cover (%) for sites RCK and DLK in the years 2014-17. Data is shown as mean + standard deviation, pooled for each 10 m interval summarizing 10 transects per site. Site topography is also shown beneath in grey.*



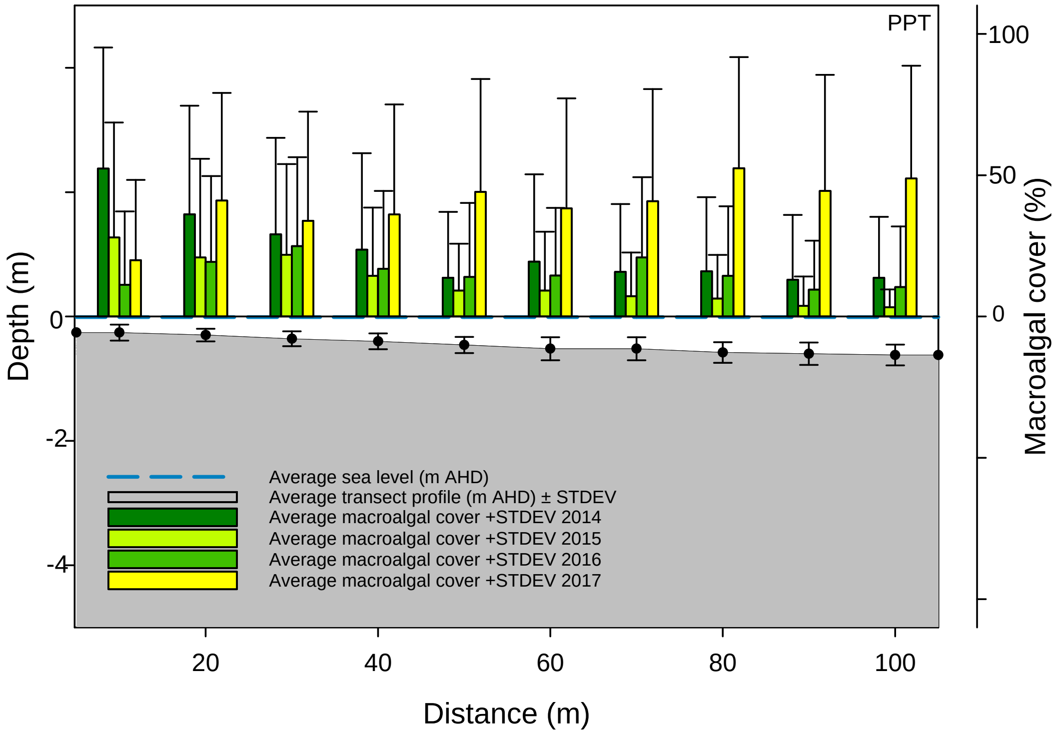
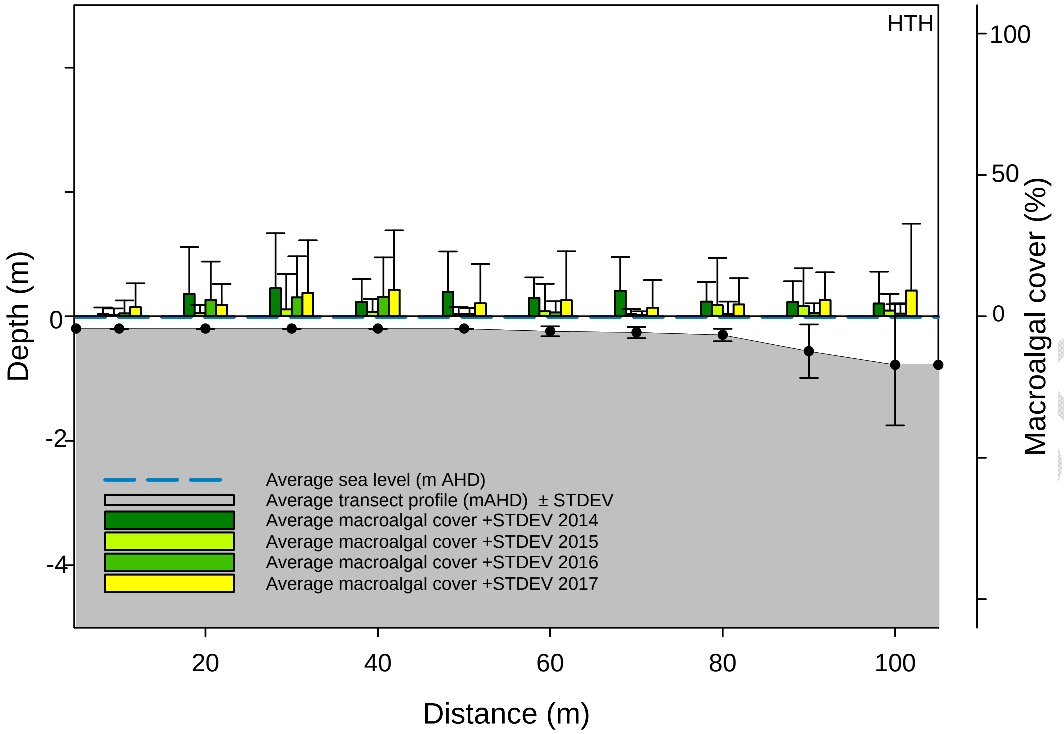


Figure A 20 *Average macroalgal cover (%) for sites LUB and PPT in the years 2014-17. Data is shown as mean + standard deviation, pooled for each 10 m interval summarizing 10 transects per site. Site topography is also shown beneath in grey.*



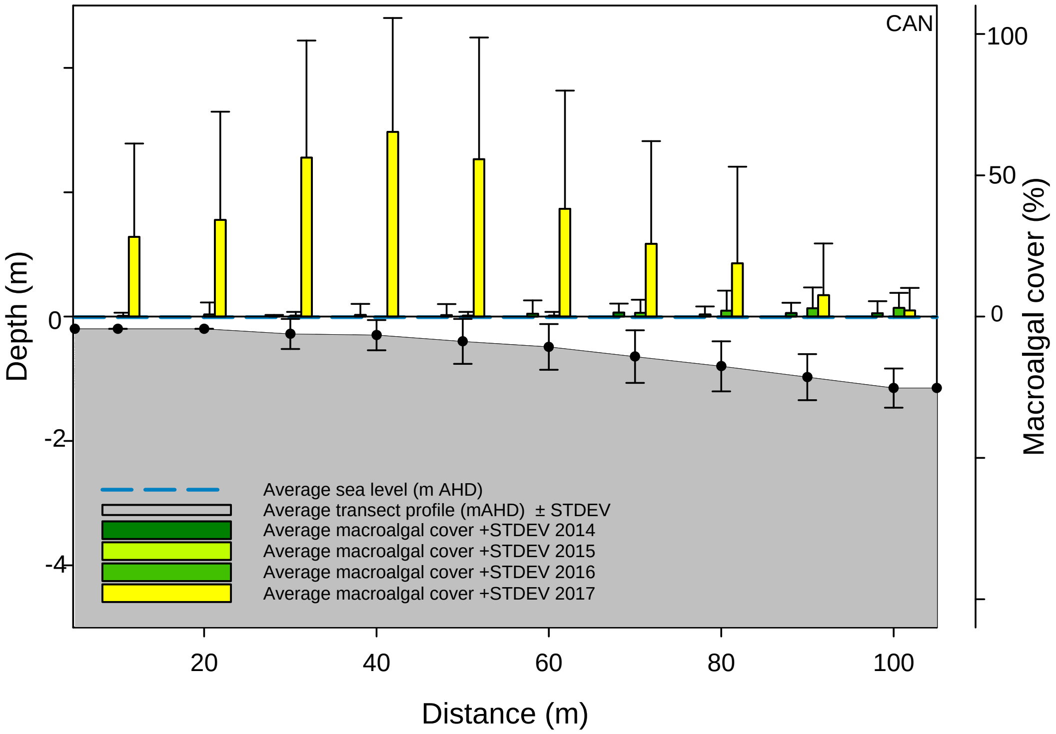


Figure A 21 *Average macroalgal cover (%) for sites HTH and CAN in the years 2014-17. Data is shown as mean + standard deviation, pooled for each 10 m interval summarizing 10 transects per site. Site topography is also shown beneath in grey.*

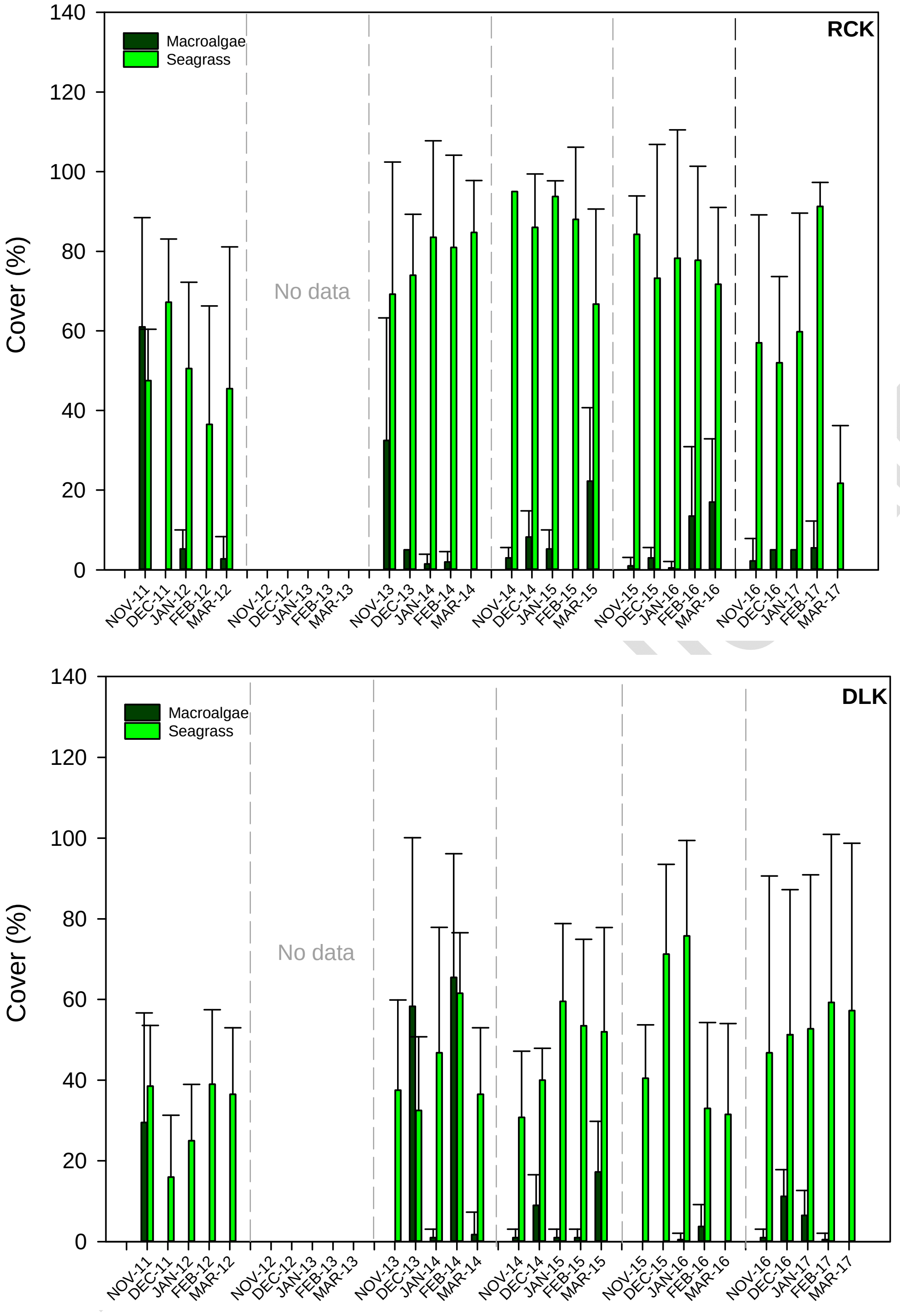


Figure A 22 *Monthly average percent cover of both macroalgae and seagrass at sites RCK and DLK from November 2011 to March 2017 (10 quadrat observations, mean + standard deviation).*

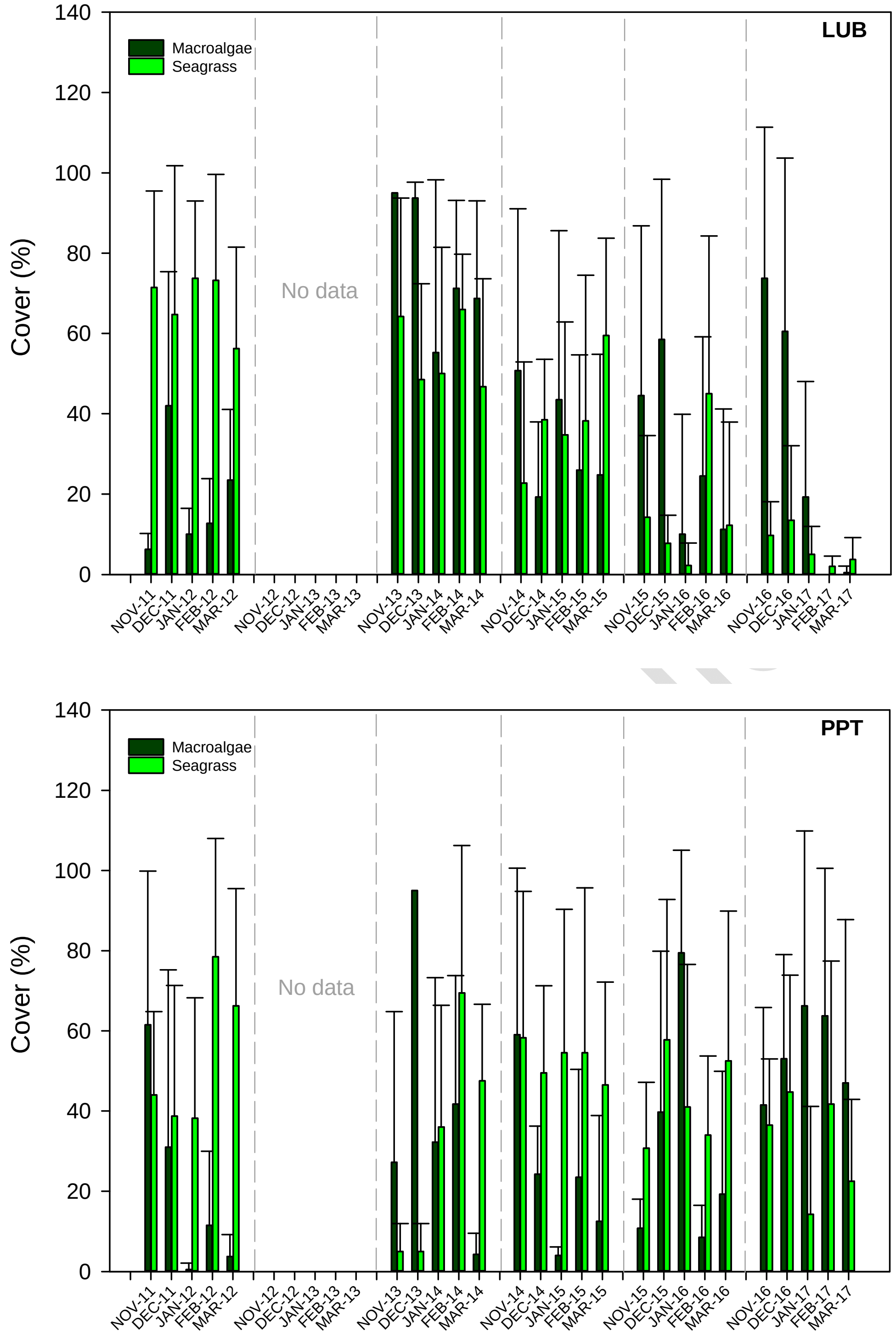


Figure A 23 *Monthly average percent cover of both macroalgae and seagrass at sites LUB and PPT from November 2011 to March 2017 (10 quadrat observations, mean + standard deviation).*

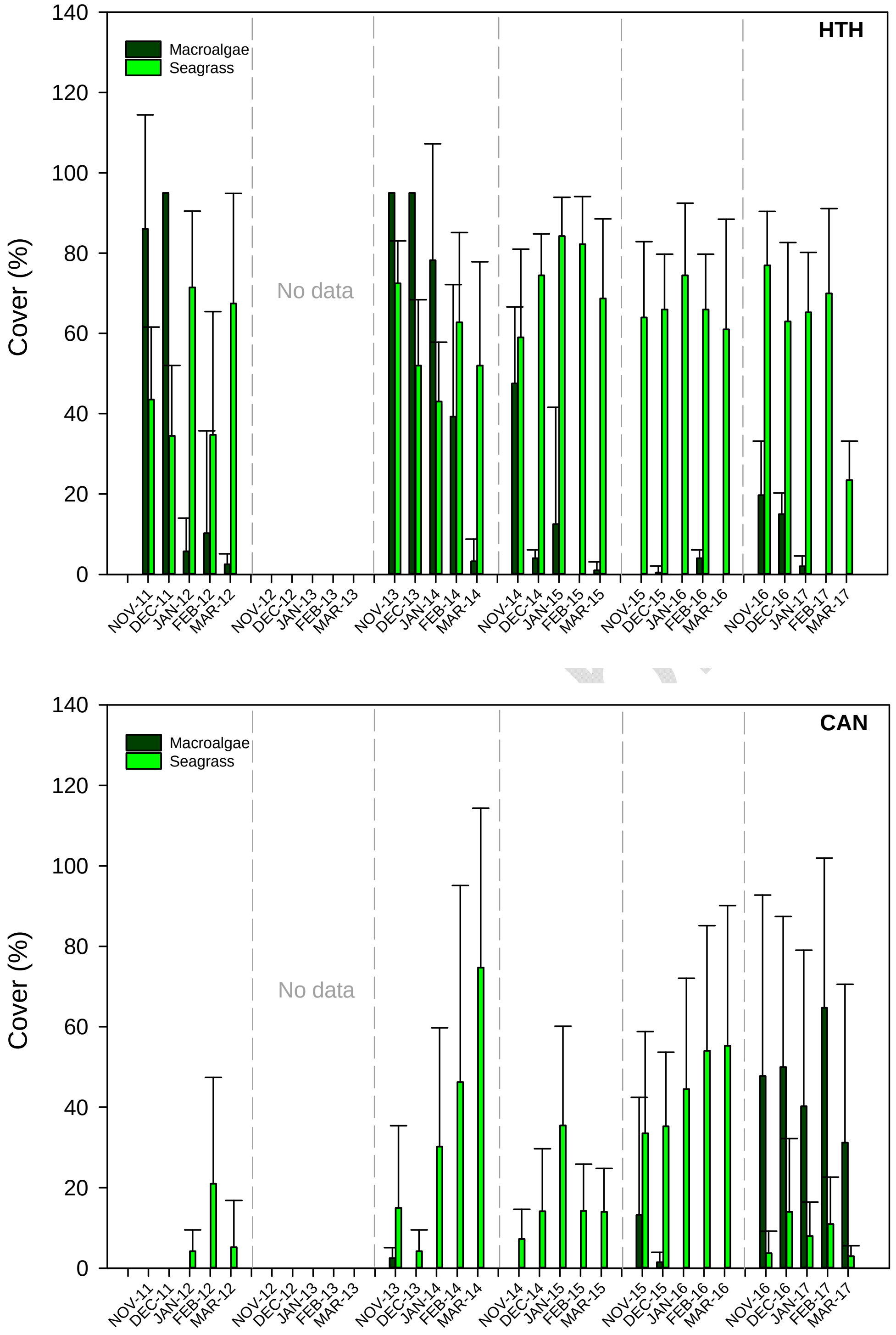


Figure A 24 *Monthly average percent cover of both macroalgae and seagrass at sites HTH and CAN from November 2011 to March 2017 (10 quadrat observations, mean + standard deviation).*

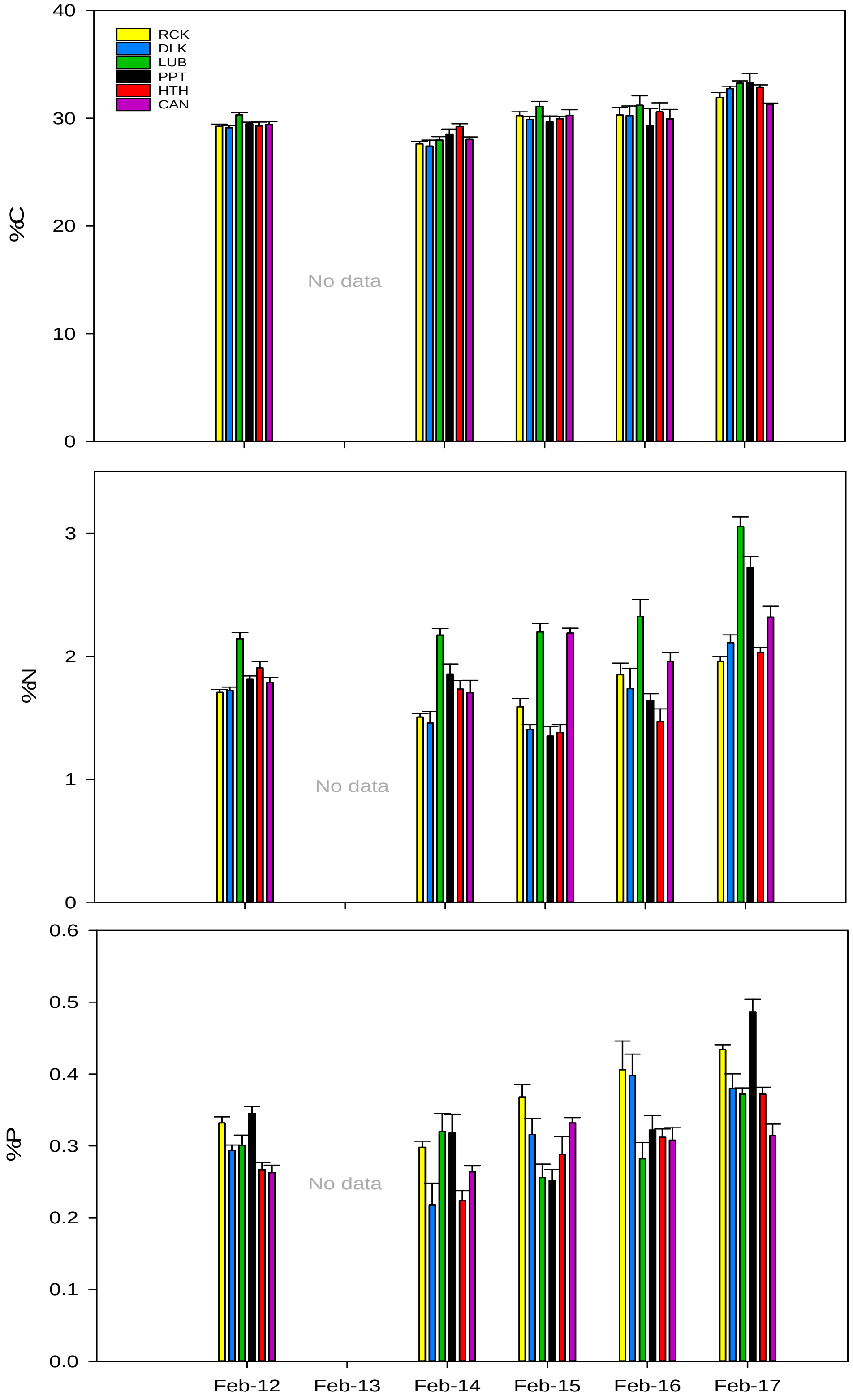


Figure A 25 *Percentage carbon, nitrogen and phosphorus within leaf tissues of Halophila ovalis measured in February across the validation period (mean + st. err., n=5).*

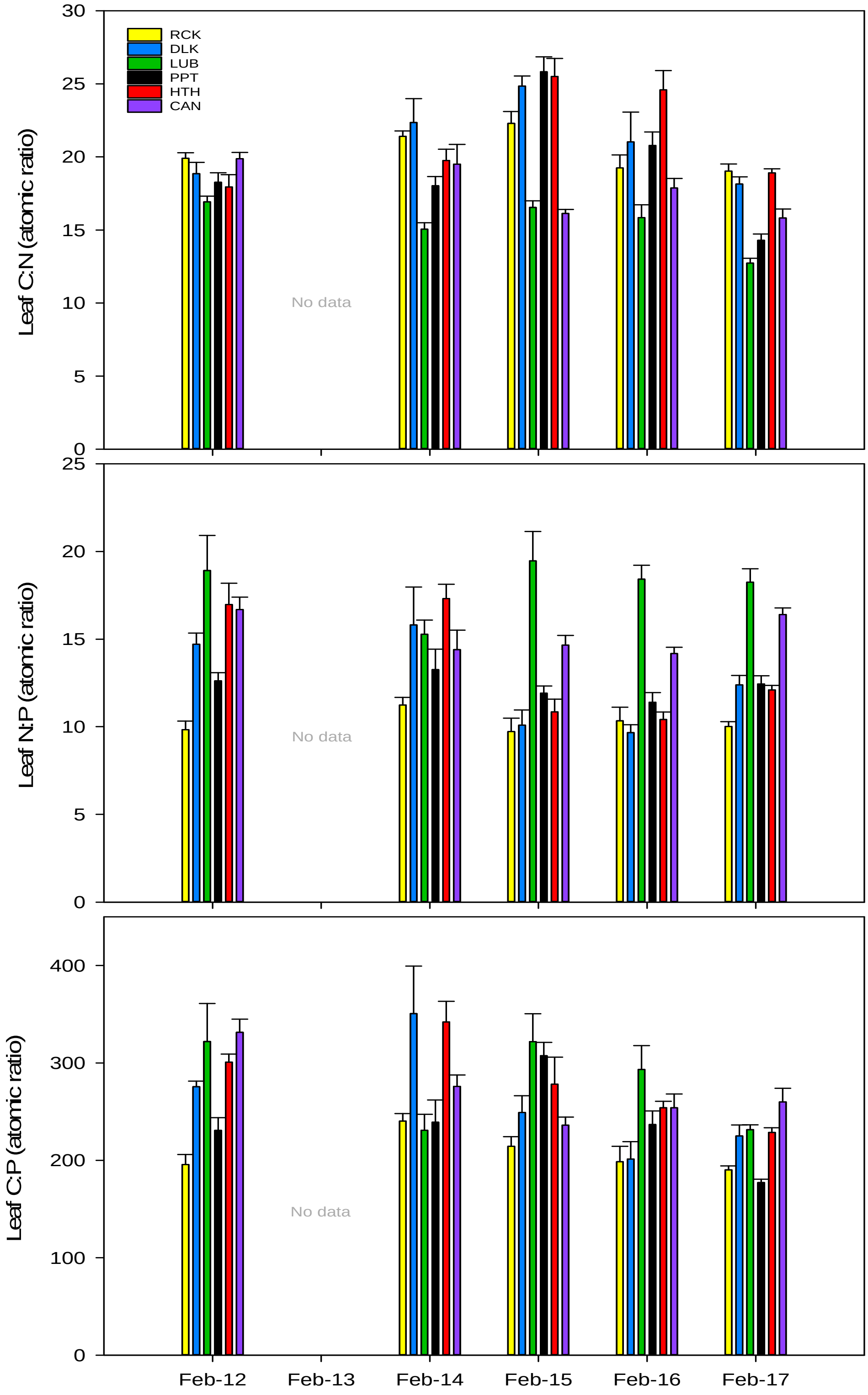


Figure A 26 *Leaf C:N, N:P and C:P atomic ratios for sites in the Swan-Canning across the validation period (mean + st. err., n=5).*

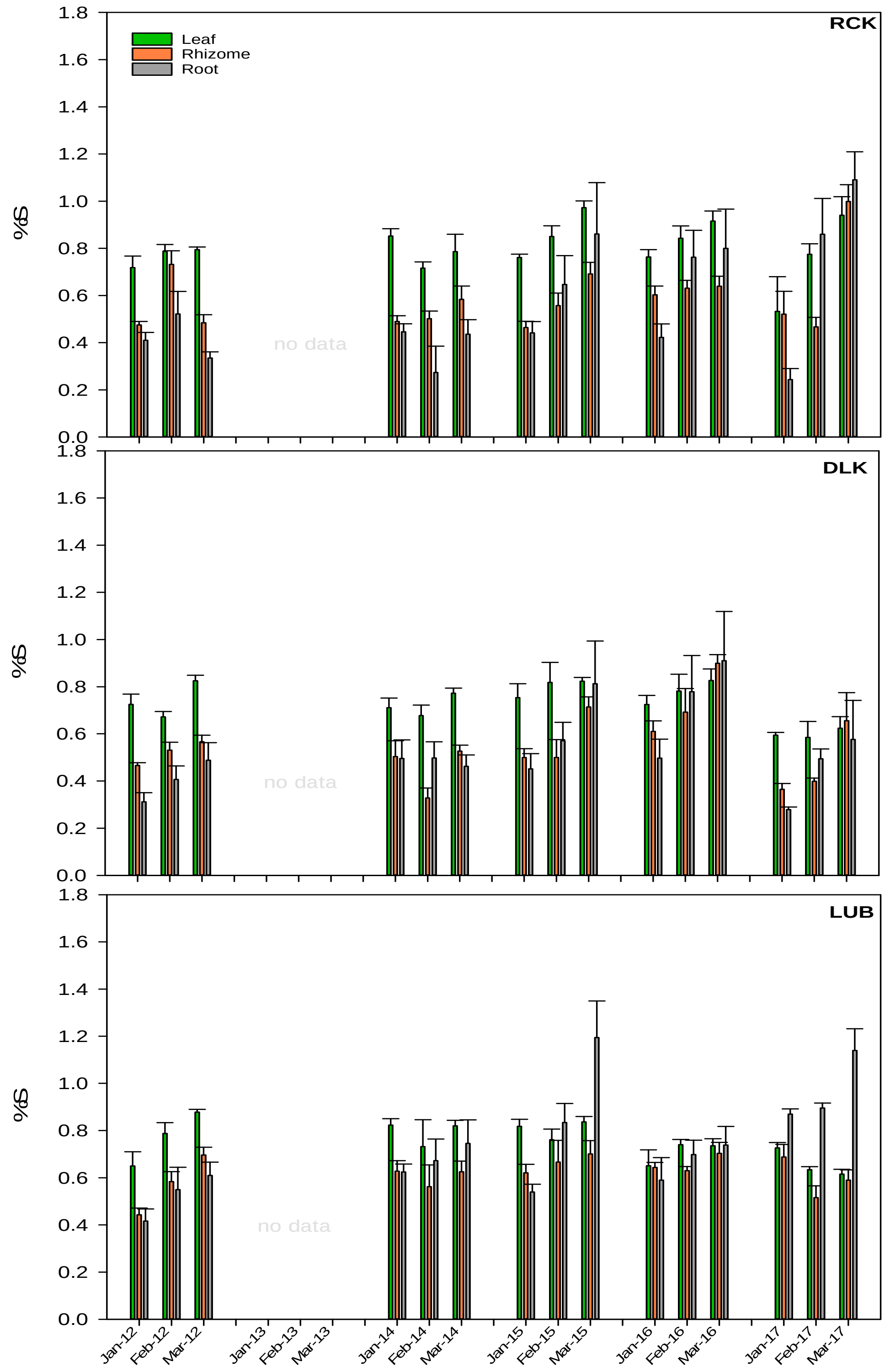


Figure A 27 *Percentage sulfur within leaves, rhizomes and roots of seagrass from January to March in each sampling year for sites RCK, DLK and LUB (mean + st. err, n=5).*

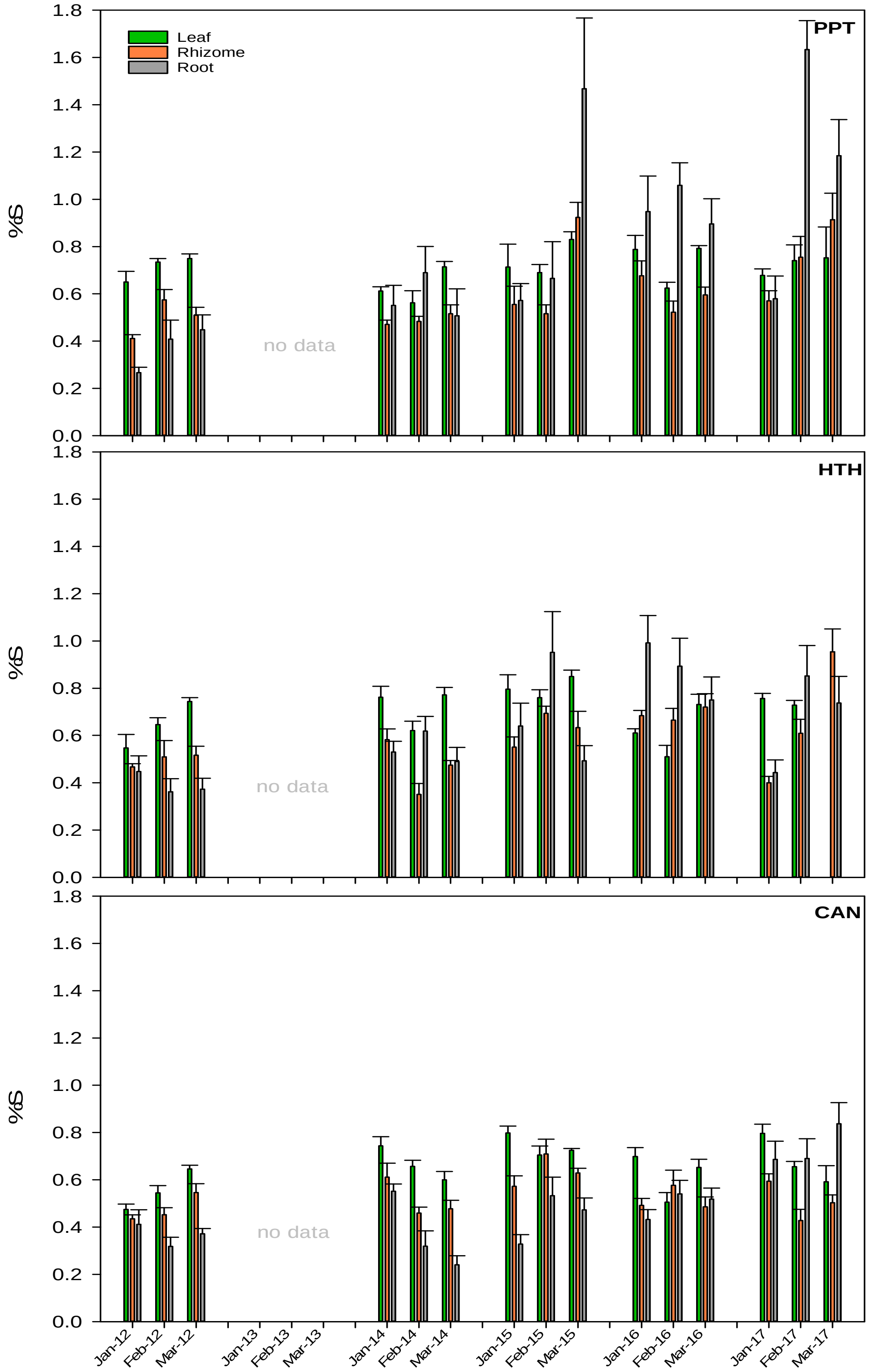


Figure A 28 *Percentage sulfur within leaves, rhizomes and roots of seagrass from January to March in each sampling year for sites PPT, HTH and CAN (mean + st. err, n=5).*

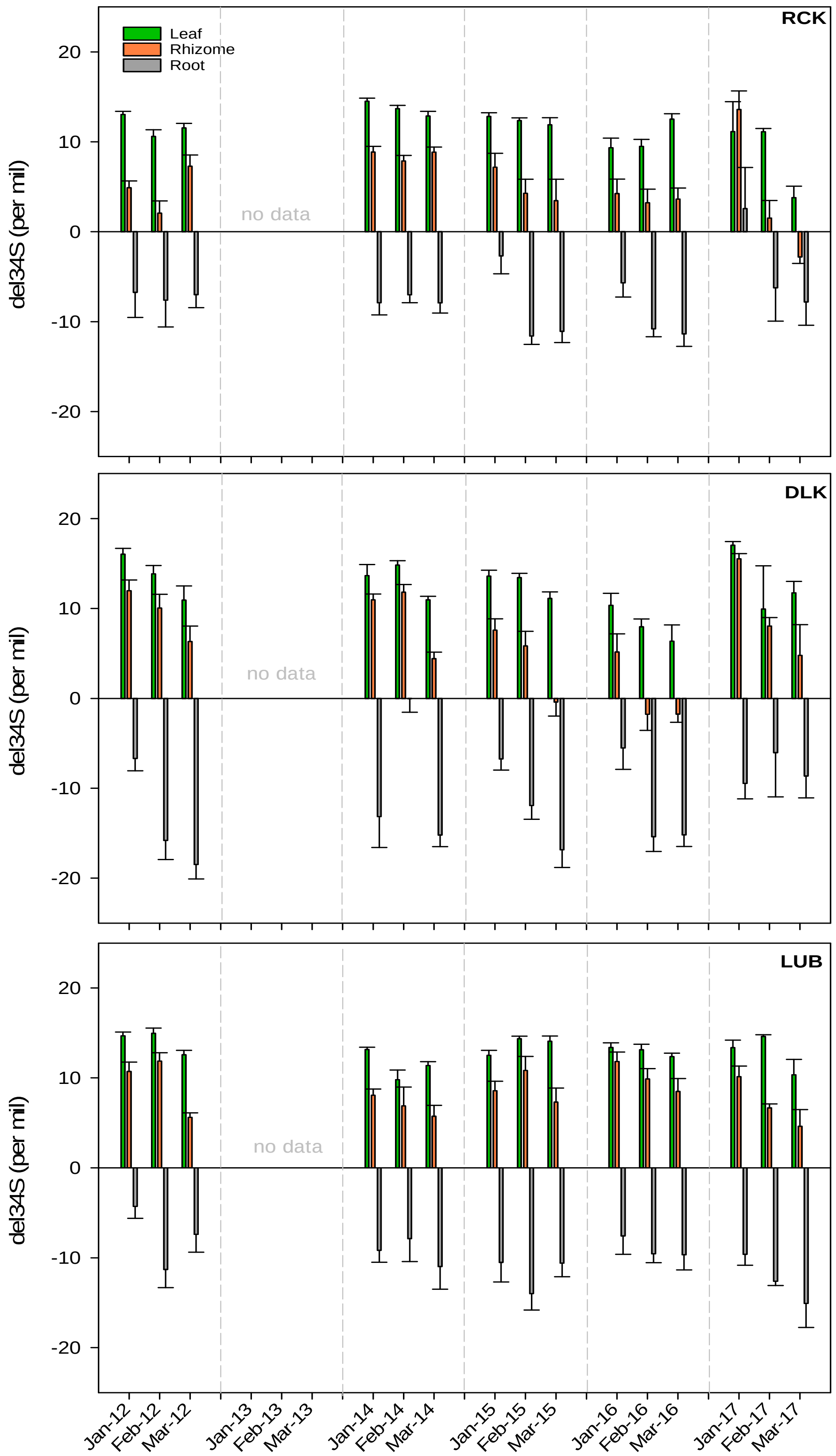


__Figure A 29__ Sulfur stable isotope ratio in leaves, rhizomes and roots for RCK, DLK and LUB across the validation period (mean + st. err, n=5).

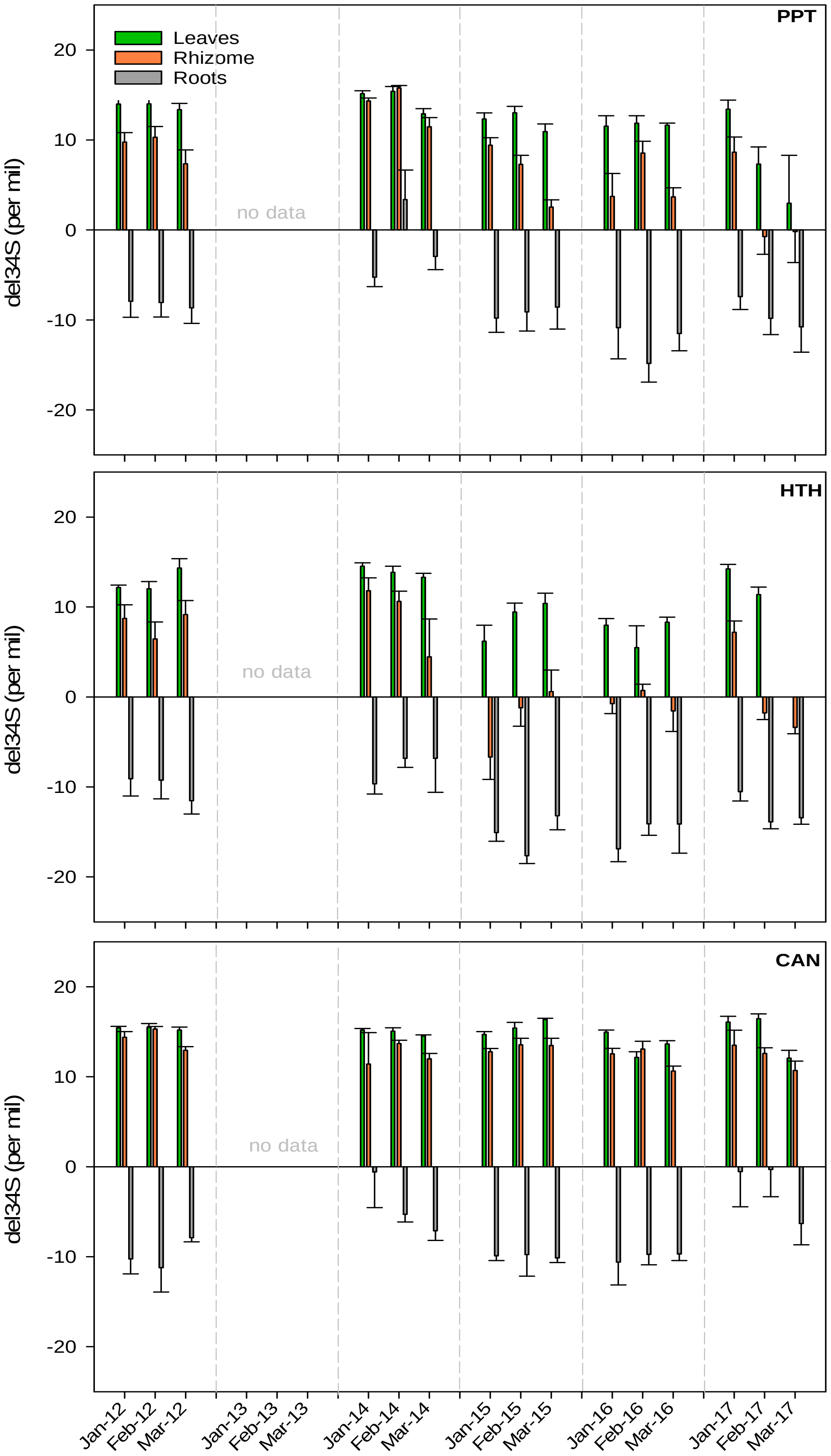
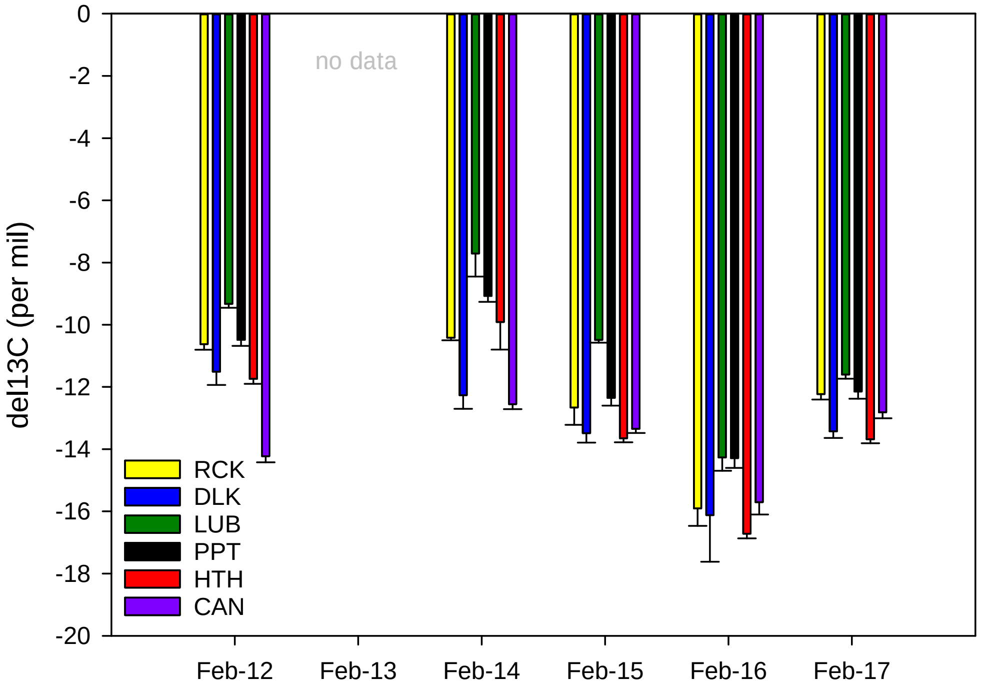


Figure A 30 *Sulfur stable isotope ratio in leaves, rhizomes and roots for PPT, HTH and CAN across the validation period (mean + st. err, n=5).*



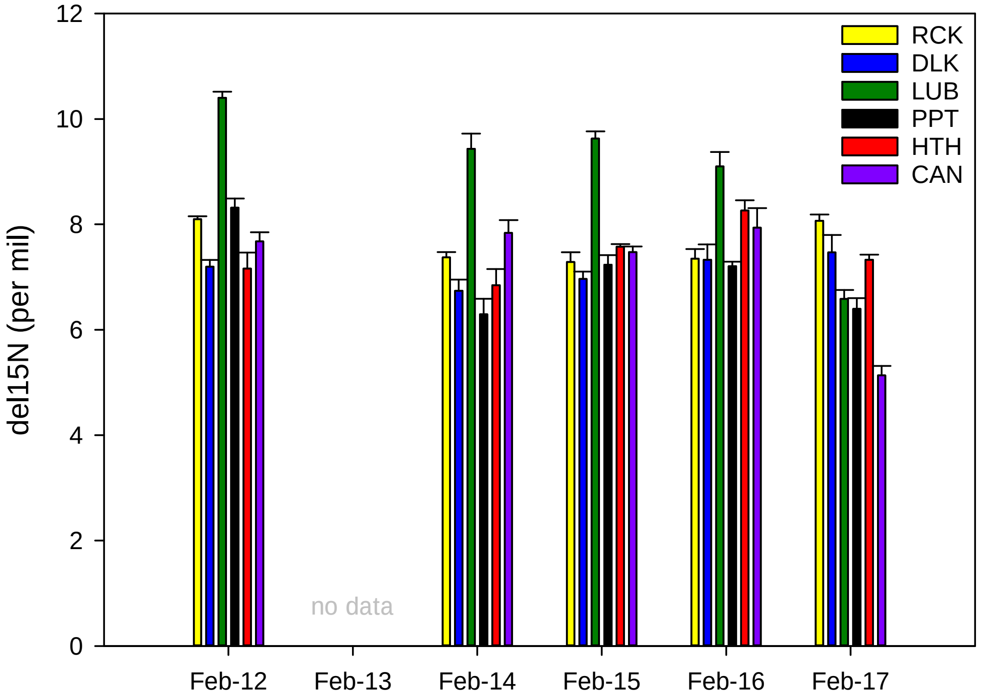


Figure A 31 *Carbon and nitrogen stable isotope ratios within leaf tissues for all sites across the validation period (mean + st. err., n=5).*

Appendix E — Statistics of meadow-scale change between years

Table A 2 *The ratio of seagrass presence (P) relative to total number of observations Wilcoxon(N^{obs}) for each site for 2012 and 2013, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass presence between 2012 and 2013.*

	2012 P/N^{obs}	2013 P/N^{obs}	Difference (2012-2013)	N	T	Z	*P* value
PPT	0.647	0.796	+0.149	63	284.500	4.953	$p<0.05$
DLK	0.726	0.817	+0.091	21	76.500	1.356	*0.175*
RCK	0.752	0.893	+0.141	35	47.000	4.390	$p<0.05$
LUB	0.747	0.937	+0.19	67	77.000	6.634	$p<0.05$
HTH	0.694	0.802	+0.108	50	113.500	5.058	$p<0.05$
CAN	0.135	0.221	+0.086	36	179.500	2.412	$p<0.05$

Table A 3 *The ratio of seagrass presence (P) relative to total number of observations Wilcoxon(N^{obs}) for each site for 2013 and 2014, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass presence between 2013 and 2014*

	2013 P/N^{obs}	2014 P/N^{obs}	Difference (2013-2014)	N	T	Z	*P* value
PPT	0.796	0.672	-0.124	66	480.000	3.996	$p<0.05$
DLK	0.817	0.629	-0.188	27	71.500	2.823	$p<0.05$
RCK	0.893	0.930	+0.037	18	19.000	2.896	$p<0.05$
LUB	0.937	0.852	-0.085	44	158.500	3.927	$p<0.05$
HTH	0.802	0.729	-0.073	50	311.000	3.152	$p<0.05$
CAN	0.221	0.282	+0.061	42	206.500	3.063	$p<0.05$

Table A 4 *The ratio of seagrass presence (P) relative to total number of observations Wilcoxon(N^{obs}) for each site for 2014 and 2015, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass presence between 2014 and 2015*

	2014 P/N ^{obs}	**2015 P/N ^{obs}**	**Difference (2014-2015)**	**N**	**T**	**Z**	***P* value**
PPT	0.672	0.622	-0.05	75	1246.500	0.943	0.346
DLK	0.629	0.685	+0.56	39	346.000	0.614	*0.539*
RCK	0.930	0.856	-0.074	14	42.500	0.628	0.530
LUB	0.852	0.781	-0.071	65	649.000	2.764	*$p<0.05$*
HTH	0.729	0.810	+0.081	50	308.500	3.176	*$p<0.05$*
CAN	0.282	0.218	-0.064	51	393.500	2.526	*$p<0.05$*

Table A 5 *The ratio of seagrass presence (P) relative to total number of observations Wilcoxon(N^{obs}) for each site for 2015 and 2016, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass presence between 2015 and 2016.*

	2015 P/N ^{obs}	**2016 P/N ^{obs}**	**Difference (2015-2016)**	**N**	**T**	**Z**	***P* value**
PPT	0.622	0.645	+0.023	62	844.000	0.929	0.353
DLK	0.685	0.738	+0.053	44	277.000	2.544	*$p<0.05$*
RCK	0.856	0.891	+0.035	26	161.500	0.356	0.722
LUB	0.781	0.846	+0.065	74	830.500	3.000	*$p<0.05$*
HTH	0.810	0.831	+0.021	39	323.500	0.928	0.353
CAN	0.218	0.406	+0.188	57	108.500	5.705	*$p<0.05$*

Table A 6 *The ratio of seagrass presence (P) relative to total number of observations Wilcoxon(N^{obs}) for each site for 2015 and 2016, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass presence between 2016 and 2017.*

	2016 P/N^{obs}	**2017 P/N^{obs}**	**Difference (2016-2017)**	**N**	**T**	**Z**	***P* value**
PPT	0.645	0.599	-0.046	81	1417.500	1.144	0.253
DLK	0.738	0.536	-0.202	64	351.500	4.604	$p<0.05$
RCK	0.891	0.933	+0.042	23	64.500	2.235	$p<0.05$
LUB	0.846	0.838	-0.008	45	486.000	0.356	*0.722*
HTH	0.831	0.899	+0.068	36	105.000	3.582	$p<0.05$
CAN	0.406	0.453	+0.047	61	787.000	1.138	*0.255*

Table A 7 *The average percentage cover of seagrass at each site for 2012 and 2013, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass cover between 2012 and 2013.*

	2012 (%)	**2013 (%)**	**Difference**	**N**	**T**	**Z**	***P* value**
PPT	37.5	55.2	+ 17.7	92	644.500	5.819	$p<0.05$
DLK	27.1	41.2	+ 14.1	69	369.000	5.013	$p<0.05$
RCK	26.5	38.8	+ 12.3	65	336.000	4.813	$p<0.05$
LUB	28.2	68.4	+ 40.2	100	86.500	8.384	$p<0.05$
HTH	23.9	64.8	+ 40.9	95	25.000	8.370	$p<0.05$
CAN	1.4	9.4	+ 8.0	39	59.000	4.619	$p<0.05$

Table A 8 *The average percentage cover of seagrass at each site for 2013 and 2014, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance ($p<0.05$ is significant) comparing seagrass cover between 2013 and 2014.*

	2013 (%)	**2014 (%)**	**Difference**	**N**	**T**	**Z**	***P* value**
PPT	55.2	39.1	-16.1	94	676.500	5.868	$p<0.05$
DLK	41.2	36.7	-4.5	69	1100.000	0.643	0.520
RCK	38.8	57.0	+18.2	67	178.000	6.003	$p<0.05$
LUB	68.4	48.0	-20.4	99	491.000	6.925	$p<0.05$
HTH	64.8	37.0	-27.8	99	201.500	7.935	$p<0.05$
CAN	9.4	13.6	+4.2	49	308.000	3.029	$p<0.05$

Table A 9 *The average percentage cover of seagrass at each site for 2014 and 2015, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance (p<0.05 is significant) comparing seagrass cover between 2014 and 2015.*

	2014 (%)	**2015 (%)**	**Difference**	**N**	**T**	**Z**	***P* value**
PPT	39.1	43.3	+4.2	96	1759.000	2.079	*p<0.05*
DLK	36.7	35.3	-1.4	73	1288.500	0.341	0.733
RCK	57.0	44.2	-12.8	72	865.000	2.520	*p<0.05*
LUB	48.0	30.1	-17.9	97	575.500	6.480	*p<0.05*
HTH	37.0	47.9	+10.9	96	1098.000	4.495	*p<0.05*
CAN	13.6	3.2	-10.4	58	156.000	5.416	*p<0.05*

Table A 10 *The average percentage cover of seagrass at each site for 2015 and 2016, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance (p<0.05 is significant) comparing seagrass cover between 2015 and 2016.*

	2015 (%)	**2016 (%)**	**Difference**	**N**	**T**	**Z**	***P* value**
PPT	43.3	30.4	-12.9	92	994	4.46	*p<0.05*
DLK	35.3	44.3	+9.0	77	505	5.06	*p<0.05*
RCK	44.2	56.6	+12.4	81	867	3.74	*p<0.05*
LUB	30.1	45.1	+15.0	98	1014	5.00	*p<0.05*
HTH	47.9	56.4	+8.5	97	1184	4.29	*p<0.05*
CAN	3.2	21.8	+18.6	62	48.5	6.51	*p<0.05*

Table A 11 *The average percentage cover of seagrass at each site for 2016 and 2017, with Wilcoxon matched pair t-test results (N, T, Z) and p-value of significance (p<0.05 is significant) comparing seagrass cover between 2016 and 2017.*

	2016 (%)	**2017 (%)**	**Difference**	**N**	**T**	**Z**	***P* value**
PPT	30.4	30.5	+0.1	94	2096.500	0.513	*0.608*
DLK	44.3	22.7	-21.6	83	499.500	5.646	*p<0.05*
RCK	56.6	58.8	+2.2	80	1404.000	1.036	0.300
LUB	45.1	33.3	-11.8	98	877.000	5.487	*p<0.05*
HTH	56.4	54.1	-2.3	96	1953.000	1.370	*0.171*
CAN	21.8	14.9	-6.9	74	775.00	3.300	*p<0.05*

Appendix F — Indicator scores

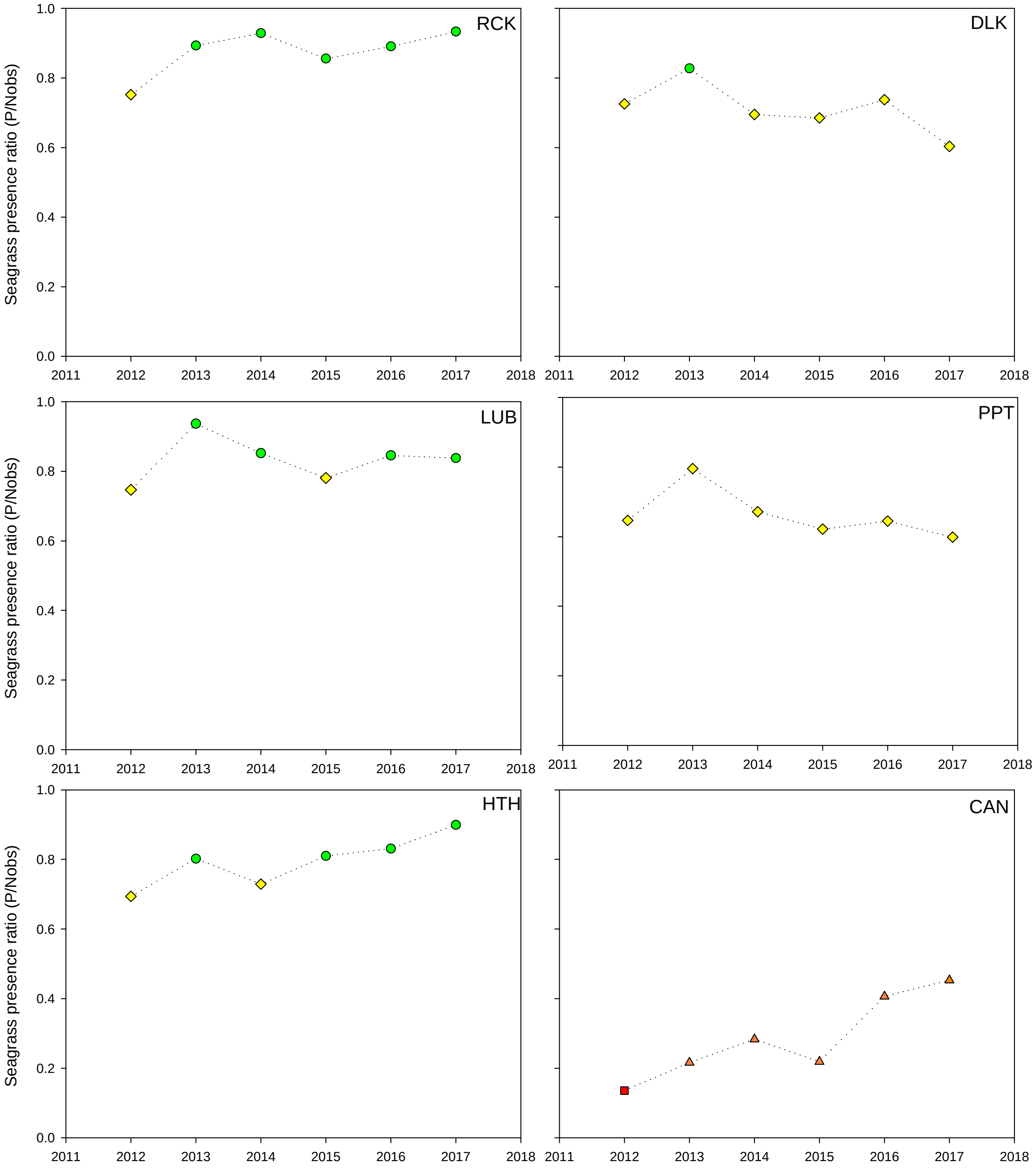


Figure A 32 *Seagrass presence indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3).*

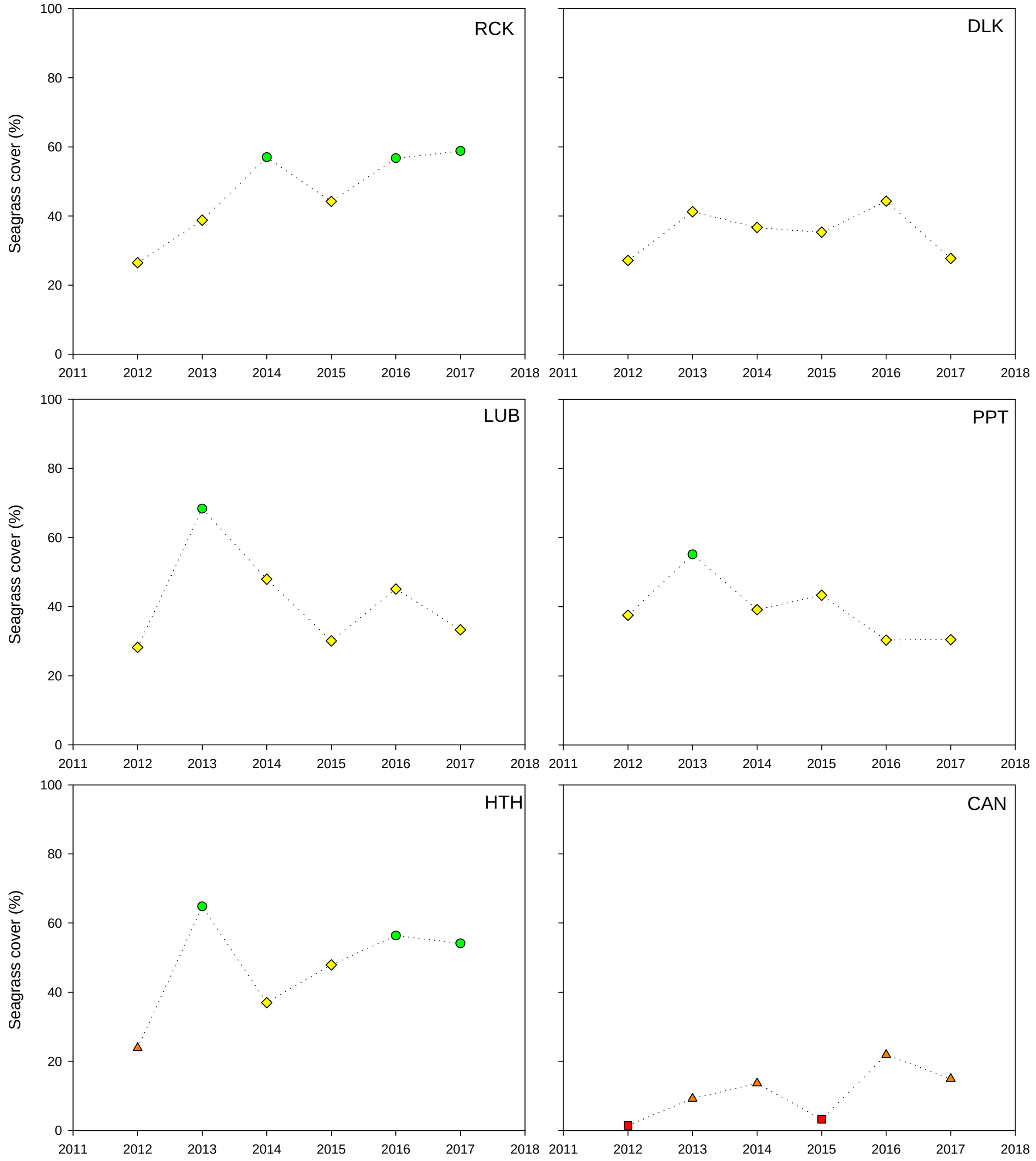


Figure A 33 *Seagrass cover indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3).*

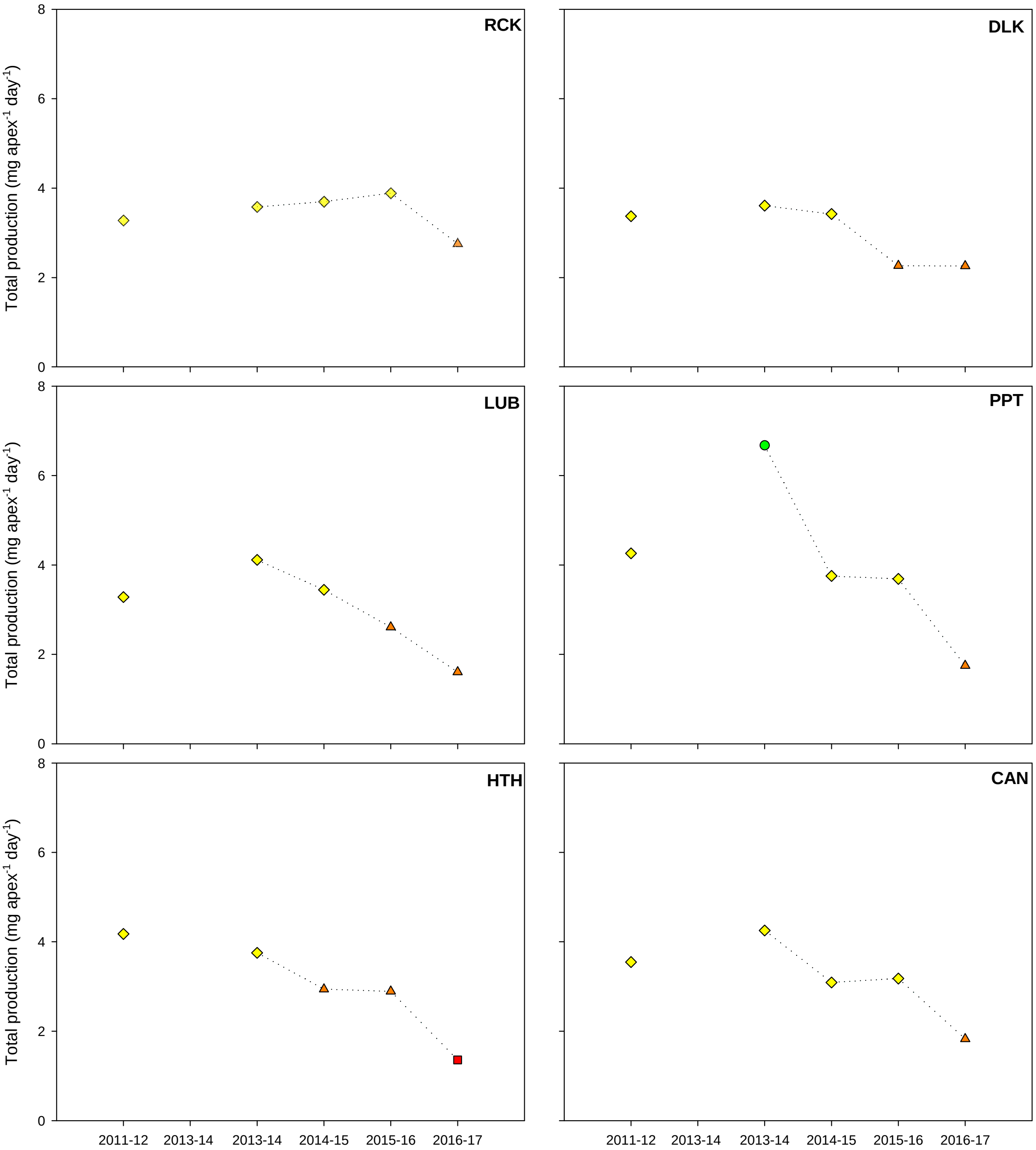


Figure A 34 *Seagrass productivity indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3).*

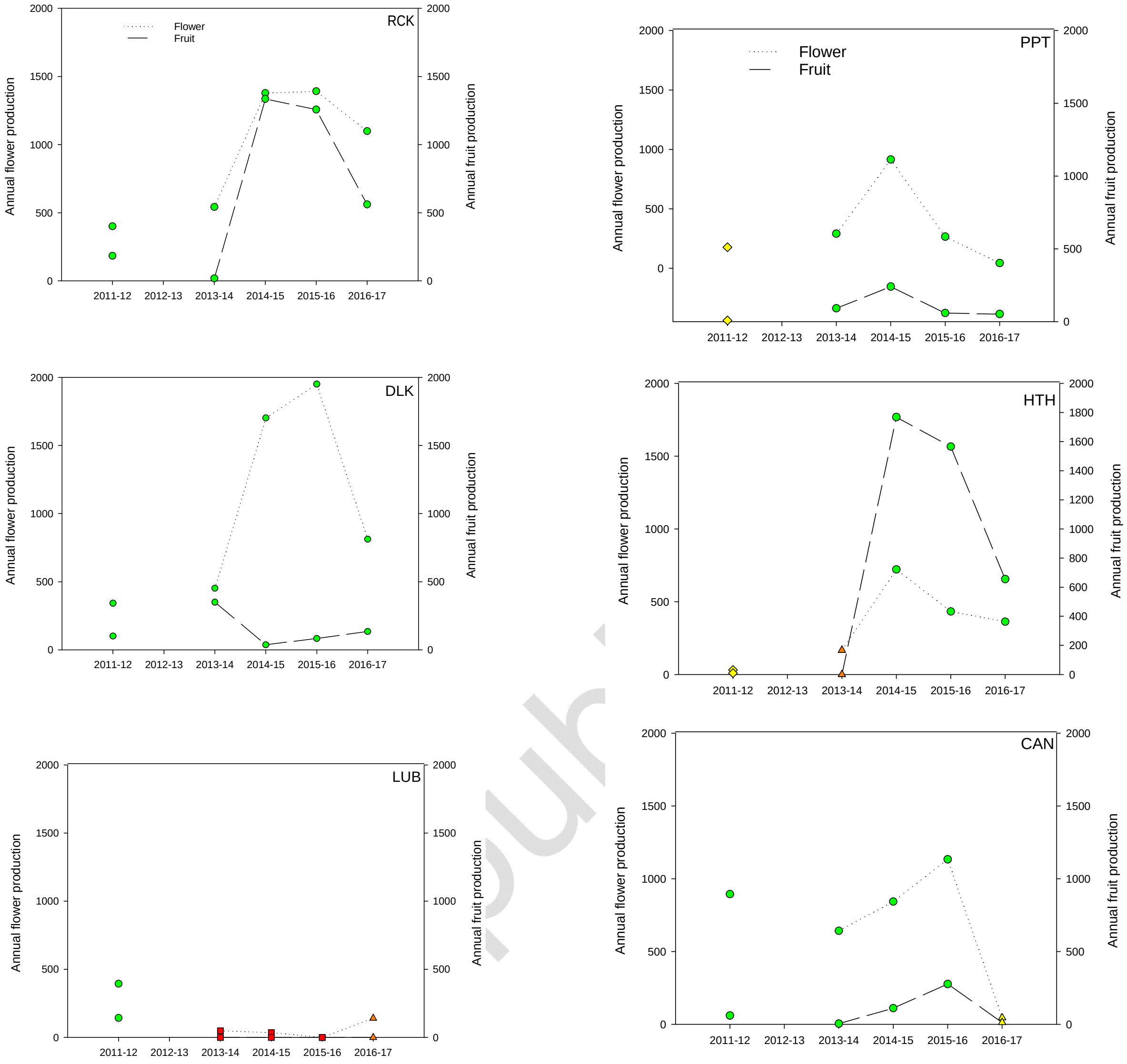


Figure A 35 *Seagrass reproduction indicator is a combination of flowering and fruiting density – shown for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3.*

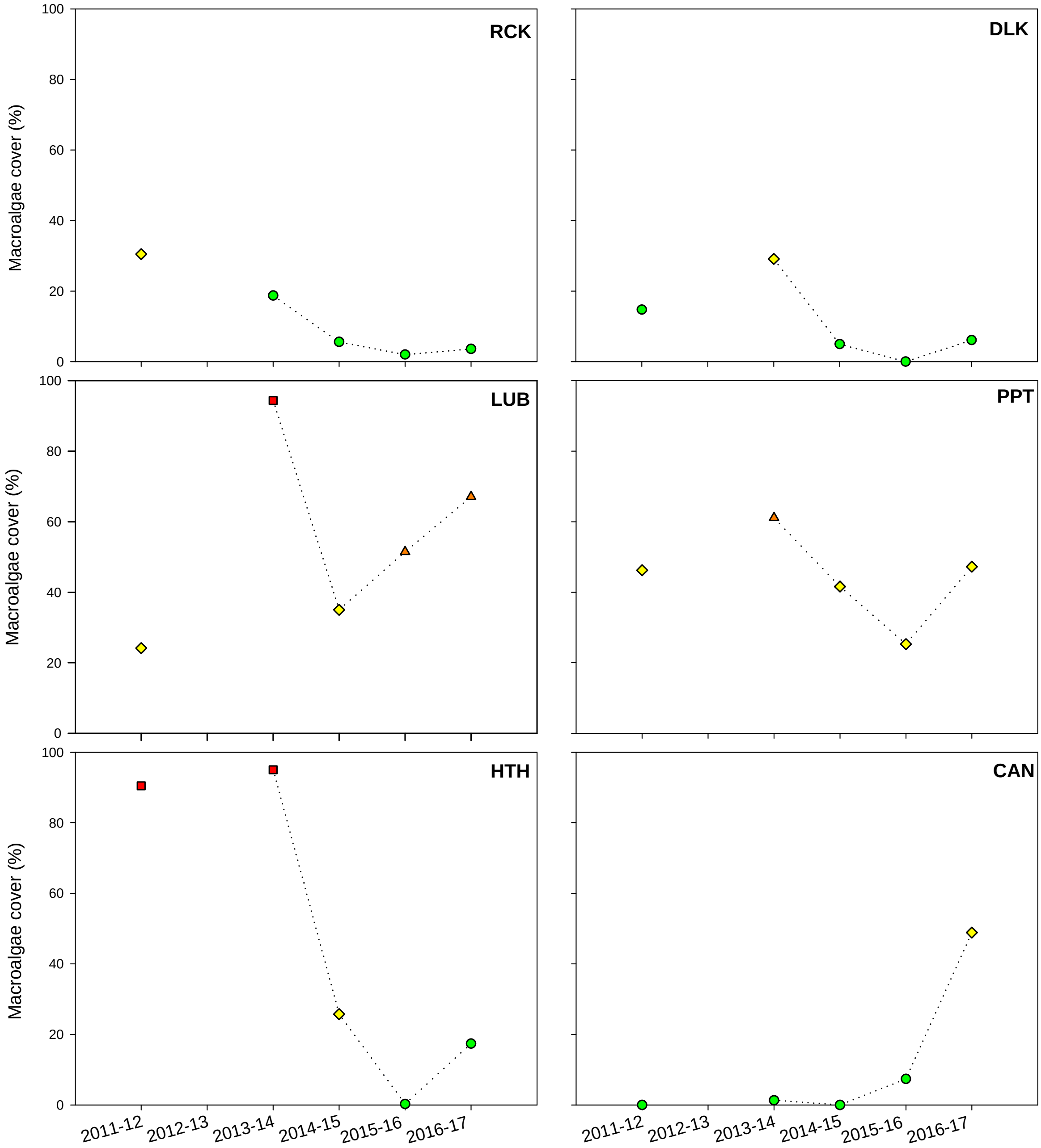


Figure A 36 *Macroalgal indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3.*

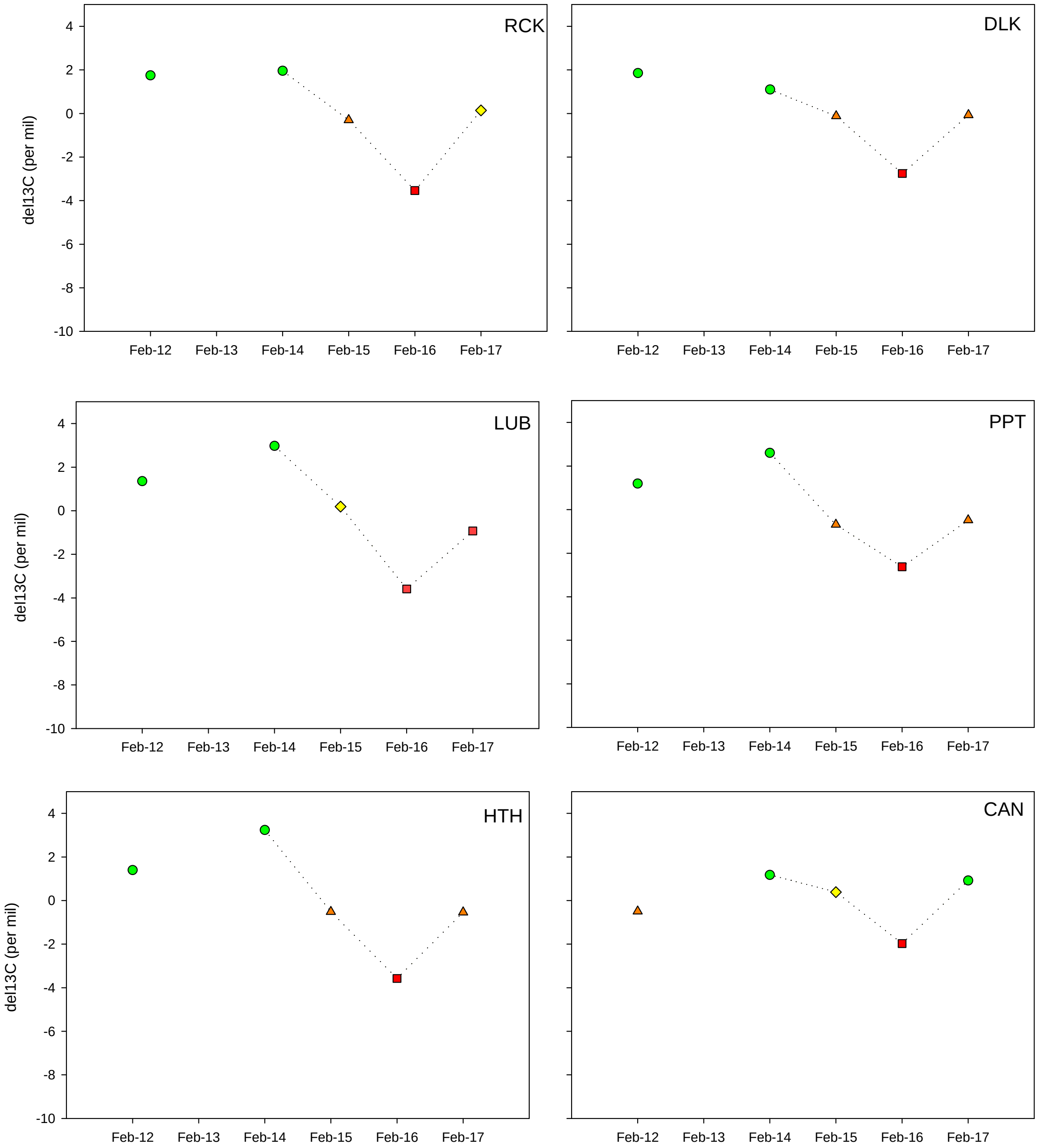


Figure A 37 *Stable carbon isotope indicator (deviation from long-term average at each site) for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3).*

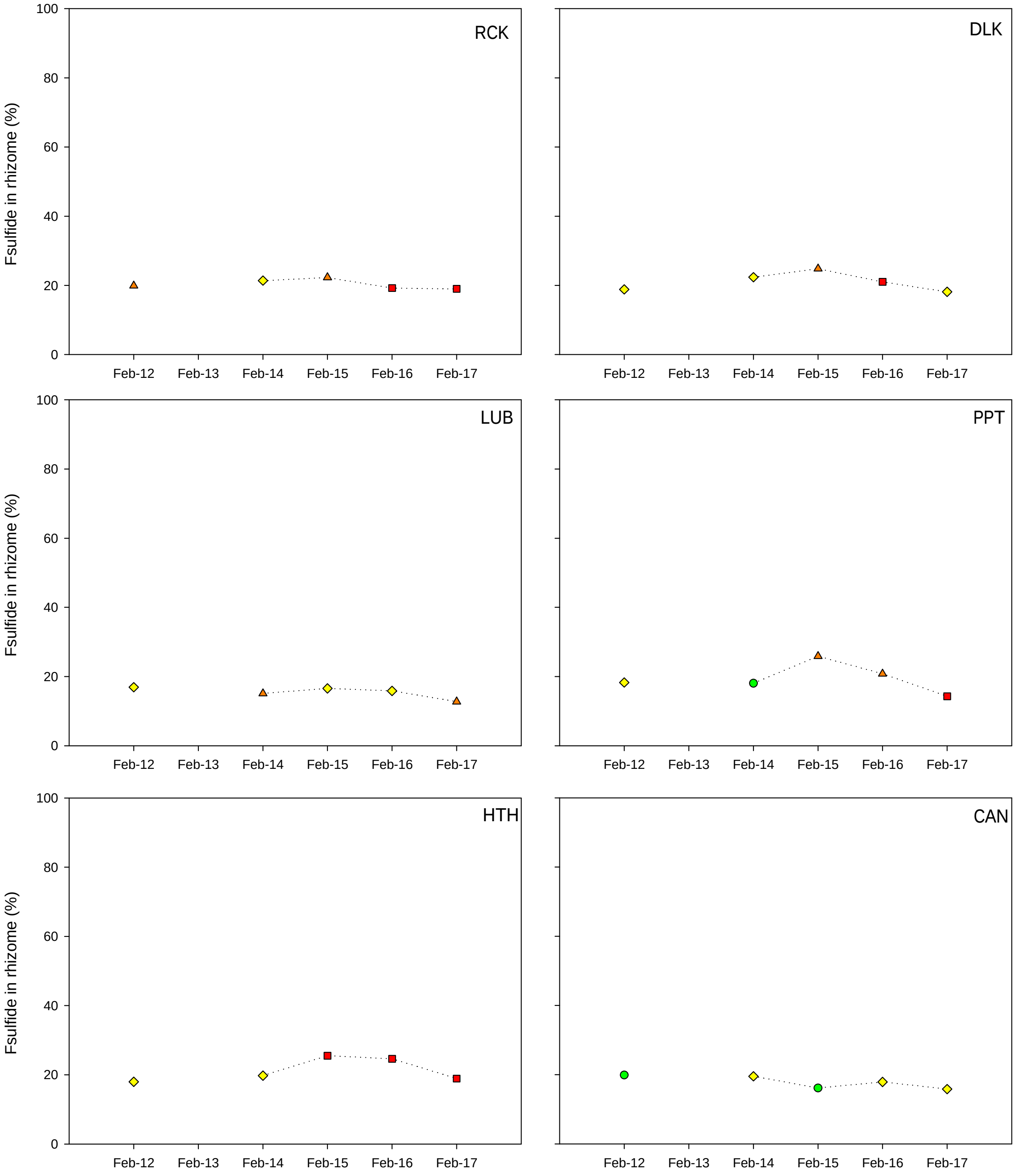


Figure A 38 *Fsulfide (sediment-stress) indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3*

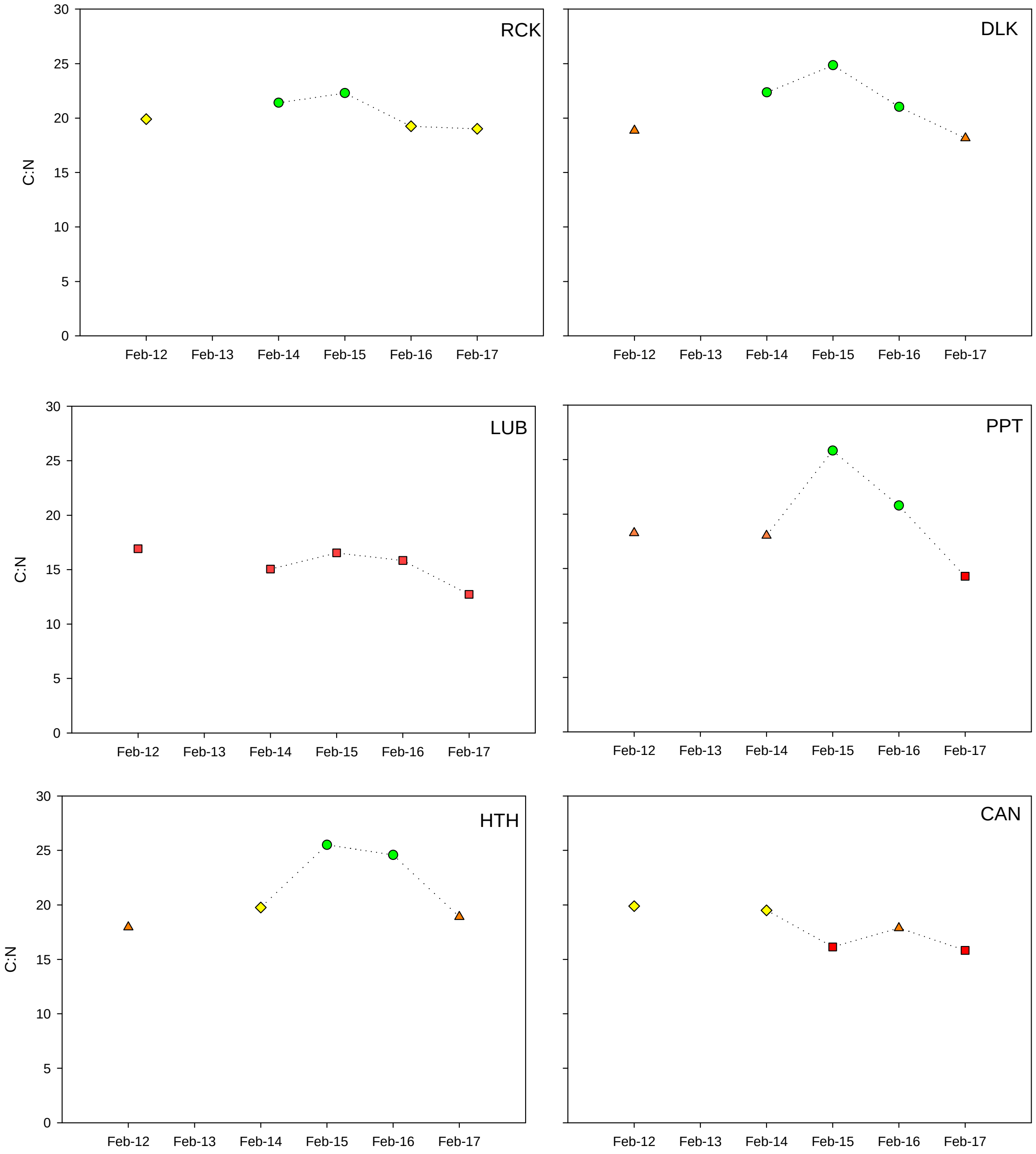


Figure A 39 *Carbon to nitrogen atomic ratio indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3.*

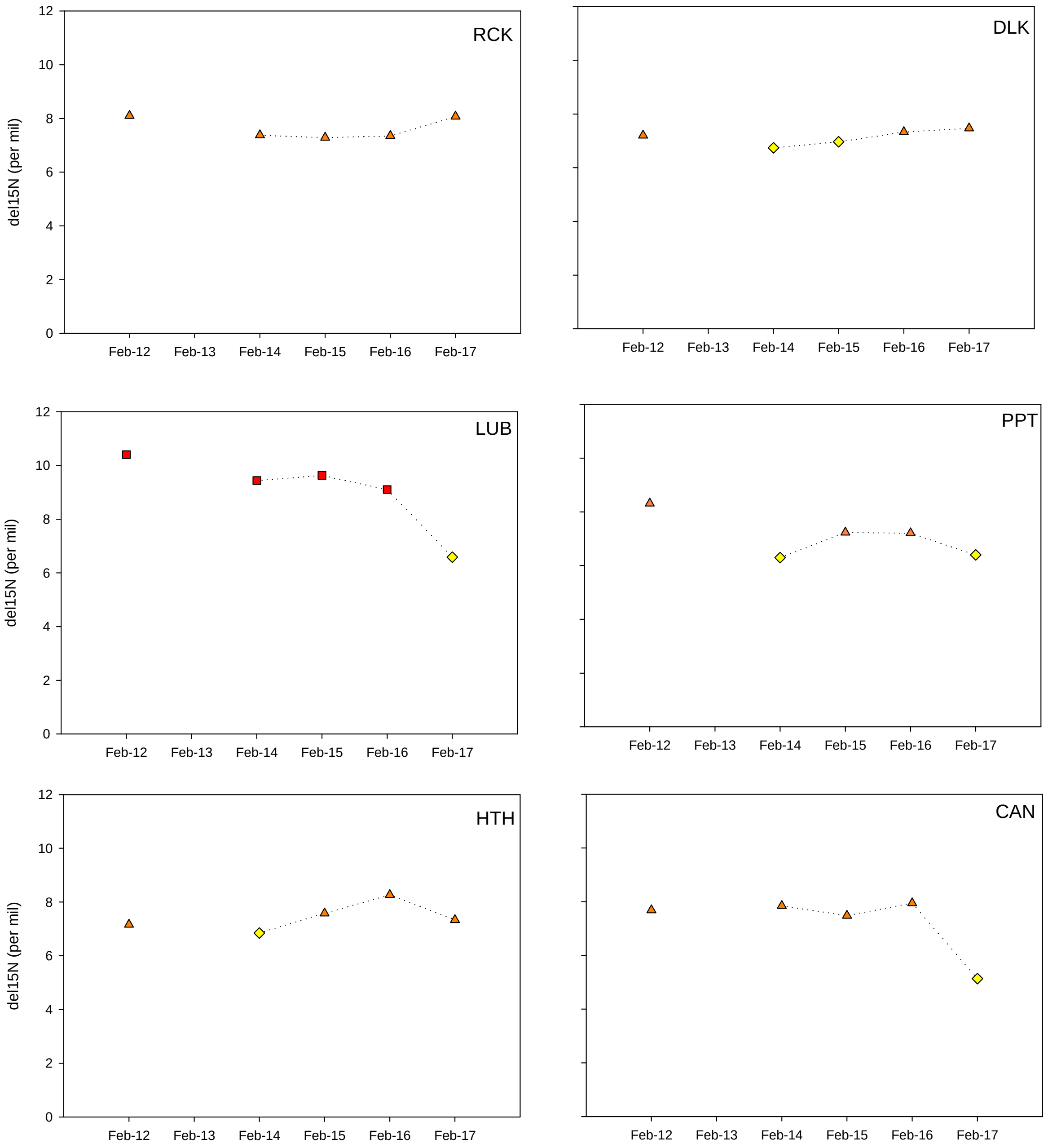


Figure A 40 *Nitrogen stable isotope ratio indicator for six sites in the Swan-Canning estuary across the validation period, where red=poor, orange=low, yellow=fair and green=good condition (as scored by categories in Table 3*

Appendix G — Evaluation and revision of indicators

Table A 12 *Revised seagrass cover indicator scores using adjusted classification cut-offs where <5% poor, 5-20% low, 20-40% fair and >40% good.*

Seagrass %cover – new cutoffs proposed						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	26.5	27.1	28.2	37.5	23.8	1.4
2012-13	38.8	41.2	68.4	55.2	64.8	9.2
2013-14	57.0	36.7	48.0	39.1	37.0	13.6
2014-15	44.2	35.3	30.1	43.3	47.9	3.2
2015-16	56.7	44.3	45.1	30.3	56.4	21.8
2016-17	58.8	27.7	33.3	30.5	54.1	14.9

Table A 13 *Comparison of scoring seagrass presence using 6 transects instead of 10*[12]*.*

Seagrass presence (P/N° observation ratio)						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	0.75	0.73	0.75	0.65	0.69	0.14
2012-13	0.89	0.83	0.94	0.80	0.80	0.22
2013-14	0.93	0.70	0.85	0.67	0.73	0.28
2014-15	0.86	0.69	0.78	0.62	0.81	0.22
2015-16	0.89	0.74	0.85	0.65	0.83	0.41
2016-17	0.93	0.60	0.84	0.60	0.90	0.45
Seagrass presence (P/N° observation ratio) – six transects only						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	0.84	0.79	0.74	0.74	0.73	0.12
2012-13	0.95	0.92	0.95	0.93	0.85	0.21
2013-14	0.95	0.77	0.86	0.78	0.76	0.28
2014-15	0.89	0.79	0.83	0.67	0.82	0.23
2015-16	0.87	0.83	0.90	0.74	0.83	0.43
2016-17	0.91	0.72	0.92	0.63	0.93	0.49

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[12] Six transects chosen to score were transect C-H of each site, as generally this encompasses the site location where plant-scale measurements are taken

Table A 14 *Comparison of scoring seagrass cover using 6 transects instead of 10*

Seagrass %cover – new cutoffs proposed

	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	26.5	27.1	28.2	37.5	23.8	1.4
2012-13	38.8	41.2	68.4	55.2	64.8	9.2
2013-14	57.0	36.7	48.0	39.1	37.0	13.6
2014-15	44.2	35.3	30.1	43.3	47.9	3.2
2015-16	56.7	44.3	45.1	30.3	56.4	21.8
2016-17	58.8	27.7	33.3	30.5	54.1	14.9

Seagrass %cover- using new cut offs

	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	34	32	29	38	27	1
2012-13	46	50	72	66	67	8
2013-14	61	43	50	47	38	13
2014-15	47	42	34	45	50	4
2015-16	56	52	50	36	57	22
2016-17	57	36	39	32	55	17

Table A 15 *Comparison of scoring seagrass reproduction by annual production of flowers (Dec-Mar) and fruit (Jan-Mar) with data from Jan-Mar only*

Seagrass reproduction (annual flowers | fruits m^{-2})

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	400 | 184	342 | 101	394 | 143	176 | 9	31 | 9	893 | 60
2012-2013						
2013-2014	542 | 18	452 | 350	48 | 0	290 | 92	166 | 0	642 | /5
2014-2015	1378 | 1335	1703 | 37	35 | 0	915 | 241	722 | 1768	843 | 111
2015-2016	1392 | 1257	1951 | 83	0 | 0	266 | 60	432 | 1566	1133 | 276
2016-2017	1099 | 560	811 | 135	144 | 0	44 |53	362 | 655	45 | 13

Seagrass reproduction (Jan to March flowers | fruits m^{-2})

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	294 | 184	230 | 92	55 |142	133 | 9	37 | 9	893 | 60
2012-2013						
2013-2014	640 | 18	529 | 350	64 | 0	386 | 92	221 | 0	851 |5
2014-2015	782 | 1335	1515 | 37	23 | 0	1025 |274	912 | 1676	1076 |117
2015-2016	787 | 1257	1515 | 83	0 | 0	101 | 59	332 | 1566	1105 | 276
2016-2017	1521 |485	861 | 128	5 |0	27 | 34	479 | 547	58 |15

Table A 16 *Trial scoring of the seagrass reproduction indicator with either single month of data (Jan and Feb) or two months of data (Jan and Feb)*

Seagrass reproduction (JAN flowers | fruits m^{-2})

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	580 | 179	234 | 234	151 | 428	193 | 14	0 |0	884 |27
2012-2013						
2013-2014	1188 | 0	676| 0	124 | 0	359 | 0	0 |0	828| 0
2014-2015	1920 | 3909	3412 | 14	69 | 0	1561 | 635	1340 | 1201	2362 | 165
2015-2016	2196 | 2265	3440 | 14	0 | 0	193 | 138	759 | 1920	1395 | 221
2016-2017	2804 | 234	1616| 28	14 | 0	82 |0	925 | 552	138 | 27

Seagrass reproduction (FEB flowers | fruits m^{-2})

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	276 | 303	221 | 27	13 |0| 0	82 |0	110 |0	1602 | 151
2012-2013						
2013-2014	428 | 55	386 | 0	55| 0	221 |0	179 | 0	1119 | 0
2014-2015	428 | 27	1132| 0	0 |0	1505 | 151	1146 | 2818	773 |147
2015-2016	96 | 1409	1091 |234	0 |0	69 |27	138 |1975	1257 | 386
2016-2017	1519 | 207	904 | 90	0| 0	0| 9	483 | 773	36|9

Seagrass reproduction (JAN and FEB flowers | fruits m^{-2})

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	428 | 242	228 | 131	83 | 214	138 | 7	55 |0	1243 | 90
2012-2013						
2013-2014	808 | 28	532 | 0	90 | 0	290 | 0	90 | 0	974 |0
2014-2015	1174 | 1969	2272 | 7	35 | 0	1533 | 393	1243 |2010	1568 | 157
2015-2016	1147 | 1837	2265 | 124	0 | 0	131 | 83	449 | 1947	1326 |304
2016-2017	2162 | 221	1261 |59	7 | 0	41 | 5	705 | 663	87 | 18

Table A 17 *Trial scoring of the seagrass productivity with different combinations of data*

Seagrass productivity (ANNUAL JAN-MARCH)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	3.3	3.4	3.3	4.3	4.2	3.5
2012-2013						
2013-2014	3.6	3.6	4.1	6.7	3.8	4.3
2014-2015	3.7	3.4	3.4	3.8	2.9	3.1
2015-2016	3.9	2.3	2.6	3.7	2.9	3.2
2016-2017	2.8	2.3	1.6	1.7	1.4	1.8

Seagrass productivity (JAN ONLY)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	3.2	2.5	3.5	5.7	6.1	
2012-2013						
2013-2014	4.5	3.8	4.4	7.4	4.8	4.5
2014-2015	5.5	4.1	4.2	6.8	3.9	4.8
2015-2016	4.5	3.7	3.2	4.5	4.4	3.8
2016-2017	3.7	3.0	1.9	2.5	1.5	1.4

Seagrass productivity (FEB ONLY)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	2.9	4.9	3.8	3.9	3.4	3.2
2012-2013						
2013-2014	3.6	3.3	4.1	7.9	3.6	5.1
2014-2015	2.9	2.8	2.8	3.4	3.1	2.2
2015-2016	4.3	1.7	2.9	3.9	1.9	2.3
2016-2017	2.1	1.4	1.8	1.5	1.4	2.1

Seagrass productivity (MARCH ONLY)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	3.6	2.7	2.5	3.2	3.0	3.9
2012-2013						
2013-2014	2.7	3.6	3.8	4.7	2.8	3.2
2014-2015	2.7	3.3	3.3	1.1	1.8	2.3
2015-2016	2.9	1.4	1.7	2.7	2.4	3.4
2016-2017	2.4	2.4	1.0	1.3	1.1	1.9

Seagrass productivity (JAN AND FEB)

	RCK	DLK	LUB	PPT	HTH	CAN
2011-2012	3.1	3.7	3.7	4.8	4.7	3.2
2012-2013						
2013-2014	4.0	3.6	4.3	7.7	4.2	4.8
2014-2015	4.2	3.5	3.5	5.1	3.5	3.5
2015-2016	4.4	2.7	3.0	4.2	3.1	3.1
2016-2017	2.9	2.2	1.9	2.0	1.5	1.8

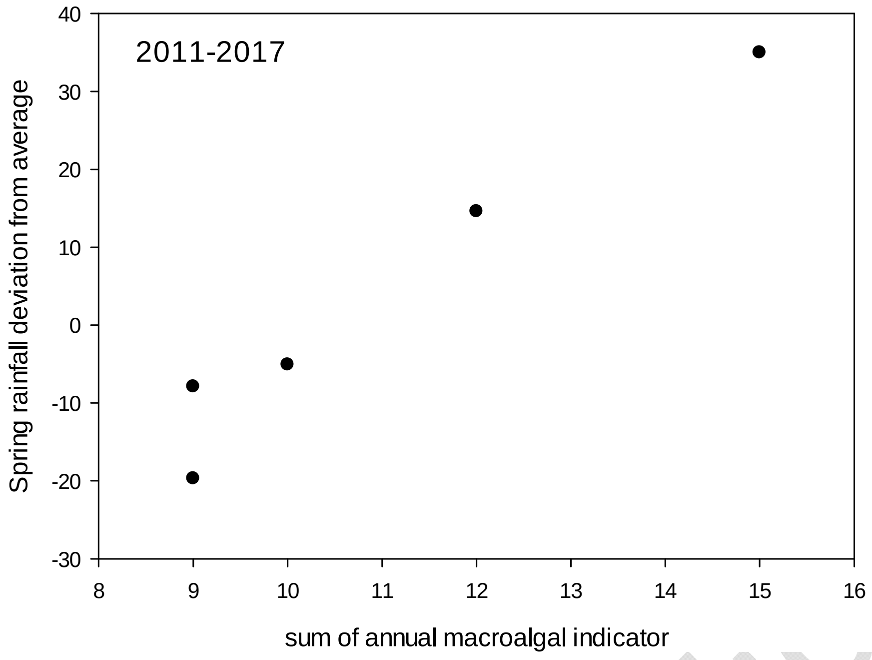

Figure A 41 *Evaluation of relationship of classification cut-offs for macroalgal indicator where spring rainfall from Table 4 is plotted against the annual sum of the indicator score for all sites pooled given in Table A 19 (where green is scored 1, yellow is scored 2, orange is scored 3, and red is scored 4).*

Table A 18 *Original site-specific value d13C indicator values*

	Indicators(using original Specific-site, calculated as the mean) (Jan to April2012 and Feb2014) (this is an average of averages, if the sample # differs between months)					
Specific value (avg of avg)	**-10.2**	**-11.6**	**-8.6**	**-9.8**	**-10.8**	**-13.3**
	Difference betw. Measured value and site-specific ref)					
	RCK	DLK	LUB	PPT	HTH	CAN
2011-12	-0.42	0.09	-0.73	-0.68	-0.94	-0.93
2013-14	-0.21	-0.67	0.89	0.73	0.89	0.74
2014-15	-2.46	-1.89	-1.88	-2.56	-2.85	-0.05
2015-16	-5.70	-4.53	-5.66	-4.49	-5.92	-2.41
2016-17	-2.03	-1.83	-3.00	-2.35	-2.88	0.48

Table A 19 *Revised indicator categorization for each site and year for light-stress (del13C)*

	δ^{13}C in leaves (February)					
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-12	-10.62	-11.51	-9.33	-10.48	-11.74	-14.23
2012-13						
2013-14	-10.41	-12.27	-7.71	-9.07	-9.91	-12.56
2014-15	-12.66	-13.49	-10.48	-12.36	-13.65	-13.35
2015-16	-15.90	-16.13	-14.26	-14.29	-16.72	-15.71
2016-17	-12.23	-13.43	-11.60	-12.15	-13.68	-12.82

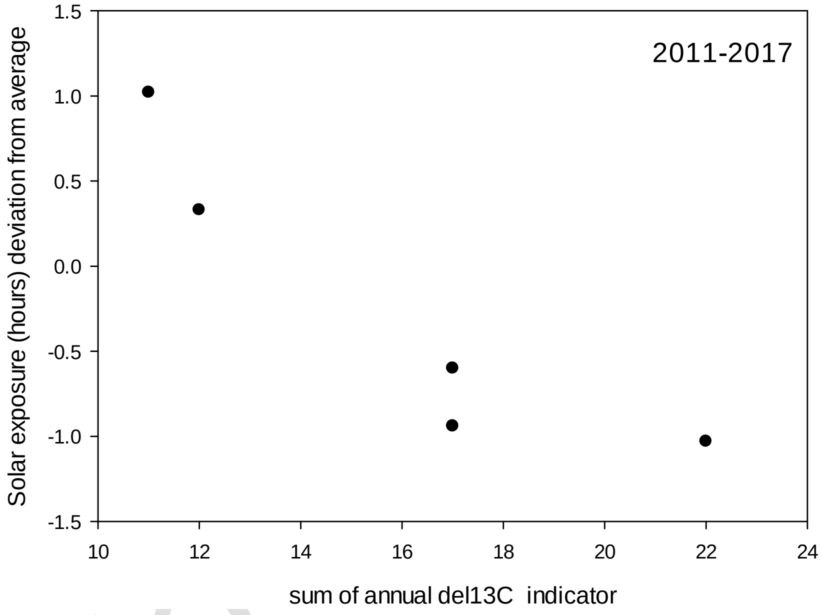

Figure A 42 *Evaluation of relationship of classification cut-offs for absolute d13C, where annual solar exposure from Table 4 is plotted against the annual sum of the indicator score for all sites pooled given in Table A 19 (where green is scored 1, yellow is scored 2, orange is scored 3, and red is scored 4).*

Table A 20 Trial scoring of Fsulfide using different subsets of data

Fsulfide in rhizome (Jan-March)						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-2012	37.04	26.98	27.08	27.65	29.85	16.83
2012-2013						
2013-2014	28.73	27.55	32.27	17.18	27.78	20.41
2014-2015	38.10	39.60	28.55	34.59	56.01	17.97
2015-2016	40.87	48.35	25.71	37.01	50.91	20.89
2016-2017	40.79	27.70	33.35	44.53	49.16	20.81
Fsulfide in rhizome (Jan-Feb)						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-2012	39.76	23.66	23.04	25.74	30.96	15.47
2012-2013						
2013-2014	29.08	22.51	31.01	14.58	22.89	20.01
2014-2015	36.26	33.85	26.62	29.89	59.69	18.21
2015-2016	40.78	45.61	23.84	35.06	49.69	19.17
2016-2017	32.35	21.99	30.27	41.16	44.19	18.89
Fsulfide in rhizome (Feb)						
	RCK	**DLK**	**LUB**	**PPT**	**HTH**	**CAN**
2011-2012	42.8	25.7	21.8	25.2	33.4	14.5
2012-2013						
2013-2014	30.1	21.6	32.3	13.04	24.2	17.55
2014-2015	39.8	36.0	23.9	32.5	53.0	17.3
2015-2016	42.0	53.9	26.2	29.3	48.0	18.5
2016-2017	47.1	31.2	34.5	52.6	55.1	20.01

Appendix H — Invasive species and other benthic invertebrates

Two invasive species are reported on within this report, *Batillaria australis* and *Didenmum perlucidum*. Observations have been made of both species during the course of the five years of sampling as they may interact with seagrass.

Didenmum perlucidum

Didenmum perlucidum (white sea squirt) is a tropical colonising ascidian which is usually found on hard substrates, such as jetties or moorings, however in the Swan estuary it has been observed growing on *Halophila ovalis.* Simpson et al. (2016) showed that this invasive ascidian enveloped the above-ground plant material, which reduced the photosynthetic performance and biomass. Simpson et al. (2016) also indicated that the presence of human infrastructure, such as boat moorings, were a strong predictor in *Didenmum perlucidum* being observed within an urban estuary.

In 2013-14 it was observed at the marine extent of the estuary at DLK and RCK, generally 40 to 50 m off shore in water depths greater than 2.5 m. In 2014-15 and 2016-17 *Didenmum perlucidum* was only observed at DLK, again over 50 m offshore and in depths typically greater than 2.5 m. In 2015-16 *Didenmum perlucidum* was observed at DLK and at PPT. Although *Didenmum perlucidum* has consistently been observed it does not appear to be having a widespread impact.

Continued long-term monitoring is recommended to assess potential further spread or harmful impacts, particularly in locations such as DLK where there several jetties and many moorings are present, as they may promote additional establishment of *Didenmum perlucidum*.

Benthic invertebrates including the invasive snail, *Batillaria australis*

Over the five years of sampling benthic fauna found within the seagrass cores were separated and identified as *Batillaria australis*, hermit crab and other (which include three gastropod species and six bivalve species). Presence of other invertebrates such as polychaetes, amphipods, isopods and crustaceans were also recorded. Abundance and distribution of species within the estuary varied monthly, seasonally and between sites due to tidal flow and river discharge, altering temperature and salinity which are key drivers in the distribution of estuarine invertebrates.

The invasive mud snail *Batillaria australis* is widely distributed in the Swan-Canning and occurs in very high numbers, orders of magnitude higher than any other native snail or gastropod. While it does not directly feed on *Halophila ovalis*, it can impact the seagrass through its burying behaviour causing bioturbation and potential uprooting. Their shells can provide a hard substrate for the attachment of nuisance algae's such as *Gracilaria spp*. to grow on. As the snails are abundant all year round, this creates additional management implications, as they can provide an attachment point for algae, which typically have high spatio-temporal variability. This may result in increased algal growth which is could interact unfavourably with seagrass habitats.

There was a general trend of increasing *Batillaria australis* numbers in years 2011-12 to 2015-16, with a slight reduction in 2016-17 (Figure A 43). *Consistent* trends show a higher abundance at the marine extent of the estuary at RCK and DLK, and the lowest abundance at CAN (Figure A 44). Since 2012 *Batillaria australis* density appears to have increased across all sites. Median snail numbers scaled by the estimated area of seagrass in the Swan-Canning estuary of 403 ha (Forbes & Kilminster 2016) show that *Batillaria australis* population in seagrass-vegetated areas are steadily increasing: ~5 billion in 2012 to just shy of 7 billion in 2016-17. The highest abundance was observed in 2015-16 with abundance exceeding 7 billion. A slight drop in density was observed after the 2017 February flooding event, however numbers appear to have increased again by March at most sites. The population at CAN has remained relatively stable and low throughout. CAN recorded high abundance of the dog whelk *Nassarius spp.* during the first few years of sampling. However, in the last two years and very noticeably in 2017, numbers have declined drastically. This seems to have coincided with an increase in the density of *Batillaria australis*.

During the five years of sampling we observed the invasive Asian Bag Mussel, *Musculista senhousia,* for the first time in February 2014 at CAN. It was first observed in the Swan-Canning in 1982 (Slack-Smith & Brealey 1987) and is native to the western Pacific coastal region. It was again seen but in higher abundance during the 2017-18 season.

Species list of invertebrates observed in the Swan-Canning from 2011-17

- Hermit crabs
- Gastropods:
    - *Nassarius* spp
    - *Bedeva* spp
    - *Mitrella* spp
    - *Batillaria australis* (non-indigenous species)
- Bivalves
    - *Tellina deltoidalis*
    - *Soletellina alba*
    - *Musculista senhousia* (non-indigenous species)
    - *Xenostrobus* spp[13]
    - *Fluviolantas subtorta*[13]
    - *Spisula spp.*
- Amphipods
- Isopods
- Brittle stars
- Crabs (Halicarcinus crabs)
- Shrimps/prawns
- Polychaetes

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[13] DWER continued monitoring at four of the monitoring sites (RCK, DLK, PPT and HTH) in 2017-18. Results are not reported directly, however we would like to make mention *Xenostrobus* and *Fluviolantas* spp, were observed during this sampling period

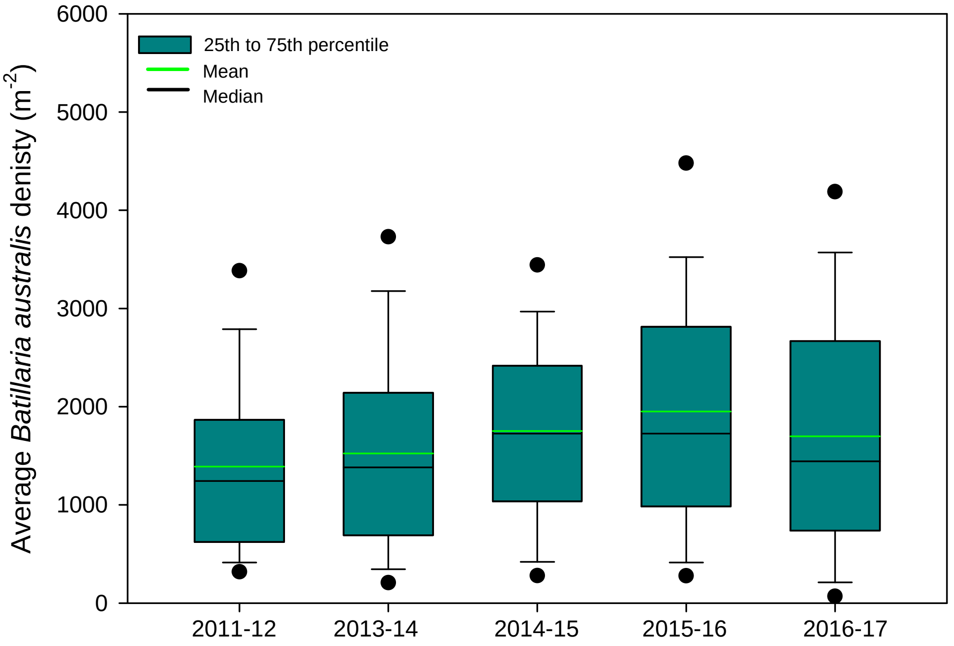

Figure A 43 *Population of Batillaria australis within seagrass meadows of the Swan-Canning, represented as boxplots of density.*

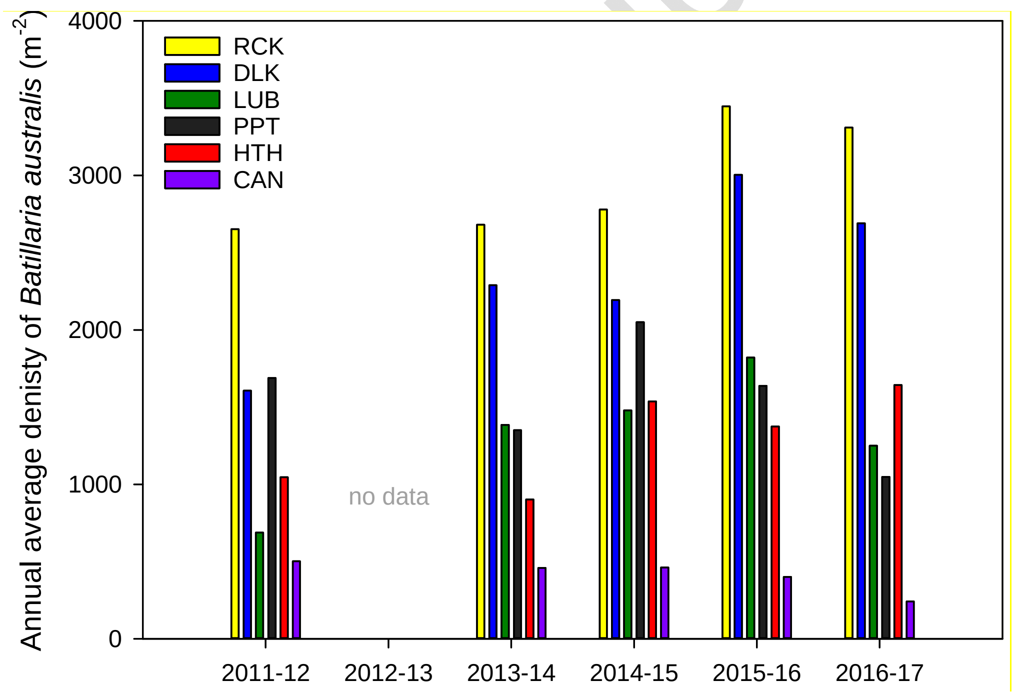

Figure A 44 *Average density of Batillaria australis at each site, for each year of study.*

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