MILESTONE PROGRESS REPORT

FRDC PROJECT NUMBER: 2018-186

1. ORIGINAL MILESTONE DATE AND DETAIL:

Milestone 3: Complete processing of field samples, analysis of biomarkers and oceanographic data
Milestone 4: First model runs completed and any additional data requirements identified. Year 1 field data analysed

2. PROGRESS AGAINST MILESTONE DETAIL:

Milestone 3: Complete processing of field samples, analysis of biomarkers and oceanographic data.

Seagrass

A total of 54 core samples were collected in May 2020, and have been processed to determine seagrass leaf morphology, biomass and density. There were no differences in any of these variables between site which will be in the predicted aquaculture plume, and those outside, as determined through generalised linear mixed modelling (Table 1). Given that aquaculture has not yet commenced, this is as expected, and indicates that the control and impact sites are comparable.

Table 1: Differences in seagrass morphology, biomass and density between aquaculture plume and control sites around Fitzgerald Bay. The treatment effect mean in all cases has a 95% confidence interval that includes zero, indicating no difference between the two groups of sites.

Treatment parameter	Control sites Mean (95% CI)	Aquaculture sites Mean (95% CI)	Treatment effect Mean (95% CI)
Leaf length (mm)	252.7 (179.7 - 346.4)	244.1 (173.8 - 334.8)	0.035 (-0.435 - 0.503)
Leaf width (mm)	7.5 (5.5 - 10.2)	8.8 (6.4 - 11.8)	0.154 (-0.282 - 0.590)
Leaf thickness (mm)	0.19 (0.15 - 0.24)	0.17 (0.13 - 0.21)	-0.137 (-0.460 - 0.186)
Leaf biomass (g)	123.1 (75.9 - 190.2)	95.0 (58.5 - 146.8)	-0.259 (-1.024 - 0.686)
Shoot biomass (g)	382.1 (236.8 - 585.8)	432.7 (262.2 - 676.8)	0.125 (-0.788 - 1.046)
Root biomass (g)	133.6 (81.2 - 211.8)	176.0 (108.4 - 279.2)	-0.277 (-0.956 - 0.391)
Epiphyte biomass (g)	19.6 (8.7 - 38.5)	16.1 (7.1 - 31.6)	-0.196 (-1.252 - 0.853)
Above-ground biomass (g)	584.8 (364.7 - 890.2)	515.8 (319.6 - 786.2)	-0.126 (-0.758 - 0.501)
Below-ground biomass (g)	485.4 (146.5 - 1202.8)	149.5 (45.6 - 373.6)	-1.178 (-2.655 - 0.318)
Leaf count	34.5 (19.3 - 57.0)	26.7 (15.0 - 44.3)	-0.255 (-1.018 - 0.511)
Shoot count	14.1 (6.6 - 26.3)	11.8 (5.6 - 22.2)	-0.176 (-1.141 - 0.802)

Biomarkers

Samples of leaf, root and epiphyte material from each individual sample were analysed for a range of biomarkers to determine if these could provide useful early indicators of impending seagrass decline. Biomarkers analysed were the percentage content of carbon, nitrogen, phosphorus and non-structural carbohydrates, along with stable isotopes of carbon nitrogen. Similar data were collected in Boston Bay under the current Aquaculture Environmental Monitoring Program undertaken by SARDI, and are included in the analyses presented here for comparison. While there were significant small-scale differences in tissue composition between samples, subsites and sites, there were no difference due to location (Port Lincoln versus Fitzgerald Bay), or between control and aquaculture sites (Table 2). There were differences between the three tissue compartments, which was expected. There did appear to be some differences in the spread of samples between control and aquaculture sites at both locations (Figure 1), which was confirmed by permutational analysis of dispersion.

Table 2: PERMANOVA results for biomarker analysis.

Source	df	SS	Pseudo-F	P(perm)
Location(Lo)	1	5477.9	0.78081	0.459
Aquaculture (Aq)	1	3517.6	0.50138	0.6015
Compartment(Co)	2	1.30E+05	39.233	**0.0001**
LoxAq	1	1762.2	0.25118	0.7957
LoxCo	2	5290.9	1.591	0.1619
AqxCo	2	5143.3	1.5466	0.1725
Si(LoxAq)	8	67525	17.326	**0.0001**
LoxAqxCo	2	4315.4	1.2977	0.2606
Su(Si(LoxAq))	24	12574	1.7048	**0.0001**
Si(LoxAq)xCo	16	29536	5.32	**0.0001**
Sa(Su(Si(LoxAq)))	72	22539	1.4015	**0.0009**
Su(Si(LoxAq))xCo	44	15695	1.597	**0.0005**
Residual	111	24794		

Si – Site, Su – Subsite, Sa - Sample

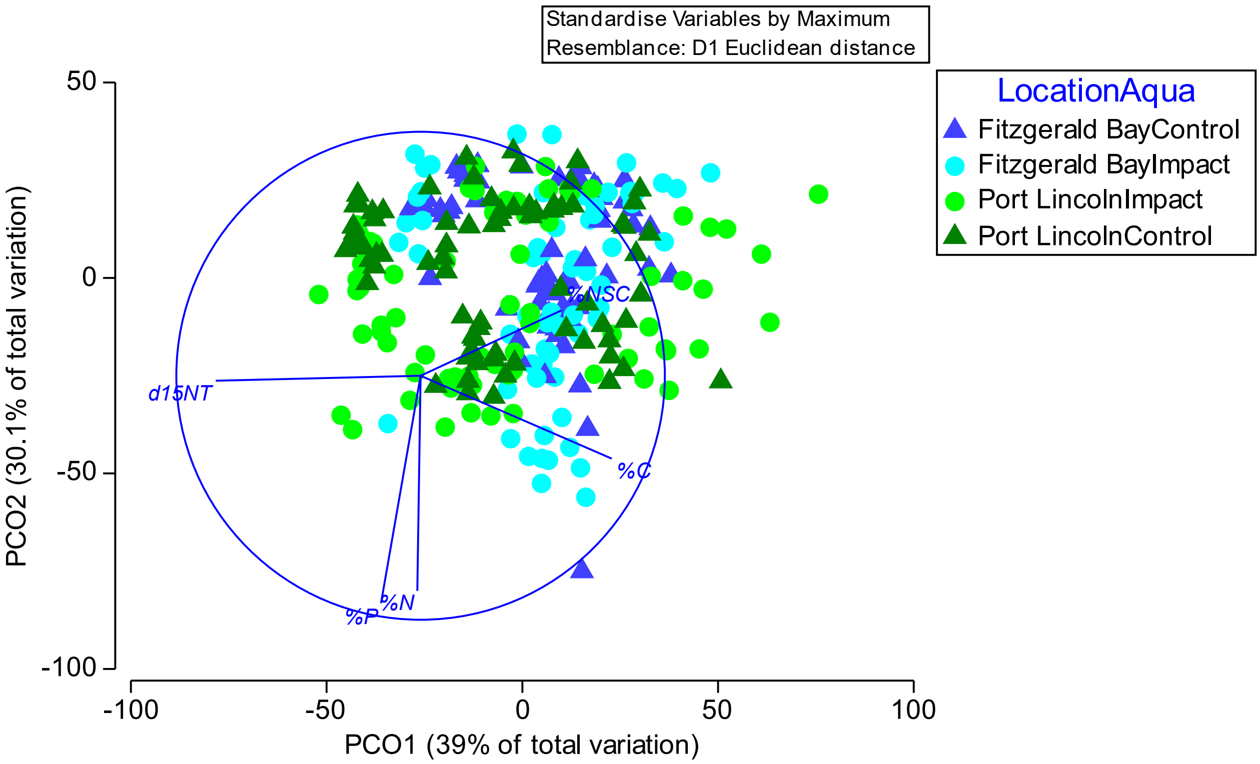

Figure 1: Principal co-ordinates analysis of biomarkers in seagrass samples from Port Lincoln and Fitzgerald Bay.

In addition, samples were analysed for a full suite of amino acids. Results were similar to those for the other biomarkers, with the exception that there was a difference between Fitzgerald Bay and Port Lincoln, and this difference varied between tissue compartments (Table 3, Figure 2).

Table 3: PERMANOVA results for amino acid analysis.

Source	df	SS	Pseudo-F	P(perm)
Location(Lo)	1	0.21123	18.38	**0.0001**
Aquaculture (Aq)	1	0.008342	0.72589	0.6457
Compartment(Co)	2	0.73489	25.076	**0.0001**
LoxAq	1	0.011535	1.0037	0.4259
LoxCo	2	0.13013	4.4402	**0.0008**
AqxCo	2	0.019558	0.66736	0.808
Si(LoxAq)	8	0.12145	3.3137	**0.0001**
LoxAqxCo	2	0.033974	1.1592	0.2987
Su(Si(LoxAq))	24	0.11937	1.6944	**0.0001**
Si(LoxAq)xCo	14	0.22432	3.608	**0.0001**
Sa(Su(Si(LoxAq)))	72	0.21259	1.127	0.0586
Su(Si(LoxAq))xCo	39	0.1786	1.7479	**0.0001**
Residual	105	0.27509		

Si – Site, Su – Subsite, Sa - Sample

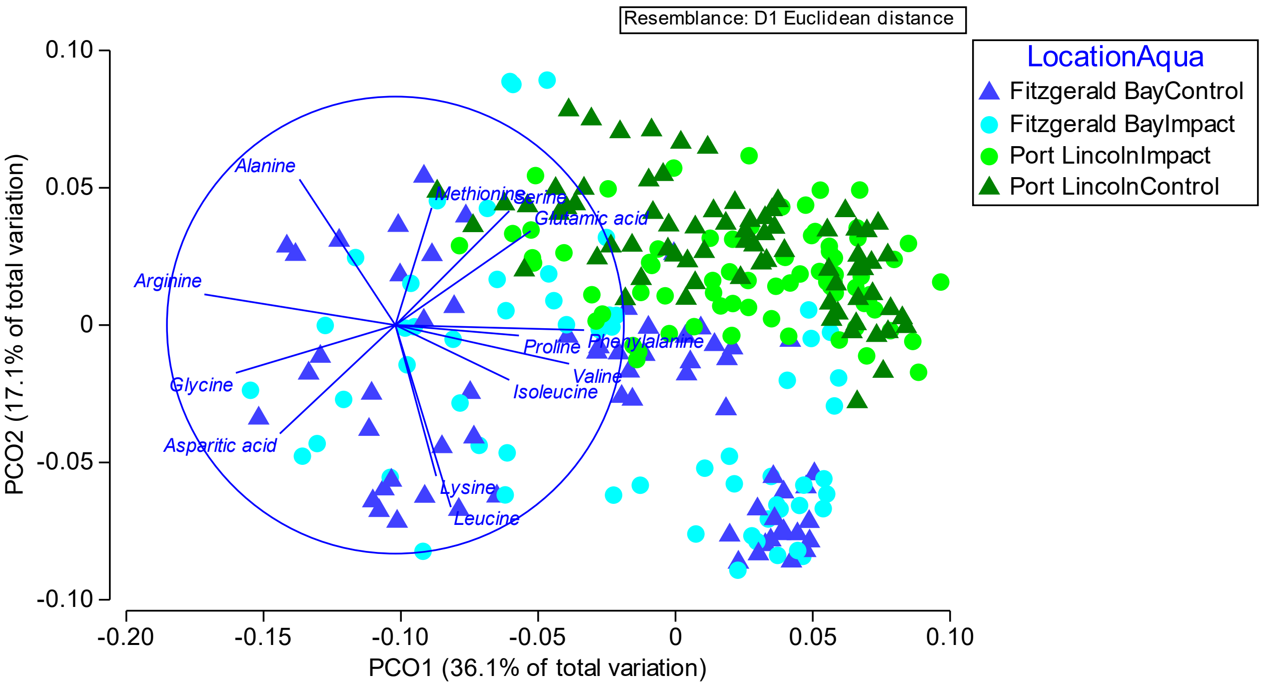

Figure 2: Principal co-ordinates analysis of amino acids in seagrass samples from Port Lincoln and Fitzgerald Bay.

Oceanography

See report on Year 1 field data below.

Milestone 4: First model runs completed and any additional data requirements identified. Year 1 field data analysed.

First seagrass model runs completed

SARDI's existing 1.5km resolution coupled hydrodynamic-biogeochemical (BGC) model of Spencer Gulf and Gulf St Vincent is based on the Regional Ocean Modelling System (ROMS) (Shchepetkin and McWilliams, 2005; Haidvogel et al., 2000, Fennel et al. 2006) and is used as the base to develop the seagrass model. Validation of the hydrodynamic-biogeochemical model has been previously presented for Spencer Gulf (Middleton et al. 2013) and more recently the LEPAZ (Tanner et al. 2020). Application of the seagrass model to South Australia required adaption of the coupled hydrodynamic-biogeochemical to run on the COAWST (Warner et al. 2010) version of ROMS. The COAWST model includes a new submerged aquatic vegetation (SAV) (Kalra et al., 2020) model which is underpinned by a spectral light model (Bisset et al., 1999) to simulate the growth/mortality of seagrass.

The SAV model was run for one-year to demonstrate the model could support a stable seagrass biomass. The model simulated conditions during 2018 using atmospheric forcing from the National Centers for Environmental Prediction (NCEP) Climate Forecast System Version 2 (CFSv2; Saha et al., 2012), lateral hydrodynamic boundary condition forcing from the Bluelink Reanalysis (BRAN; Oke et al., 2008) with biological boundary conditions from the CSIRO Atlas of Regional Seas (CARS; Ridgway et al. 2001).

Two model scenario runs were performed with the SAV model activated, one with anthropogenic inputs and one without. Anthropogenic nutrients included those from tuna and finfish aquaculture located in the LEPAZ, wastewater treatment plants located in both gulfs and the Whyalla steelworks. These initial simulations were designed to test if differences in anthropogenic inputs would show up in the seagrass response.

Seagrass Model Input Parameters

The SAV module has a large number of input parameters, a summary of the inputs is given in Table 4. For the initial runs, the default values from the published simulation of West Falmouth Harbor, Massachusetts, USA (Kalra et al., 2020) were used and provided a stable seagrass population for both runs.

Table 4. Submerged Aquatic Vegetation model input parameters

Parameter	Description	Units	Value
KI	Half saturation for light	E m^{-2} s^{-1}	100.0
KNWC	Half saturation coef. water-colmn. N uptake	mmol	5.71
KNSED	Half saturation coef. for sediment N uptake	mmol	100.0
LMBMAX	Maximum AGB in self-shading formulation	mmol N m^{-2}	475.0
GMODopt	Growth rate options [1 or 2]		2
SCL	SAV growth fraction for growth model 1		0.08
SCL2	SAV growth fraction for growth model 2		0.2
THTA	Temperature growth theta for growth model 1		0.0633
THTA2	Temperature growth theta for growth model 2		1.010
TOPT	Optimum temperature for SAV growth	C	15.0
TCRIT	Critical temp. for development of AGB	C	10.0
KL_EPB	Half saturation light limitation (epiphytes)	E m^{-2} s^{-1}	50.0
KN_EPB	Half saturation nutrient limitation(epiphytes)	mmol	10.0
LMBMAX_EPB	Maximum EPB in self-shading formulation	mmol N m^{-2}	475.0
SCL2_EPB	Maximum growth fraction (epiphytes)		0.2
THTA2_EPB	Temp. growth theta for growth model (epiphytes)		1.08
TOPT_EPB	Optimum temperature (epiphytes)	C	25.0
ARSC_EPB	Max fraction of photosynthesis (epiphytes)		0.01
ARC_EPB	Active respiration coefficient (epiphytes)		0.0633

BSRC_EPB	Maximum fraction of epiphyte biomass respired		0.0015
BRC_EPB	Basal respiration coefficient (epiphytes)		0.069
KMORT_EPB	Mortality rate (epiphytes)	$days^{-1}$	0.001
GRZMX_EPB	Maximum grazing rate (epiphytes)	$days^{-1}$	0.10
GRZK_EPB	Grazing coefficient (epiphytes)		0.01
KMAG	Above biomass ground mortality rate		0.0005
ARSC	Maximum fraction of primary production respired		0. 1
ARC	Active respiration coefficient	$days^{-1}$	0.01
BSRC	Maximum fraction of biomass required		0.0015
BRC	Basal respiration coefficient	$days^{-1}$	0.069
RTStTL	Seasonal root storage coefficient	$days^{-1}$	0.02
DOWNt	Downward translocation coefficient		0.1
TRNS	Upward translocation coefficient		0.02
KM	Below ground biomass mortality	$days^{-1}$	0.005

Seagrass Model Results

Figure 3 shows the distribution of the modelled seagrass on day 291, the seagrass distribution shows reasonable agreement with known seagrass coverage and density in the South Australian gulfs (Figure 4). This result, as expected, shows the distribution of seagrass is strongly influenced by light availability. Spatial comparison of the seagrass distribution shows little variation between the run with and without anthropogenic inputs. The maximum difference occurred on day 291 of the run corresponding to Oct 18, 2018. Looking at the differences between the two model runs on day 291 (Figure 5) it is apparent that the model has generated additional growth in the areas where anthropogenic inputs are occurring, specifically around Boston Bay and at the outflow of the wastewater treatment facilities.

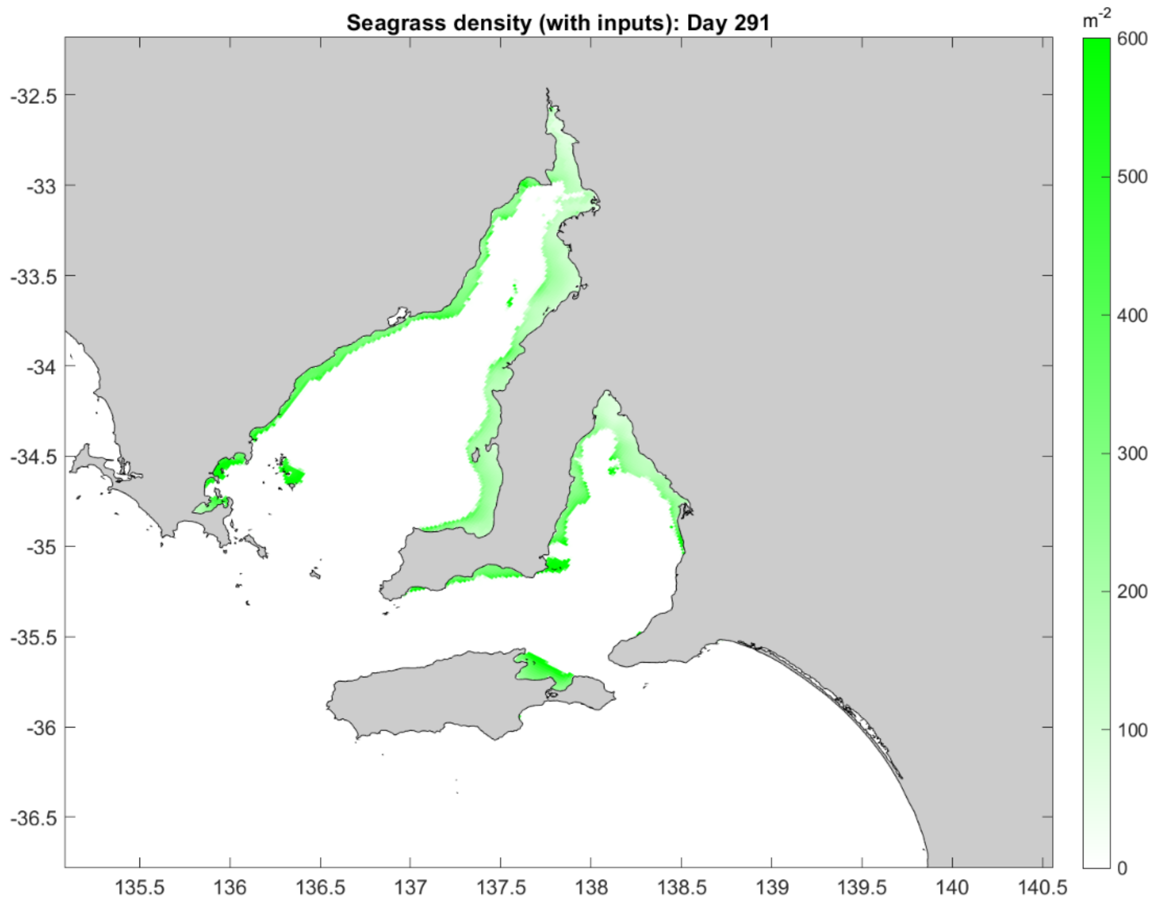

Figure 3. Plant density (plant/m2) for model run with anthropogenic inputs Oct 18 2018.

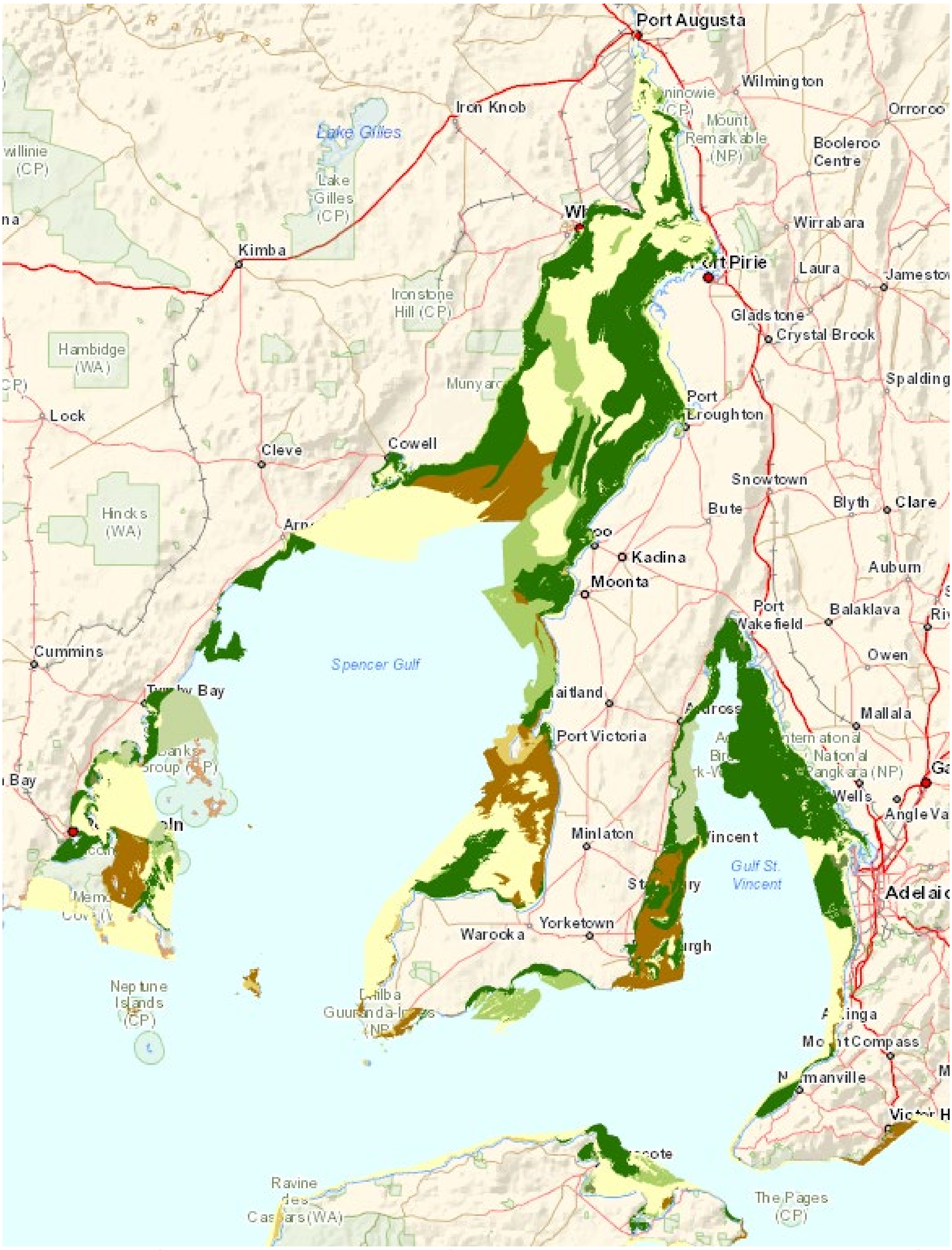

Figure 4. Image of marine benthic habitats obtained from the NatureMaps website showing the distribution of seagrass (green shades) in the South Australian gulfs (source: https://data.environment.sa.gov.au/NatureMaps/Pages/default.aspx).

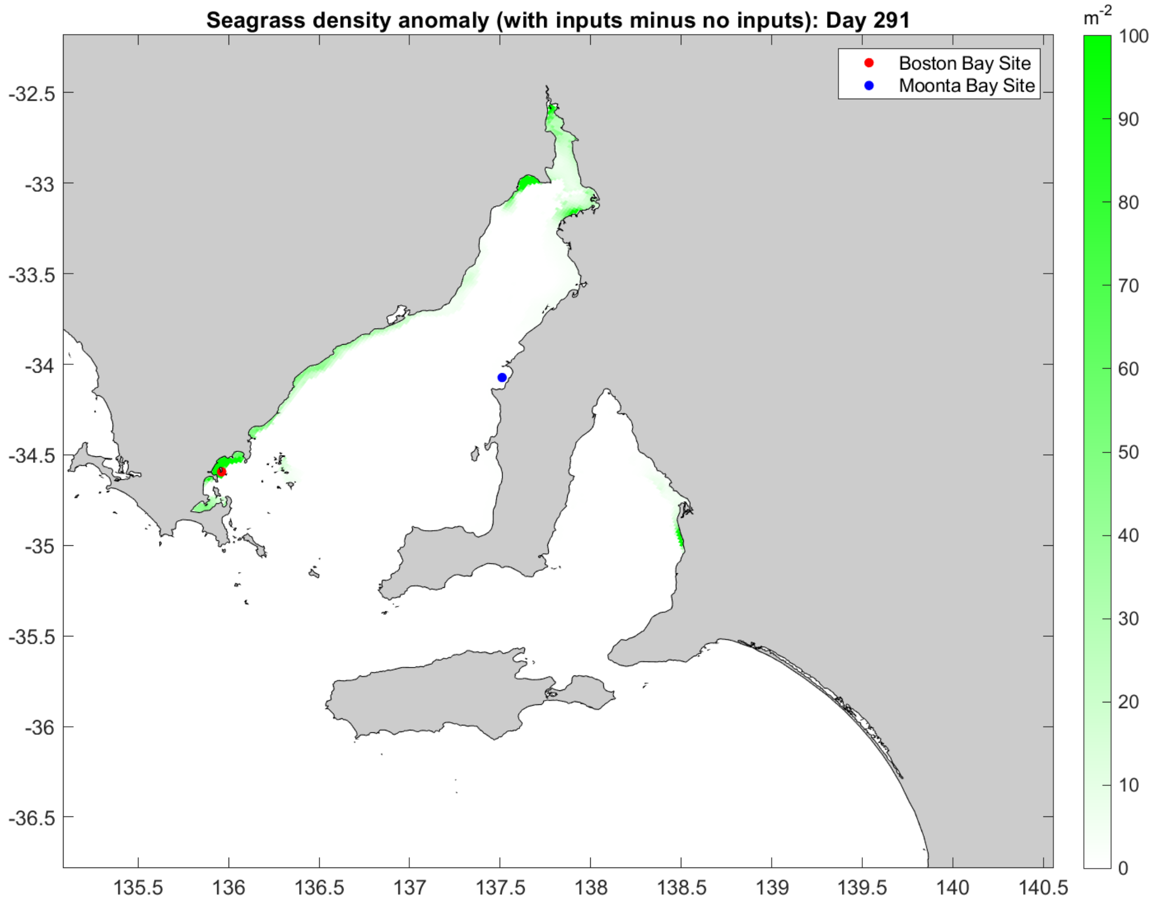

Figure 5. Plant density anomaly (plant/m2) for model run with anthropogenic inputs minus without anthropogenic inputs, two sites in Boston Bay (red marker) and Moonta (blue marker) for time series comparison are plotted.

A further comparison can be made between the development of seagrass density at two sites with moderate initial seagrass coverage, one in Boston Bay, an area which receives relatively large anthropogenic inputs of NH_4, and one near Moonta Bay, where there has been little change in seagrass density between the two runs. Figure 6 shows that in Boston Bay, seagrass density increases strongly compared to the no-input scenario. Seagrass growth commences at the start of April when NH_4 levels start to rise due to aquaculture emissions in the LEPAZ. In contrast, there is almost no difference in seagrass density throughout the year at the Moonta Bay site. There is also an increase in plant density at all sites starting in October. This is due to a seasonal change in the translocation threshold of nitrogen that is hard coded into the SAV model. This change occurs after day 273 which is Oct 1. The timing of this seasonal translocation was changed from the original Northern Hemisphere model to account for the seasonal difference in the Southern Hemisphere.

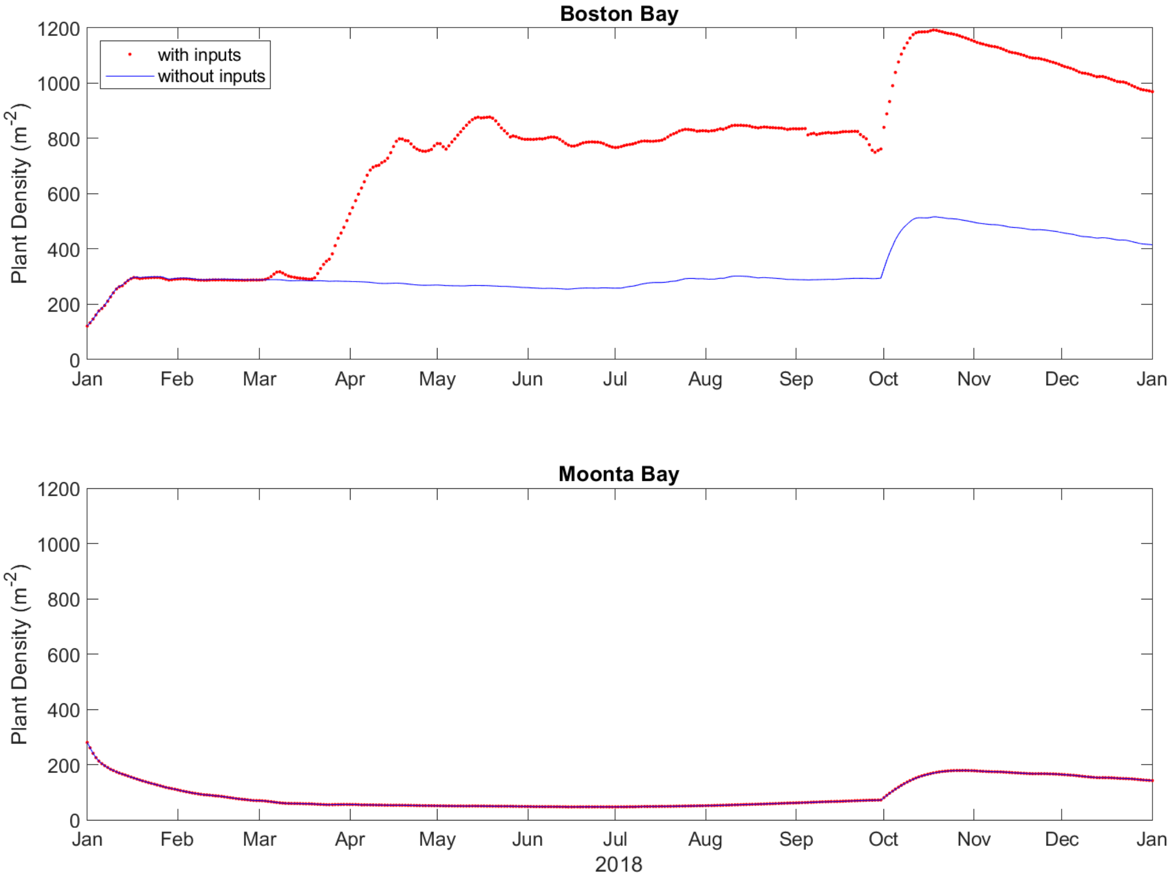

Figure 6 Time series of seagrass density at Boston Bay and Moonta Bay over the 2018 model simulation. Red dots represent seagrass density with anthropogenic inputs, the blue line, without.

Additional data and model development requirements identified

The seagrass model has been implemented within the ROMS COAWST modelling system. Since seagrass plant density is expected to remain near a long-term equilibrium level, the fact that the seagrass density does not change dramatically during a year of model simulation is a positive result. Initial tests demonstrate the seagrass responds to the input of additional nutrients; however the system needs local parameters to be adopted to provide a meaningful simulation of the seagrass in the South Australian gulfs. For example, based on field observations of seagrass condition in the presence of elevated NH_4 discharges in Spencer Gulf, such as those associated aquaculture emissions in the LEPAZ, we may have expected a stronger epiphyte response which will act to limit seagrass growth. However, initial test runs completed with locally derived parameter values quickly led to the entire seagrass population rapidly dying off.

Due to the large number of parameters needed to run the seagrass model, sensitivity studies are needed to see which parameters have a significant effect on the seagrass and epiphyte growth rates and then to test local values on a parameter by parameter basis. To expediate this process, a one-dimensional version of the SAV has been developed. It is anticipated that sensitivity studies using the one-dimensional model will help refine parameter values needed to better characterise local seagrass in the full 3D model, as well as identify local parameters for which additional information (data/studies) is required. Suitable parameter values can then be implemented to better understand the long-term impacts of anthropogenic nutrient inputs on seagrass growth at the regional scale.

Since changes in seagrass plant density and distribution are expected to occur slowly over timescales of months to years, long-term coupled hydrodynamic-BGC simulations with anthropogenic inputs from aquaculture and other major industrial sources within the gulfs have now been completed for the period 2016-2019 in preparation for future seagrass model runs. In addition, in preparation for seagrass model scenario runs focussing on understanding the potential impact of nutrient discharges associated with the re-introduction of finfish aquaculture in the FBAZ, feed schedules needed to estimate nutrient loads associated with the expected peak production of ~3000 tonnes of finfish have been provided by Cleanseas.

Finally, considering one of the dominant mechanisms through which anthropogenic nutrient discharges impact seagrasses is through modification of the underwater light environment due to generating increased organic matter loads via primary production, improved bathymetry data is being sourced from the Australian Hydrographic Office. It is anticipated this will improve the model grid on which simulations of seagrass growth/distribution are conducted. These improvements are also expected to improve the hydrodynamic and BGC predictions made by the model.

Year 1 Field data analysed

An oceanographic mooring (Figure 7) was deployed between July 21 and September 18, 2020 in the western sector (32°57.255 S, 137°48.227 E) of the Fitzgerald Bay Aquaculture Zone (FBAZ). The mooring consisted of a Nortek Signature 100 Acoustic Doppler Current Profiler (ADCP) to measure current speed and directions at 1m intervals throughout the water column. Near bottom measures of pressure (depth), temperature, salinity, chlorophyll *a* (chl-a), turbidity and dissolved oxygen saturation were made using a Seabird 16plus SeaCAT CTD sensor package. JFE DEFI2-L photometers for the measurement of photosynthetically active radiation (PAR) light intensity were attached to the mooring frame at 0.55 m and 1.35 m above the seabed and an additional light meter was located at the SA Water Whyalla site to provide measures of the surface light intensity. All data collected by the mooring was processed following standard Integrated Marine Observing System (IMOS) QA/QC protocols. Following the mooring deployment and recovery, near surface and bottom samples were taken using a Niskin bottle to form a composite sample from which sub-samples for multiple water quality parameters were processed and sent to the SARDI analytical laboratory for analysis.

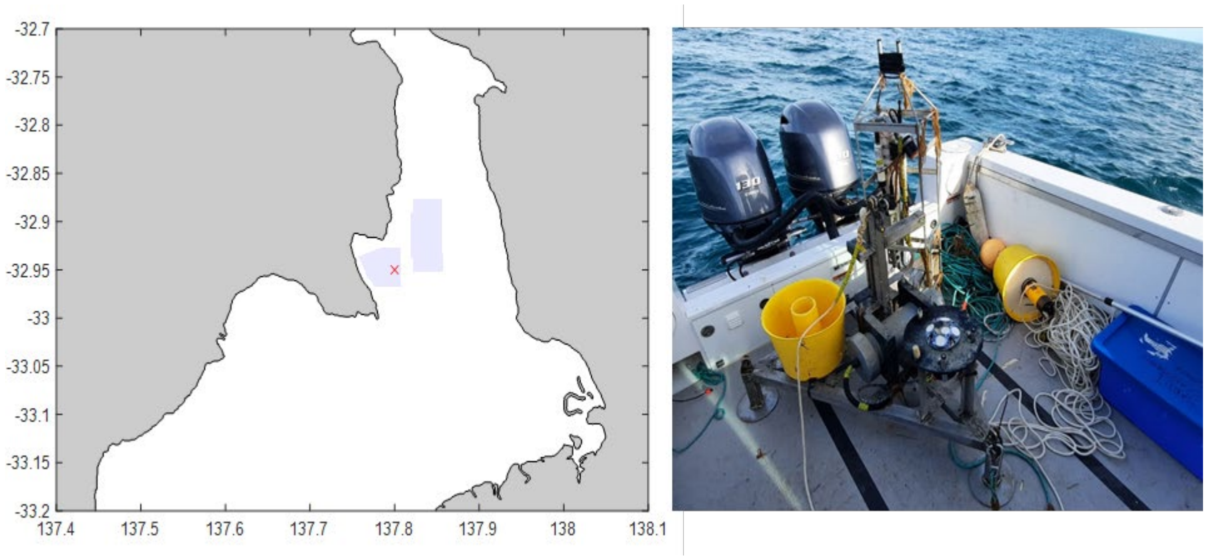

Figure 7. (Left) Location of the Fitzgerald Bay aquaculture zone in upper Spencer Gulf (blue shaded region) showing the location of the oceanographic mooring and water sampling site (red cross). (Right) Picture of the ocean mooring post recovery.

The concentrations of water quality parameters determined from water samples are shown in Table 5. In general, the observed concentrations were comparable to those previously measured in upper Spencer Gulf (Middleton et al. 2013). Dissolved inorganic nitrogen concentrations were generally low (NH_4 and NO_x < 8 µg/L) and similar to those observed in offshore waters of the Lower Eyre Peninsula Aquaculture Zone (LEPAZ) in May during periods of peak anthropogenic inputs and exchange with shelf waters (Tanner et al. 2020). Although low, the observed NH_4 and NO_x concentrations are greater than what might be expected considering the distance and timescales of connectivity with lower Spencer Gulf assuming nutrients are sourced from the shelf. This suggests other nutrient cycling such as denitrification (Middleton et al. 2013) and/or nitrification (Messer et al. 2021) are likely to influence dissolved nitrogen concentrations in upper Spencer Gulf. Silicate concentrations in the northern gulf region are high and exceed those measured in the LEPAZ. Measured phosphorus concentrations (<0.5 µg/L) were below the detection limit and are expected to be limiting phytoplankton (or seagrass) growth.

Measures of total nitrogen and total phosphorus levels include inorganic and organic components that may be sourced naturally (e.g. plant and animal material) or from man-made emissions (e.g. wastewater treatment, fertilizers and run-off). Observed total nitrogen concentrations were high (<130 µg/L) and above the ANZECC water guideline concentrations recommended for coastal waters (60 µg/L) or embayment's (100 µg/L). High total nitrogen concentrations are often associated with algal blooms and provide an indicator of eutrophication. However, like inorganic nutrients, low observed (below detection level) total phosphorus concentrations are expected to limit phytoplankton and seagrass growth. Observed chl-a concentrations (~0.4 µg/L) were low and were approximately half that observed in the LEPAZ. Total suspended solid (TSS) concentrations (~4 µg/L) were also low and are comparable to those observed in the LEPAZ.

Table 5. Mean and standard deviation winter and spring water quality concentrations determined from water sampling in the western sector of the Fitzgerald Bay Aquaculture Zone. Measured parameters included ammonium (NH_4,) oxides of nitrogen (NO_x), phosphorus (PO_4) and silicate (Si), total nitrogen (TN), total phosphorus (TP), chlorophyll a (chl-a) and total suspended solids (TSS). * indicates values at the detection level.

Parameter	**21-Jul-2020 (µg/L)**	**15-Sep-2020 (µg/L)**
chl-a	0.39 ± 0.1	0.41 ± 0
NH_4	7.7 ± 2.1	5.0 ± 0
NO_X	7.0 ± 1.0	7.7 ± 2.5
PO_4	0.5 ± 0*	0.5 ± 0*
Si	70.0 ± 0	40.0 ± 0
TN	180.0 ± 17.3	136.7 ± 5.8
TP	10.0 ± 0*	10.0 ± 0*
TSS	4.0 ± 0	4.3 ± 0.6

Time-series plots from the mooring (Figure 8) show the water temperature increased from a low of ~12-13°C in late July and early August up to nearly 16°C by mid-September. Salinities decreased over the first half of the mooring deployment consistent with the observed cooling and low evaporation rates expected for this time of the year. Baseline chl-a concentrations remained low and were consistent with the concentrations derived from water samples. Periodic increases in chl-a concentrations greater than 1 µg/L above the baseline were observed over timescales of several hours to several days. The frequency and intensity of these events increased during the later half of the deployment as temperatures warmed. These increases also coincided with increased measures of turbidity. Dissolved oxygen saturation concentrations remained high (>94%), but like salinity, reliable measures were not recorded in the second half of mooring deployment due to the likely biofouling of sensors.

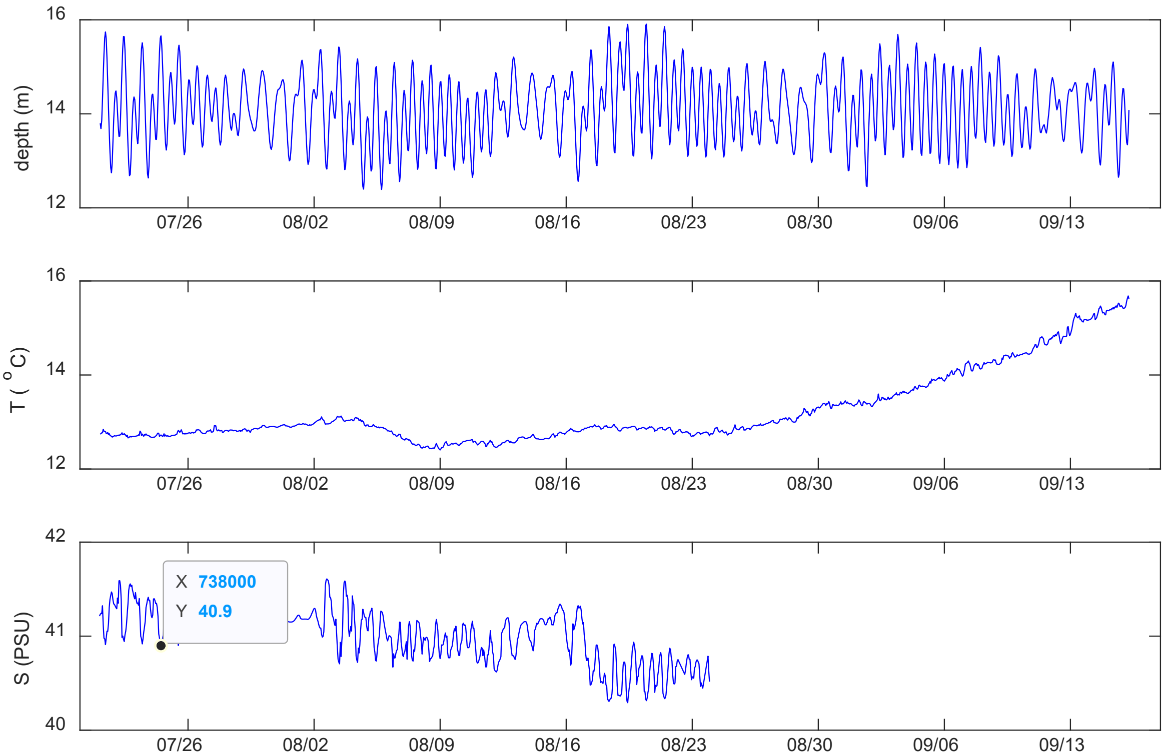

Figure 8. Time series of physical and water quality parameters measured by the ocean mooring. Red triangles show the chla concentrations determined from water samples shown in Table 1. (continued on next page).

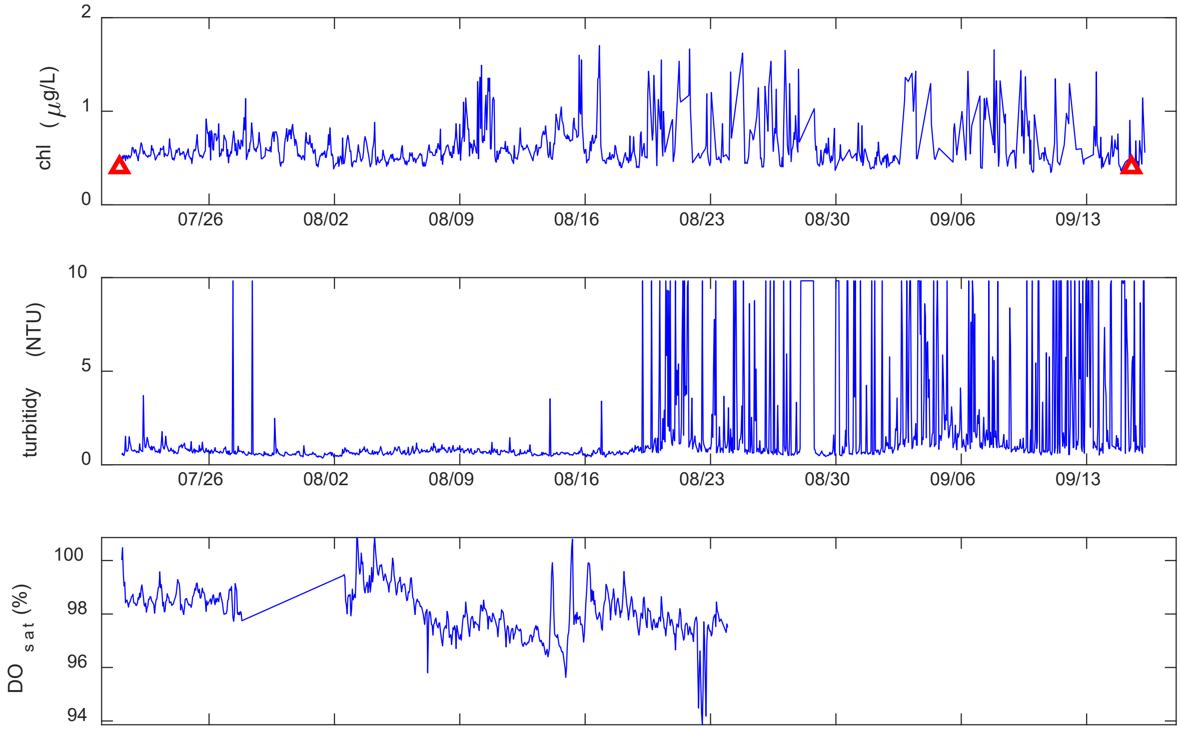

Figure 8. Time series of physical and water quality parameters measured by the ocean mooring. Red triangles show the chla concentrations determined from water samples shown in Table 1.

Daily estimates of the light attenuation due to seawater (K_d) between 12-1 pm were obtained from PAR light intensity measures by applying the Beer-Lambert Law (Figure 9). K_d estimates ranged between 0.16 and 0.28. The mean K_d value of 0.24 is slightly greater than the gulf-average value of 0.15 currently used for the biogeochemical model parameterisation (Middleton et al. 2013) but less than those previously estimated for northern Gulf Saint Vincent along the Adelaide metropolitan coast (K_d ≥0.34, Petrusevics 2005).

PAR and K_d values were then used to determine the euphotic depth (Z_{eu}). For seagrass Z_{eu} is calculated as the depth where the light intensity is 12% of its surface value (Gordon 1994). Z_{eu} estimates ranged from 7.7 to 12.8 m with a mean value of 9.0 m and provides an indication of the depth range at which seagrasses receive their minimum light requirements for survival in upper Spencer Gulf.

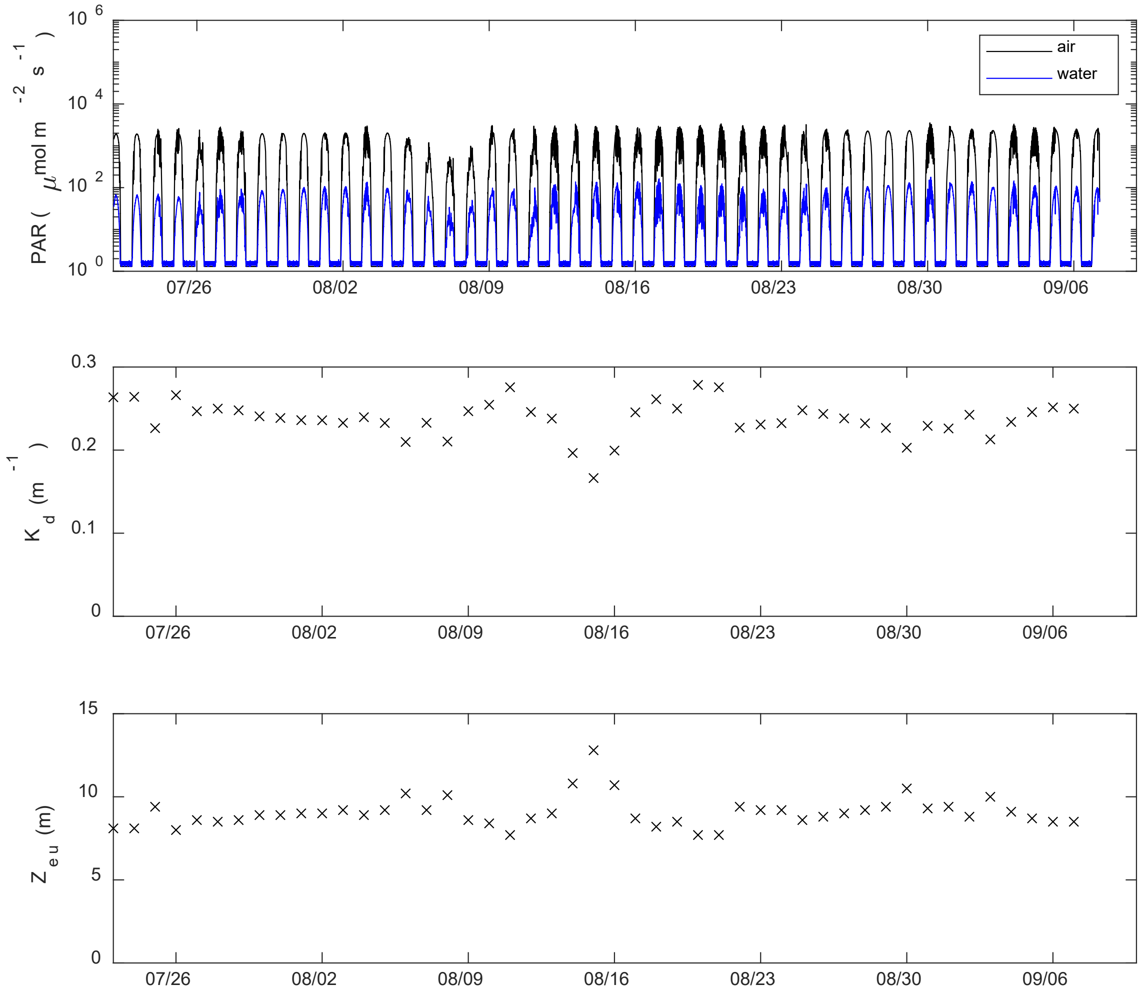

Figure 9. Time series of PAR light intensity measured in the air and at ~14 m depth (top panel). Corresponding daily estimates of the light attenuation coefficient (K_d) (middle panel) and euphotic zone depth (Z_{eu}) (bottom panel).

Strong tidal currents and associated shear are expected to dominate flushing processes and the dispersion of anthropogenic point source nutrient emissions in upper Spencer Gulf and the FBAZ (Middleton et al. 2014). Moored observations of currents have been used to validate the hydrodynamic model predictions for the FBAZ. Figure 10 shows comparison of the observed and modelled speed and direction of tidal currents. The model correctly predicts current direction but overestimates current speeds by up to a factor of 2. The over prediction is likely due to model bathymetry limitations, as the modelled depth at the mooring location (5 m) is much shallower than the actual mooring deployment depth (~13-14 m). This suggests that while the orientation of any modelled anthropogenic nutrient discharges, such as those from the reintroduction of finfish aquaculture is likely to be representative, the spatial extent of the discharge, or other modelled water quality parameters, may be overestimated.

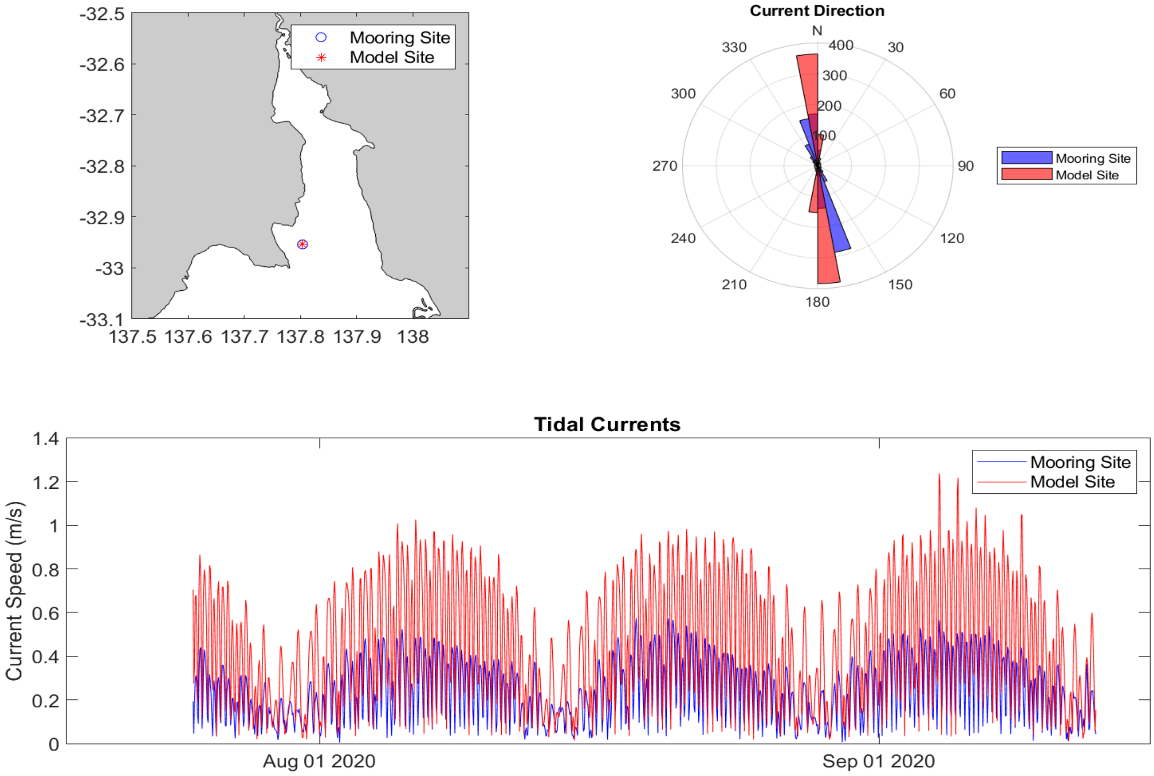

Figure 10. Comparison of observed (blue) and model (red) tidal current speeds and directions at the mooring location in the FBAZ.