Environment Protection Authority

Outer Harbor channel widening dredging

Seagrass condition assessment

Outer Harbor channel widening dredging – seagrass condition assessment

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Summary

With the emergence of the wider Post Panamax ships, Flinders Ports proposed to widen the Outer Harbor shipping channel in the Port of Adelaide to ensure unrestricted use throughout all tide and wind conditions, maintaining their competiveness against other southern Australian ports. This required a large capital dredge campaign of approximately 2 million m$_2$ of sediment and disposing the sediment to the existing dredge material placement area located 30 km offshore, in Gulf St Vincent.

The nearshore waters of Gulf St Vincent are dominated by the long-lived and slow-growing seagrass genera *Posidonia*. This seagrass is ecologically very important as a foundation species, forming the habitats that structure our nearshore communities. They also provide services to society including enhancement of fisheries production, protection of shorelines from erosion, regulation of our climate through the sequestration of carbon, and treat of our wastes. Adelaide’s metropolitan coast has lost over 6,500 ha of seagrass since the 1940s as a result of nutrient enrichment from wastewater and industrial discharges, and a reduction in the light passing through the water due to turbidity from stormwater and dredging discharges. Once lost, seagrass can take decades to recover, if at all.

Dredging generates suspended sediment in the water column reducing the light available at the seafloor. Large dredge campaigns adjacent to seagrass habitats are considered a high risk of impacting seagrass. The Environment Protection Authority’s (EPA) response to the development application required the risk to seagrass to be addressed and after changes to the dredge method, the proposal was approved and subsequently an EPA licence was granted in 2019.

The EPA licence focused on requiring Flinders Ports to undertake all reasonable and practicable measures to ensure the predicted loss through the development application process is minimised as far as possible. Strict turbidity thresholds are set for the dredge program and Flinders Ports is required to monitor seagrass condition. In addition to this, the EPA independently monitored seagrass condition at various sites adjacent to and distant from the actual dredging excavation.

The results of the EPA seagrass audit demonstrate that there was no difference between the seagrass at the sites monitored in the zone of influence compared to the locations distant from the dredging, before and after the dredge program. It is noted that there can be a delayed response and further monitoring will be undertaken in 2021.

1 Introduction

The Port of Adelaide is the largest shipping port in South Australia located approximately 14 km from the Adelaide City centre. Increasingly, Post Panamax ships are visiting the Port. These larger ships require the entrance channel and swing basin to be widened. Flinders Ports submitted a development application to the government in 2017, this was approved in 2018 and an EPA licence issued in 2019.

Nearshore environments throughout Gulf St Vincent are typically dominated by dense and continuous seagrass meadows dominated by *Posidonia* genera. Posidonia seagrass is long lived and slow growing, and has large carbohydrate reserves making it resilient to periods of reduced light (Collier *et al* 2008), but is very slow to recover from loss (Irving 2013). Seagrass meadows are extremely important ecologically as a foundation species (in the sense of Dayton 1972) forming the habitats that structure our nearshore communities. Seagrass also provides services that benefit society by increasing productivity of commercial and recreational species including Blue Swimmer Crab, King George Whiting and Southern Calamari.

Seagrass stabilises sand, resulting in less erosion on our beaches and its longevity results in long-term sequestration of carbon, in some cases more than terrestrial habitats of the same area. Seagrass also assimilates nutrients discharged by sewage treatment plants and other sources, treating our wastes (Gaylard *et al* 2020).

Adelaide’s metropolitan coast has lost over 6,500 ha of seagrass since the 1940s, as a result of poor light climate caused by nutrient discharges from wastewater treatment plants and industries, and due to suspended sediments from urban stormwater and dredging activities (Fox *et al* 2007). Substantial investment by all levels of government and industries has been made to improve water quality, including via the Adelaide Coastal Water Quality Improvement Plan, which set out targets for discharges to improve water quality and facilitate seagrass recovery (McDowell and Pfennig 2013).

Large capital dredge campaigns typically cause potentially high concentrations of suspended sediment in the water column. These can block light from reaching aquatic vegetation on the seafloor, and in areas where water slows down, sediment can accumulate over time. The best practice environment management for large dredge programs requires hydrodynamic modelling to predict areas of potential impact as a result of turbidity and sediment deposition.

Generally there are three zones that are predicted by the models and compared to known ecological thresholds (Figure 1). These are the zone of high impact (ZoH), which is where there will be a long-term environmental impact. A zone of low to moderate impact (ZoLM) is the predicted area that is likely to result in environmental impacts that would likely recover within two years. Finally a zone of influence (ZoI) which is the zone predicted to be influenced by elevated turbidity or sediment deposition but below an ecological threshold, meaning that there is no long-term ecological impact.

In many cases, total prevention of seagrass loss is not achievable or reasonable in large capital dredge campaigns. As such, the Environment Protection Authority (EPA) licence focused on requiring Flinders Ports to undertake all reasonable and practicable measures to ensure the predicted loss of seagrass through the development application process was minimised as far as possible. To inform the assessment of risk to seagrass, Flinders Ports submitted modelling results that predicted areas of potential impact to seagrass during the licence application process (Figure 2). With the exception of the four hectares of permanent seagrass loss directly in the dredge footprint, the majority of the potential risk is from indirect loss of seagrass through turbidity impacts. The area of potential loss in the ZoH and ZoLM was predicted at 158 ha (Table 1), while there is extensive seagrass within the ZoI that could be affected in the event of excessive turbidity generated.

Table 1 **Estimate of seagrass loss (hectares) from direct and indirect dredge plumes approved in the Vegetation Clearance Permit (BMT 2020)**

Classification category	Coverage	Direct Impact	Total Area (ha) within the High to Medium Impact Area (Winter)
Moderate to dense seagrass including Amphibolis and/or Posideonia.	Moderate to dense (35-100%)	-	*0.02*
Sparse seagrass including Halophila australis and/or very sparse Posidonia.	Sparse (1-35%)	*4*	*0.2*
Seagrass dominated by *Heterozostera*	Moderate to dense (35-100%)	-	158
Total		**4**	**158**

This survey did not assess seagrass loss in the areas that were approved for loss (ie ZoLM and ZoI). These will be the subject of monitoring by Flinders Ports to establish compliance with the Native Vegetation Council clearance permit.

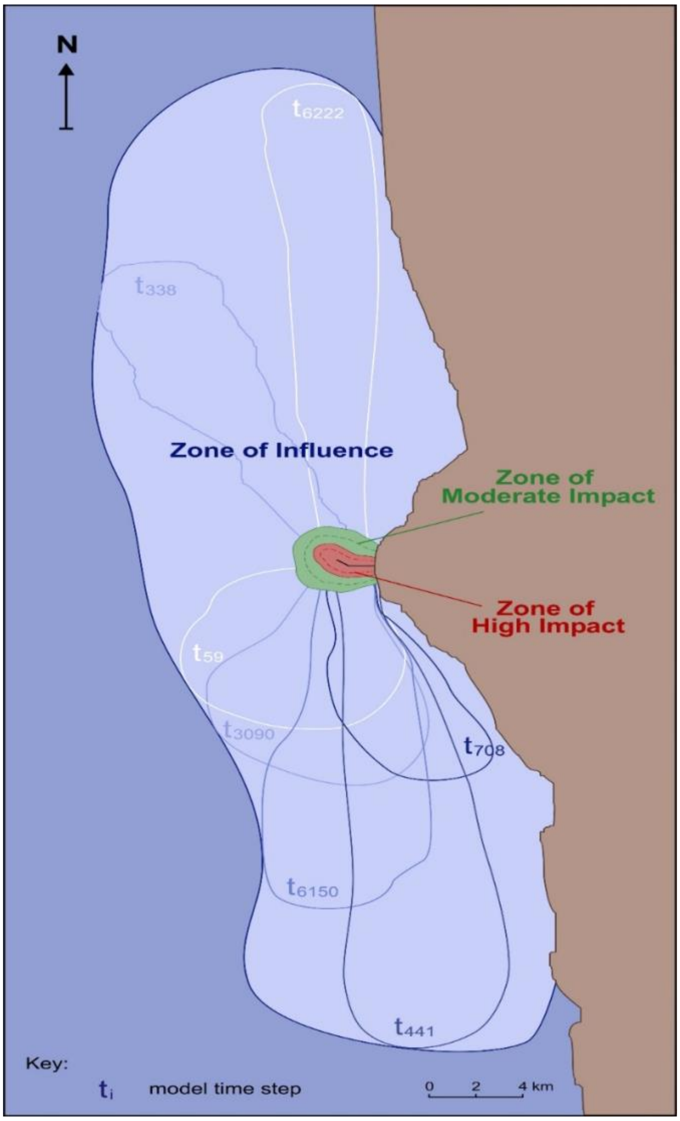

Figure 1 **A schematic from the West Australian Technical Advice for dredging indicating hypothetical spatial zones represented from modelled dredging related impacts where red represents the Zone of High Impact, green represents the Zone of Moderate Impact and pale blue represents the Zone of Influence (EPA 2016).**

The EPA required Flinders Ports to monitor seagrass before (2019) and after (2020) dredging and to undertake a further assessment in 2022 to determine whether seagrass has been lost in areas outside of the predicted footprint. The results of the Flinders Ports (and contractors) seagrass surveys are documented on the EPA website[1].

Independent seagrass surveys were conducted by EPA marine scientists at four locations, to assess seagrass extent and condition using a before and after, control and impact (BACI) design (Figure 2). The aim of these surveys was to provide further confidence in results and incorporate different metrics at a finer resolution than the *Flinders Ports Seagrass Environment Monitoring Program*. This document describes the results of the independent EPA assessment.

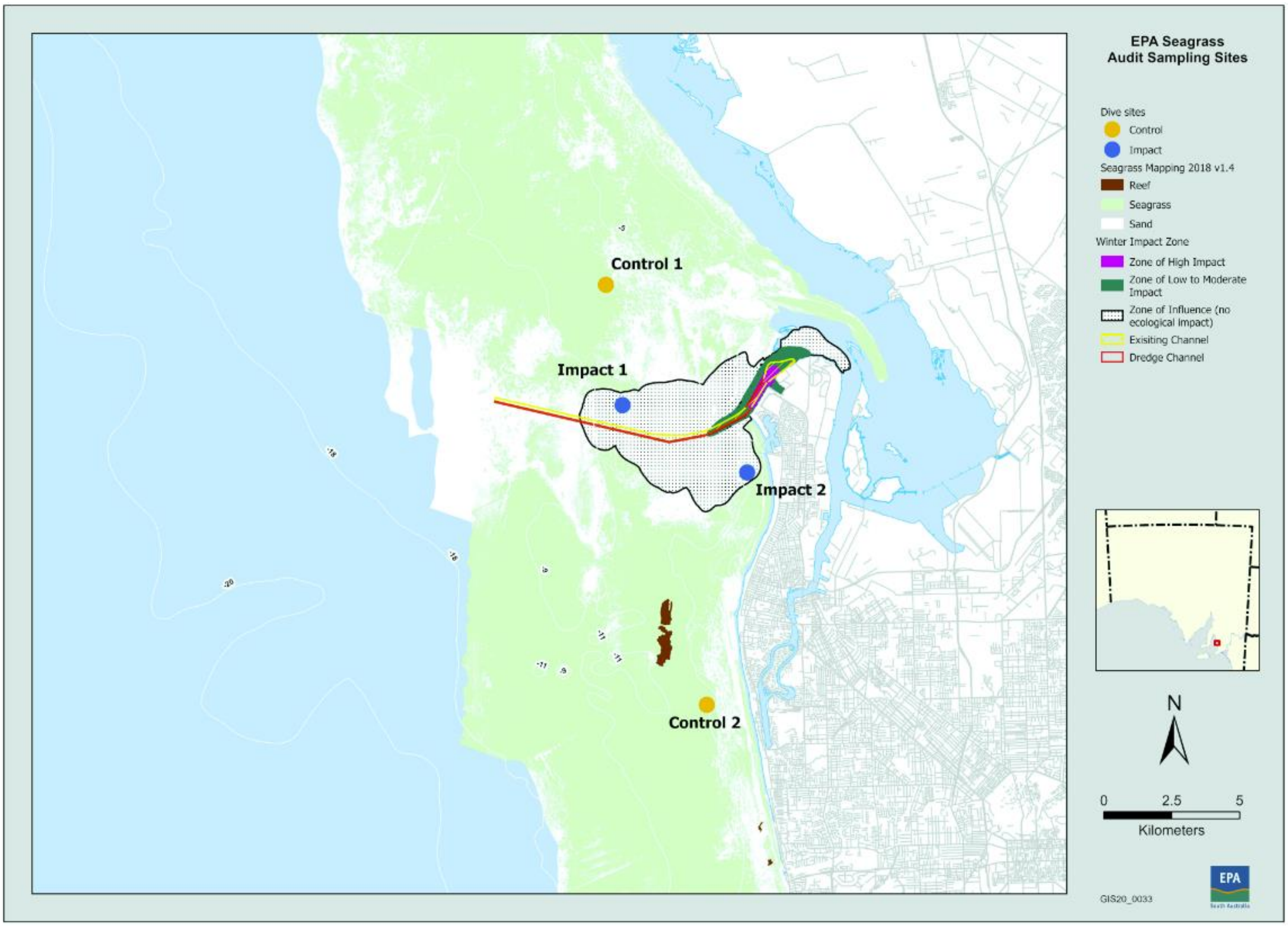

Figure 2 **Predicted dredge impact zones from Flinders Ports modelling of the Outer Harbor channel widening project (BMT 2020). Seagrass cover determined by aerial photography overlaid (Clarke *et al* 2018) with locations of control and impact sites for seagrass condition assessment.**

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[1] https://www.epa.sa.gov.au/community/stay-informed/flinders-ports

2 Methods

The zone of influence (ZoI) intersects with large areas of seagrass, which is considered to be at risk from impacts of turbidity and sediment deposition. The EPA monitoring program has adopted two control and two impact sites to ensure that the spatial extent and variability in plume trajectory was considered. Sites were selected to minimise variability between the control and impact, however in the naturally variable marine environment, there can be some subtle differences.

The seagrass surveys used two methods to assess potential change in seagrass cover. One method uses towed underwater video, evaluating less detailed information over larger spatial scales (100s of metres). While the second employed SCUBA divers to collect data at relatively small scales (10s of metres). The large and small scale surveys are designed as 'Before and After, Control and Impact' (BACI) experiments. BACI experiments are designed to examine changes in biological communities due to a particular disturbance, while accounting for natural background variability (Underwood 1992).

Posidonia seagrass sheds some of its leaves during winter to reduce its energy demands during the cooler and shorter day length period. In order to take this into account, the before and after surveys were undertaken as close as practicable to the same season (autumn) to avoid recording differences as a result of seasonal effects.

The study sites were selected from existing seagrass meadows allocated as two control and two potentially impacted sites north and south of the dredge footprint. Control sites were located distant to the ZoI with no predicted effect from the dredging, while impact sites were inside the ZoI near the ZoLM impact from the hydrodynamic model produced by Flinders Ports in the Outer Harbor channel widening project licence application (selected scenario) (Figure 1). This was considered appropriate as the approval outlines the expectation that there will be no loss of seagrass within this zone. As such, the intent of this program is to test whether there is a statistically significant loss of seagrass within the impact sites as compared to the control sites located outside of any influence of the dredging. All sites were located in approximately 7 m of water.

Towed underwater video

Towed underwater video allows a larger area of seafloor to be assessed to provide a broader indication of seagrass presence or absence and an estimate of cover as a percentage of seafloor (Gaylard *et al* 2013b). Three underwater video belt transects (80–120 m) were undertaken randomly at each site using a geo-referenced digital video camera (Scielex) angled at 90° to the seafloor, in a custom-made housing. A live video feed to a surface screen viewed by a trained operator ran directly from the camera into an audio and video encoding system (Geostamp) which overlays a GPS location, direction, speed, date and time to the video and records to a hard drive.

The surface screen and trained operator allowed the camera to be positioned approximately 1 m from the substrate in order to maximise image quality and resolution. This set-up provided a field of view of approximately 1 m$_2$, whereby each belt transect equates to approximately 80–120 m$_2$. Simultaneous full high definition video (GoPro) is collected at each site, to provide higher resolution for taxonomic identification or finer detailed analysis. Videos were analysed upon return from the field using an in-house video analysis software package. Variables recorded were seagrass species, seagrass percent cover, epiphyte load, opportunistic macroalgae and any other notable observation (eg marine debris).

Diver surveys

Five replicate cores (0.045 m^2) were taken within seagrass meadows at each site by SCUBA divers in February 2019 and March 2020. In the laboratory, cores were separated into the above and below ground components, and epiphytes (ie plants and animals living on the leaves) were removed from seagrass leaves using a plastic scraper. The number of shoots were counted, leaf length measured and samples dried at 80°C until constant weight to ascertain above and below ground biomass and biomass of epiphytes.

Statistical analysis

Seagrass data was compared using a univariate PERMANOVA test on untransformed data using Primer v7 and the PERMANOVA add on (Clarke and Warwick 2001). Essentially the design is a nested design with Site (Control 1, Control 2 etc), nested within Test (Control, Impact) which is examined across Time (Before and After). Resemblance matrices were developed using the Euclidian distance and tested for significance initially using Site nested within Test as random factors and Time as a fixed factor. Where there was no significant difference between the Site(test) x time interaction, site data was pooled and the PERMANOVA test carried out for the Test x time interaction. If this test was significant then differences can be identified using the pairwise tests. This process examines the variables at control and impact sites across the before and after surveys. In this instance the Site and Time interactions alone are less important.

In order to be conservative, p value significance of less than 0.01 was used in all tests, rather than the traditional $p < 0.05$.

3 Results

Sites were surveyed in February 2019 (Before) and March 2020 (After) to determine seagrass cover and various plant scale metrics (morphometrics), and how these metrics may have changed as a result of the dredge program.

Seagrass cover – towed underwater video

The benthic video showed that all sites were dominated by *Posidonia* seagrass and some smaller amounts of *Amphibolis* (Figure 3). The control sites had more seagrass than the impact sites at all times ($p < 0.01$) and less epiphytes ($p < 0.01$). Seagrass cover was patchy at impact sites compared to the control sites (Figure 4).

The PERMANOVA test of total seagrass cover from the benthic video showed no significant difference within the Site (Test) x Time interaction ($p = 0.015$; Appendix 1). This result means that the control sites can be pooled together and the two impact sites pooled to increase the statistical power (ability to detect a change). The PERMANOVA test (Test x Time) indicates that there was a significant difference in seagrass cover between the control and at the impact sites ($p < 0.01$) and this change was an increase in seagrass cover at impact sites, which increased from 45.6 % to 60% cover, but this was still less than at the control site (Figure 5).

Seagrass epiphytes observed by the benthic video were significantly different between the control and impact sites before and after the dredge program [Site (Test) x Time $p < 0.01$]. Pairwise tests indicated statistically significant differences at all sites between before and after the dredging (Appendix 1). However these significant findings do not demonstrate a clear pattern (Figure 3, Appendix 1).

Seagrass morphometrics

Shoot density measures the number of shoots within a defined area, this has been shown to be a good measure of seagrass health (Wood and Lavery 2000). The control sites had higher shoot density, leaf length and biomass compared to the impact sites (Figures 5 and 6), which is consistent with the higher percent cover from the towed underwater video.

The control and impact sites showed a small decline in seagrass shoot density between the before and after dredging surveys (Figure 6a). The PERMANOVA test [Site (Test) x Time] for both seagrass shoot density and leaf length were not significant and the two control sites and two impact sites were pooled for each variable (Appendix 1). The Test x Time PERMANOVA showed no significant difference on shoot density before and after dredging ($p = 0.8259$) indicating no impact due to dredging. That is, the change between the control is similar to the change in the impact site suggesting a regional scale effect (Figure 6). Similarly, the comparison of leaf length showed no significant difference between control and impact sites before and after dredging ($p = 0.149$).

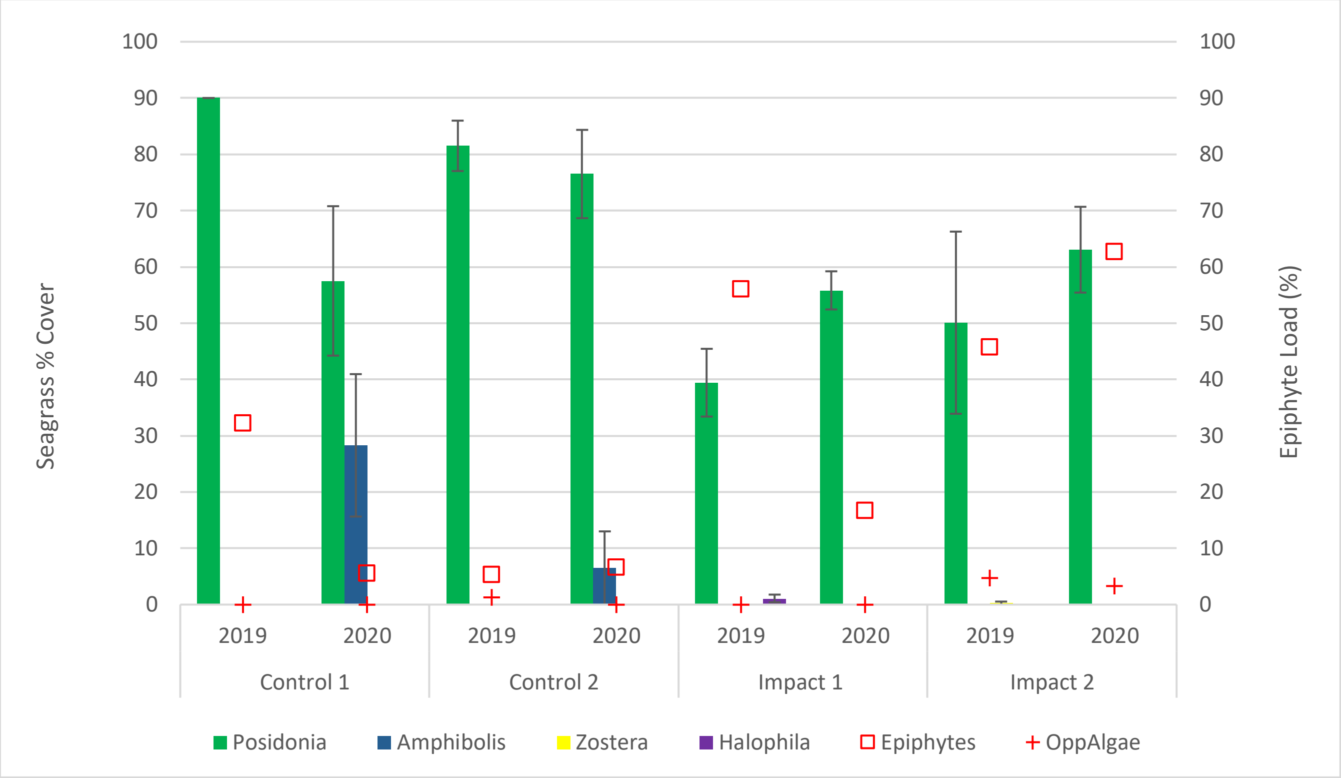

Figure 3 **Benthic habitat characterisation for control and impact sites sampled in 2019 (before) and 2020 (after) the Outer Harbor channel widening dredging. Error bars represent standard error.**

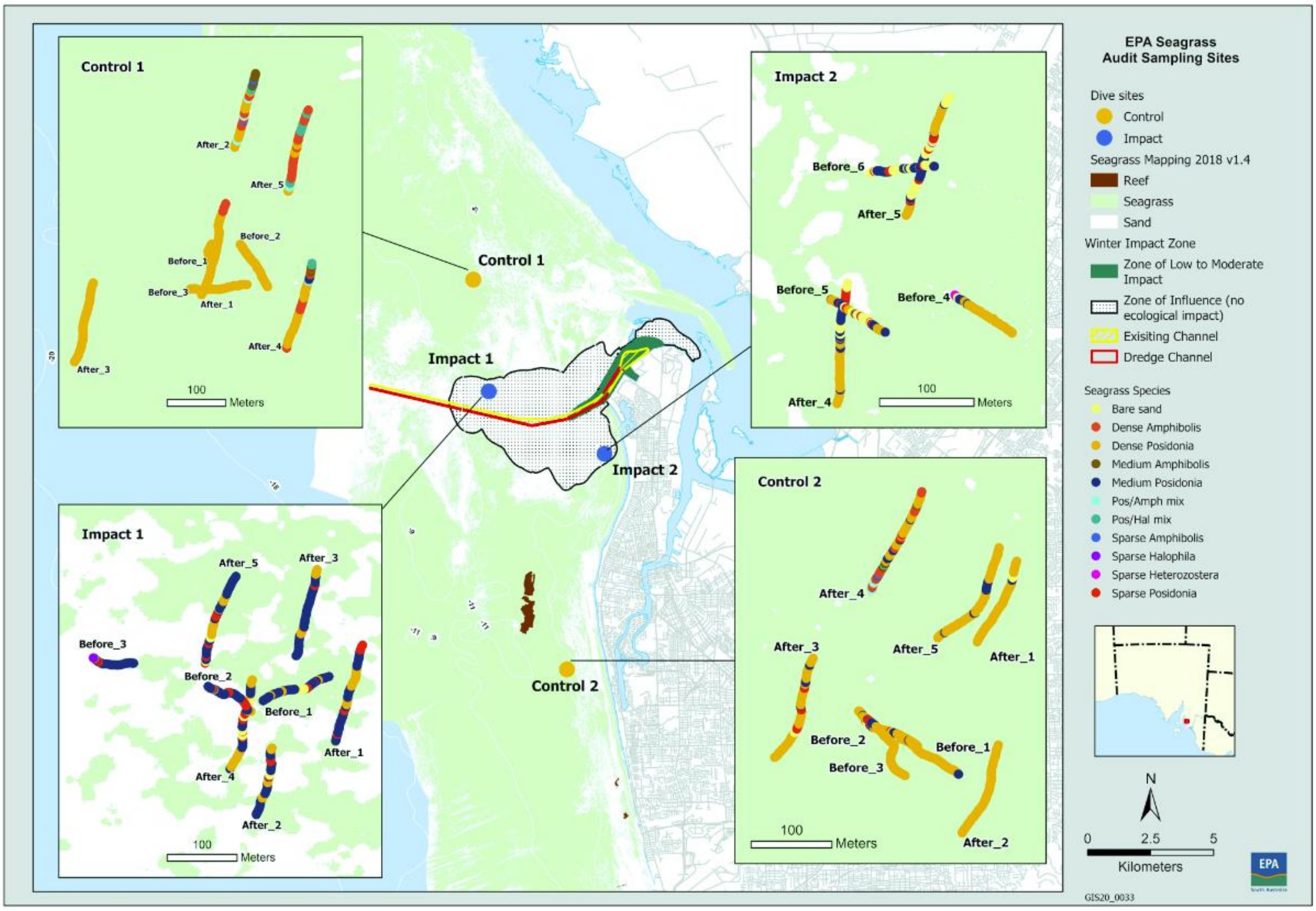

Figure **4** Image of benthic video survey results for before (2019) and after (2020) surveys at both control and impact sites

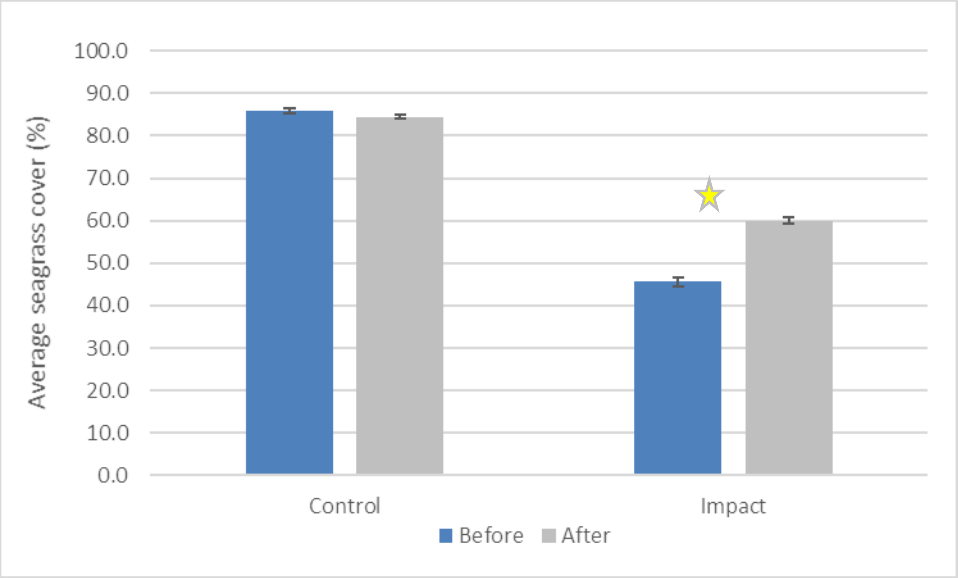

Figure 5 Average seagrass cover (%) at the control and impact sites measured using towed underwater video before (2019) and after (2020) dredging. Error bars represent standard error. Star represents statistical significant at $p < 0.01$.

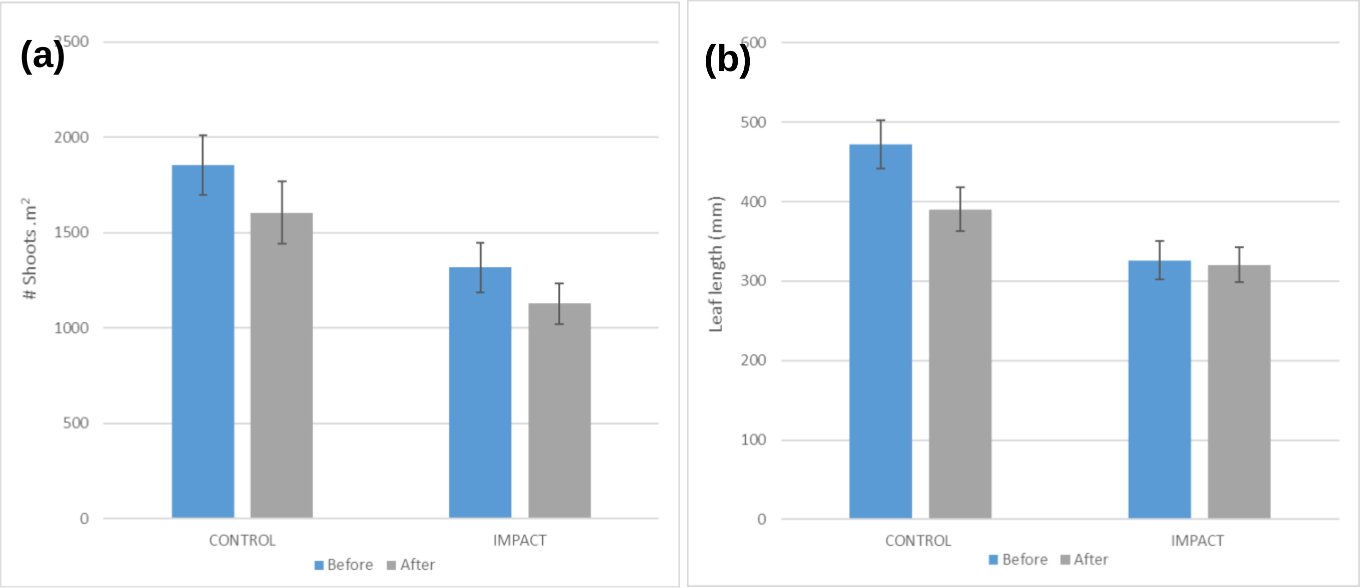

Figure 6 (a) Shoot density between pooled control and impact sites before (2019) and after (2020) dredging, (b) Leaf length between control and impact sites before (2019) and after (2020) dredging. Error bars represent standard error.

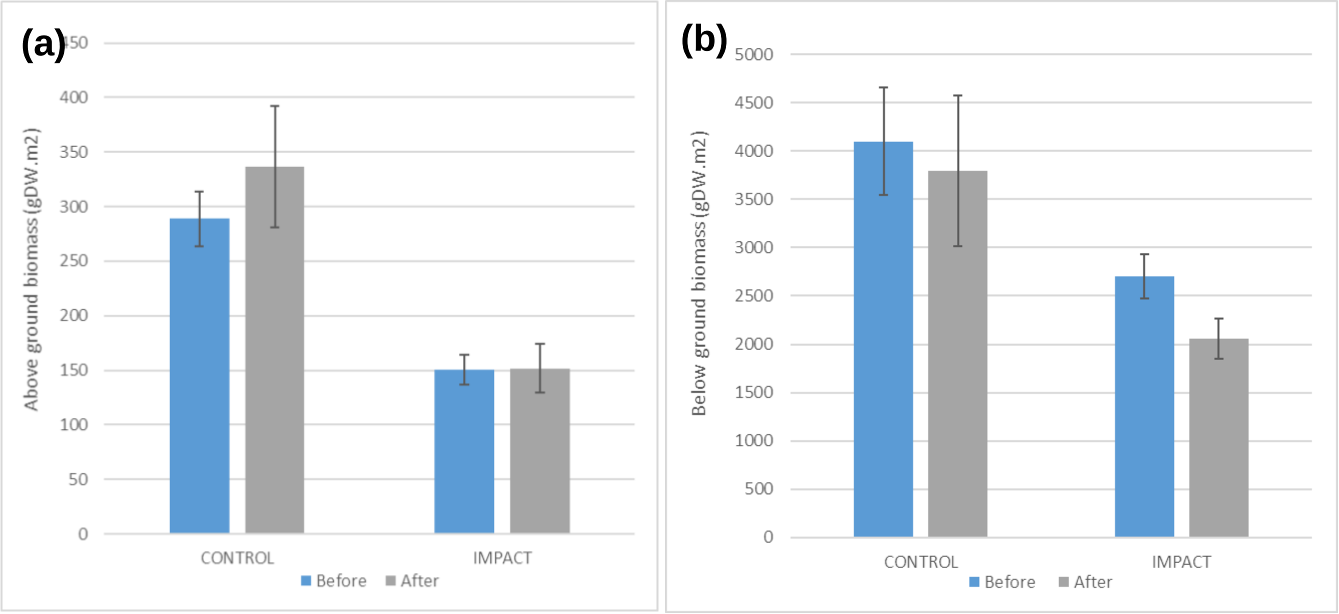

Figure 7 (a) Above ground biomass between pooled control and impact sites before and after dredging, (b) Below ground biomass between control and impact sites before (2019) and after (2020) dredging. Error bars represent standard error.

Above and below ground biomass measures the dry weight of the sample as a measure of condition. Consistent with other metrics, there was no difference between the two control and two impact sites allowing their pooling. The above and below ground biomass Test x Time PERMANOVA tests also showed no significant impact between control and impact sites as a result of the dredging ($p_{above} = 0.5106$; $p_{below} = 0.7374$).

The analysis indicates that epiphytes abundance on seagrass leaves was not significantly different for the test x time interaction ($p_{epiphytes} = 0.4054$). There were however, apparent differences between the sites and between times with the impact sites consistently higher in epiphytes compared to the control sites, while the 'after' time period was consistently higher than the 'before' survey. Pairwise differences were tested statistically, which showed the difference between epiphytes between before and after was significant at the control site ($p < 0.01$), but the impact site was not significant owing to the very large variability ($p = 0.06$, Appendix 1).

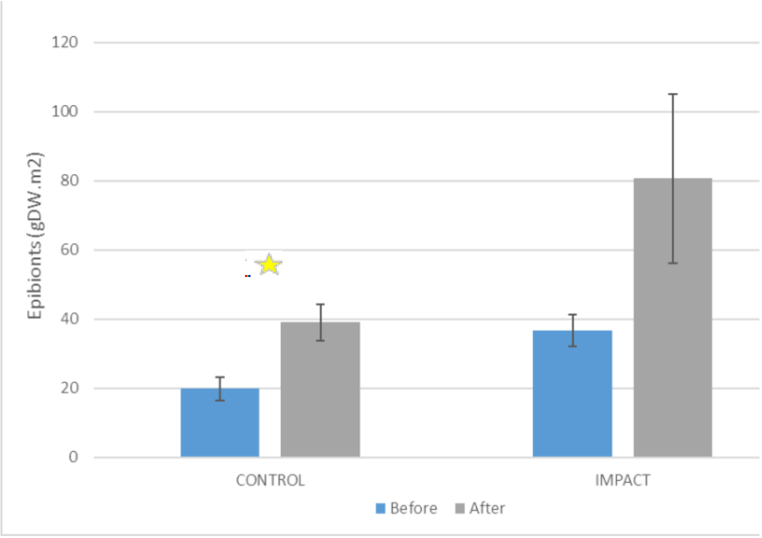

Figure 8 Epiphytes on seagrass leaves between pooled control and impact sites before (2019) and after (2020) dredging. Error bars represent standard error. Star denotes pairwise difference at control site x time.

4 Discussion

The dredging of approximately 2 million m$_2$ of sediment has significant risk of increased local turbidity and risks seagrass health (Erftemeijer and Robin Lewis 2006). *Posidonia* seagrass is the dominant species throughout Gulf St Vincent and it has been shown to have high resilience to light disturbance (O’Brien *et al* 2018a), but if impacted, is very slow to recover, if at all (Irving 2013). Other species, including *Zostera* sp. (formerly *Heterozostera*), are present inside the Outer Harbor shipping channel and are less resilient to disturbance, but are much quicker to recover (O’Brien *et al* 2018a).

The seagrass data demonstrates that prior to the dredging there were differences between the control and impact sites in terms of seagrass cover. This is likely an artefact of the proximity to the shipping channel and the long history of pollution exiting the Port River (Gaylard *et al* 2013a). Notwithstanding this, the seagrass cover at the impact sites was considered to be moderate to dense cover and in Fair to Good condition using the EPA Aquatic Ecosystem Condition Report system of classification (Gaylard *et al* 2013b), and is within the typical range of *Posidonia* meadows in southern Australia (Fernandes *et al* 2009, Bryars *et al* 2011, Nayar *et al* 2012).

Regardless of this difference in cover between sites prior to the dredging, the BACI statistical tests the Site (or Test) x Time interaction to examine the change over time in relation to the control and impact sites. This small condition difference is likely to have negligible effect on the results. There may be an argument that seagrass habitats in poorer condition may be less resilient to disturbance (O’Brien *et al* 2018a), however the results indicate no significant difference providing a further degree of confidence that the dredging did not have an impact at the sites tested.

Epiphytic assemblages are a diverse mixture of coralline and filamentous algae, sponges and other organisms which compete for space on the seagrass leaves (Borowitzka *et al* 2006). These epiphytic assemblages have different sizes, shapes and volumes which result in differences between visual estimates of cover compared to biomass contributing to small scale variability. The epiphyte biomass results show that while the Site x Time interaction was not significant to demonstrate an effect due to the dredging, there was apparent difference between the epiphytes at the control site and the impact site, particularly in the ‘After’ time step.

This site demonstrated substantial variability (Figure 8), which is common with surveys of epiphytes (Bryars *et al* 2011). Local scale hydrodynamics, proximity to nutrient sources, the particular mix of epiphytic species and the proximity to other seagrass or reef habitats are all factors that can influence small scale variability in epiphytic growth on seagrass and the survey results (Bryars *et al* 2011). Remobilisation of nutrients from dredged sediments also cannot be ruled out.

Under light stress, *Posidonia* seagrass can survive for extended periods of time by using their large carbohydrate stored in the rhizomes (O’Brien *et al* 2018b). It is possible that the seagrass surveyed in this study have used existing substantial carbohydrate reserves, this may mean they will be less resilient to any further disturbance (Unsworth *et al* 2015). It is advised that caution is exercised as declines in seagrass cover may occur into the future due to the lack of carbohydrate reserves. Further monitoring is required and planned for 2022, but as time goes on, ability to differentiate impacts from dredging compared to other factors becomes more difficult. Understanding changes in stored carbohydrates (and other biomarkers) within seagrass will help to understand the impact of reduced light on plant physiology, providing a fine scale measure of impact before the seagrass is lost. This will define useful thresholds for South Australian *Posidonia* seagrass under light stress and subsequent recovery.

5 Conclusion

The EPA undertook seagrass monitoring to independently assess the impact of a large capital dredge campaign on seagrass condition at Outer Harbor in 2019. The survey used a before after control and impact design to assess whether the turbidity generated from the dredge program had an impact on seagrass condition. Seagrass was assessed in similar seasons in 2019 and compared to 2020.

While further surveys will be undertaken in 2022, it is apparent that up until March 2020, there has been no detectable seagrass loss using multiple lines of evidence and a variety of different methods within the zone of influence as a result of the dredging. This survey did not assess seagrass loss in the areas that were approved for loss (ie ZoLM and ZoI) as this is covered by the Native Vegetation Council permit system.

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Appendix 1 Statistical outputs

Total Seagrass Cover Towed underwater video

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ti	1	49526	49526	17.56	0.0913	4205
Te	1	1.2571E+06	1.2571E+06	35.924	0.0001	6
Si(Te)	2	69995	34997	52.106	0.0001	9957
TixTe	1	72166	72166	25.588	0.0677	4343
TixSi(Te)	2	5641.2	2820.6	4.1995	0.015	9951
Res	4996	3.3556E+06	671.65			
Total	5003	4.7344E+06				

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Te	1	1.2553E+06	1.2553E+06	1831.3	0.001	998
Ti	1	51843	51843	75.632	0.001	997
TexTi	1	74965	74965	109.36	**0.001**	998
Res	5000	3.4273E+06	685.46			
Total	5003	4.7344E+06				

Shows significant test x time interaction

PAIR-WISE TESTS

Term 'TexTi' for pairs of levels of factor 'Time'

Within level 'Control' of factor 'Test'

Groups	t	P(perm)	Unique perms
After, Before	1.8294	0.06	511

Within level 'Impact' of factor 'Test'

Groups	t	P(perm)	Unique perms
After, Before	10.858	**0.001**	894

Seagrass epiphytes (Towed underwater video)

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ti	1	1.7365E+05	1.7365E+05	0.58602	0.516	917
Te	1	1.3067E+06	1.3067E+06	8.8705	0.359	6
Si(Te)	2	2.9465E+05	1.4733E+05	447.91	0.001	998
TixTe	1	708.11	708.11	0.0023896	0.96	903
TixSi(Te)	2	5.9272E+05	2.9636E+05	901.01	**0.001**	999
Res	4996	1.6433E+06	328.92			
Total	5003	4.2448E+06				

Shows Time x Site nested in Test is significant = test all sites individually.

PAIR-WISE TESTS

Term 'TixSi(Te)' for pairs of levels of factor 'Time'

Within level 'Control' of factor 'Test'

Within level 'Control1' of factor 'Site'

Groups	**t**	**P(perm)**	**Unique perms**
After, Before	46.663	**0.001**	796

Within level 'Control' of factor 'Test'

Within level 'Control2' of factor 'Site'

Groups	t	P(perm)	Unique perms
After, Before	6.4481	**0.001**	444

Within level 'Impact' of factor 'Test'

Within level 'Impact1' of factor 'Site'

Groups	**t**	**P(perm)**	**Unique perms**
After, Before	40.185	**0.001**	863

Within level 'Impact' of factor 'Test'

Within level 'Impact2' of factor 'Site'

Groups	**t**	**P(perm)**	**Unique perms**
After, Before	9.6913	**0.001**	706

Shoot density

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Ti	1	4.8394E+05	4.8394E+05	9.031	0.0992	1226
Te	1	2.5746E+06	2.5746E+06	25.319	0.3288	3
Si(Te)	2	2.0337E+05	1.0169E+05	0.47848	0.6283	9946
TixTe	1	8908.7	8908.7	0.16625	0.7057	1226
TixSi(Te)	2	1.0717E+05	53587	0.25215	0.7867	9955
Res	32	6.8006E+06	2.1252E+05			
Total	39	1.0179E+07				

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Te	1	2.5746E+06	2.5746E+06	13.034	0.0012	9542
Ti	1	4.8394E+05	4.8394E+05	2.4499	0.1252	9574
TexTi	1	8908.7	8908.7	0.0451	0.8259	9589
Res	36	7.1112E+06	1.9753E+05			
Total	39	1.0179E+07				

Demonstrates that Test x time is not significant therefore no difference between BACI design.

Leaf length

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**	**P(MC)**
Ti	1	18831	18831	1.6742	0.3236	2473	0.3213
Te	1	1.1643E+05	1.1643E+05	4.9876	0.3244	3	0.1571
Si(Te)	2	46690	23345	4.252	0.0229	9946	0.023
TixTe	1	14489	14489	1.2882	0.3807	2470	0.378
TixSi(Te)	2	22496	11248	2.0487	0.1464	9940	0.1493
Res	32	1.7569E+05	5490.4				
Total	39	3.9463E+05					

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**	**P(MC)**
Te	1	1.1643E+05	1.1643E+05	17.117	0.0002	9822	0.0004
Ti	1	18831	18831	2.7684	0.1062	9833	0.1107
TexTi	1	14489	14489	2.1301	0.1569	9821	0.149
Res	36	2.4488E+05	6802.2				
Total	39	3.9463E+05					

Demonstrates that Test x time is not significant therefore no difference between BACI design.

Above ground biomass

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Ti	1	5960.5	5960.5	0.66957	0.5015	2463
Te	1	2.601E+05	2.601E+05	68.119	0.3369	3
Si(Te)	2	7636.7	3818.4	0.32842	0.7466	9955
TixTe	1	5445.3	5445.3	0.6117	0.5102	2464
TixSi(Te)	2	17804	8901.9	0.76566	0.4837	9951
Res	32	3.7205E+05	11626			
Total	39	6.6899E+05				

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Te	1	2.601E+05	2.601E+05	23.557	0.0001	9844
Ti	1	5960.5	5960.5	0.53984	0.4855	9866
TexTi	1	5445.3	5445.3	0.49318	0.5106	9870
Res	36	3.9749E+05	11041			
Total	39	6.6899E+05				

Demonstrates that Test x time is not significant therefore no difference between BACI design.

Below ground biomass

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ti	1	2.2469E+06	2.2469E+06	1.7523	0.3118	2463
Te	1	2.4576E+07	2.4576E+07	35.984	0.3414	3
Si(Te)	2	1.3659E+06	6.8297E+05	0.253	0.7814	9957
TixTe	1	2.8685E+05	2.8685E+05	0.22371	0.6649	2457
TixSi(Te)	2	2.5645E+06	1.2822E+06	0.475	0.641	9957
Res	32	8.6383E+07	2.6995E+06			
Total	39	1.1742E+08				

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Te	1	2.4576E+07	2.4576E+07	9.7964	0.0021	9866
Ti	1	2.2469E+06	2.2469E+06	0.89565	0.3637	9861
TexTi	1	2.8685E+05	2.8685E+05	0.11434	0.7374	9821
Res	36	9.0314E+07	2.5087E+06			
Total	39	1.1742E+08				

Demonstrates that Test x time is not significant therefore no difference between BACI design.

Epiphyte biomass

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ti	1	10013	10013	2.8332	0.1168	2475
Te	1	8545.6	8545.6	3.6457	0.3248	3
Si(Te)	2	4688	2344	1.5806	0.2111	9961
TixTe	1	1549.4	1549.4	0.43841	0.7654	2467
TixSi(Te)	2	7068.1	3534	2.383	0.074	9950
Res	32	47456	1483			
Total	39	79320				

Shows Time x Site nested in Test is not significant = pool control 1 and 2

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Te	1	8545.6	8545.6	5.1956	**0.0086**	9892
Ti	1	10013	10013	6.0875	0.0031	9896
TexTi	1	1549.4	1549.4	0.94199	0.4054	9893
Res	36	59212	1644.8			
Total	39	79320				

Demonstrates that Test x time is not significant therefore no difference between BACI design. However the Test factor is significant meaning there is a difference between before and after at the Control sites.

Within level 'IMPACT' of factor 'Test'

Groups	**t**	**P(perm)**	**Unique perms**
BEFORE, AFTER	1.7734	0.0604	8049

Within level 'CONTROL' of factor 'Test'

Groups	**t**	**P(perm)**	**Unique perms**
BEFORE, AFTER	3.0437	**0.0064**	5807