SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Scientific Inquiry

Year 11-12

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Macroalgae as Bioremediators: *Photosynthesis and Nitrogen Absorption of South Australian Macroalgae*

To what extent do select macroalgae species (*Gracilaria; Ulva lactuca; Sargassum horneri; Codium fragile; Hormosira banksii*) from West Beach, South Australia, reduce seawater nutrients as measured by ammonia (NH_3 mg/L) and nitrite (NO_2 mg/L) concentrations, and perform photosynthesis as measured by seawater acidity (pH), and what does this indicate about their potential for eutrophication bioremediation?

Word Count[1]: 1,978, in-text references 93w.
However, this includes Table 11.

Just fill out another risk assessment or fake logbook

Note: Justifications are in red text

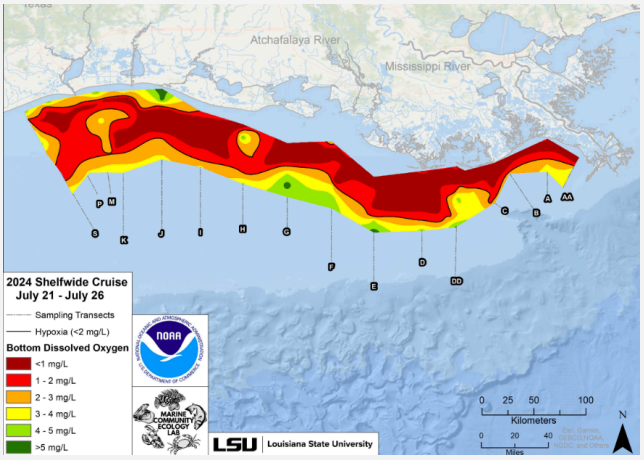
[1] Excluding titles, subheadings, data tables, equations, captions,footnotes and in-text referencing

BACKGROUND

Waste management is a complex global environmental threat which has become a global concern due to anthropogenic activities. Algal blooms, caused by excess nutrients in the water, trigger anoxic[2] conditions in a process called eutrophication[3] (National Ocean Service, 2021). Oceanic eutrophication, predominantly caused by polluted water discharged by aquaculture or estuaries.

From a technocentric, anthropocentric or ecocentric perspective, eutrophication is desirable to mitigate due to severe economic and environmental ramifications. In 2024, the annual hypoxic zone in the Gulf of Mexico caused by nutrient-discharge from the Mississippi River covered making it inhospitable for marine life, Figure 1 (National Oceanic and Atmospheric Administration, 2024).

Figure 1: Map of Hypoxic Zone in the Gulf of Mexico in 2024 (US Department of Commerce, 2024)



Another severe example of eutrophication is the algal blooms in Lake Taihu, China that have suspended drinking water for millions of people for decades (Donghao, W. et al., 2022). Annual costs from eutrophication have been estimated at around $1 billion for European coasts and $2.4 billion for US freshwater.

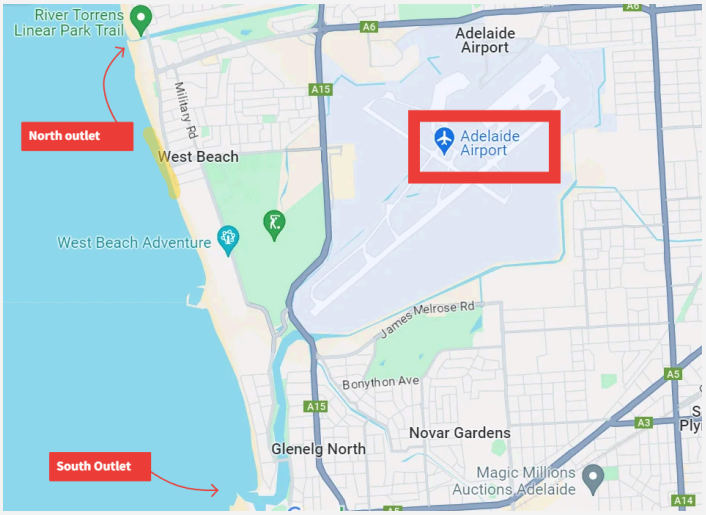
Macroalgae have high nutrient bioextraction capabilities making them effective bioremediators for eutrophic waters (Xiao et al. 2017; Aquilino et al. 2020), but their current application is limited. In order to investigate the effectiveness of macroalgae as bioremediators, the NH_3 and NO_2 absorption, and photosynthesising abilities of macroalgae from South Australia (SA) will be investigated. The findings of this study would inform species-specific environmental policy and management practices surrounding the use of macroalgae as biofilters.

[2] See Glossary.
[3] See Glossary.

SITE DESCRIPTION

Sampling occurred at West Beach, SA. Despite having globally significant levels of diverse and endemic species (Womersley 1981a, 1990; Huisman 2000) and a history of Indigenous cultivation, SA does not currently utilise macroalgae. Prominent features that influence water-quality are noted in red in Figure 2. The north outlet is at the end of the River Torrens, which flows through the city-centre and is highly polluted.

Figure 2: Map of West Beach, Gulf of St Vincent, South Australia



QUESTIONING

To what extent do select macroalgae species (*Gracilaria*; *Ulva lactuca*; *Sargassum horneri*; *Codium fragile*; *Hormosira banksii*) from West Beach, South Australia, reduce nutrients in seawater as measured by ammonia (NH_3 mg/L) and nitrite (NO_2 mg/L) concentrations, and perform photosynthesis as measured by seawater acidity (pH), and what does this indicate about their potential for eutrophication bioremediation?

PREDICTING

Macroalgae can be classified into three taxonomic groups (green; red; brown) based on photosynthetic pigments that correspond with the different light-waves received at the depth of each species' natural habitat (Gorsuch, H. n.d.). All algae have chlorophyll, but red (phycobiliproteins) and brown (carotenoids) have additional pigments to absorb light waves that penetrate deeper into water.

Three main factors affecting the nutrient removal rate of macroalgae include: biological characteristics (growth-rate, species, structure); nutrient availability (loading-rate, type of nitrogen (N)); physical conditions (light, pH, carbon dioxide, dissolved-oxygen) (Ramli, N.M., 2020). Hence, the different macroalgae species will have varying abilities to photosynthesise and absorb nutrients from seawater.

VARIABLES

1. Independent Variable

Minimal macroalgae biomass used to reduce waste, but enough for the macroalgae to survive.

1.1 Macroalgae species

- 0.5 grams (g) of macroalgae per sample in 20 millilitres (mL) of artificial-seawater
- 0.3g of macroalgae per sample in 3mL of HCO3 indicator

HCO_3 Indication had smaller biomass because of shorter experiment duration and smaller test-tubes used

Table 1: Macroalgae Species

				
Gracilaria (G) "Irish-moss"	***Ulva lactuca (UL)*** "Sea-lettuce"	***Sargassum horneri (SH)*** "Devil-weed"	***Codium fragile (CF)*** "Dead-man's fingers"	***Hormosira banksii (HB)*** "Neptune's-necklace"

Chosen according to species abundant in SA, and to have a range of pigment types for variation. Minimum of 5 species to satisfy experimental design.

2. Dependent Variables

Accessible in a secondary school environment and provide quick results with minimal complexity or margin for error

2.1 Nutrient absorption

- Measure change in NH_3 and NO_2 concentration using API-testing-kit (mg/L) and colorimeter (AU[4])

2.2 Photosynthesis

- Measure change in CO2 using HCO_3 indicator (mg/L) and colorimeter (AU)

Colorimeter produces a qualitative reading which improves accuracy by removing human error, rather than comparing the sample to a colour chart

3. Controlled Variables

Table 2: Controlled Variables

Variable	***Effect on results***	***Method of control***
Water temperature (°C)	Affects algal metabolism (Pedersen, A. et al., 2004) and therefore nutrient uptake. Extreme temperatures <5°C or >30°C (DPIR, 2022) harmful.	Water-bath ⟶ consistent water temperature (20°C).
Sample mass (g)	Greater biomass ⟶ larger surface-area ⟶ absorbs more nutrients.	Biomass ⟶ electronic scale
Seawater / HCO_3 indicator volume per sample (mL)	Determines nutrient volume	Seawater ⟶ 25mL measuring cylinder HCO_3 indicator ⟶ 3mL syringe
NH_3, NO_2, pH, salinity (mg/L) concentrations	Inconsistent initial concentrations affect final concentrations. Extreme concentrations are harmful.	Single batch of artificial-seawater with standard concentration
Experiment duration (hours)	Longer duration ⟶ increased absorption	Remove macroalgae from test-tubes simultaneously.
Light intensity	Affects photosynthesis and nutrient absorption.	Grow-light
Acclimatisation	No acclimation ⟶ stressed macroalgae / unregulated nutrient absorption due to temperature changes (Geppi, E.F., 2022)	1 week artificial-seawater and experimental temperature exposure before data collection.

Chosen by accounting for the optimal temperature range for each species and the winter sea temperature (experiment conducted in June/July) in SA

Ensures time is controlled equally across samples so macroalgae no longer continues absorbing nutrients during testing

Means light intensity is consistent, and non-reliant on uncontrollable factors such as daylight cycles

[4] (Absorbance) A measure of the amount of light captured by a substance at a particular wavelength. Unit is AU.

SAMPLING STRATEGY

Location chosen due to accessibility and credibility: referenced in various sources for macroalgae sampling (Edyvane, K. 2008).

Time chosen to coincide with low tide.

Reduces harm / stress such as drying

Researched native macroalgae species abundant during sampling season. Sampled 500m of shoreline north of the Henley Sailing Club at West Beach, SA on 26/06/24 at 7:30 - 8am. Beached macroalgae submerged in bucket of seawater. In lab, samples washed, sorted and checked for live organisms (none found). Species identified using factsheet from State Herbarium (flora.sa.gov.au, n.d).

If live organisms are found ensure ethical guidelines are followed by immediately returning them to sample location

Table 3: Materials

Quantity	*Specification*	*Equipment*	*Uncertainty*
1	-	Water-bath	±0.2°C
1	-	Electronic-scale	±0.01g
1	-	Vernier-colorimeter	±0.005AU
1	-	Thermometer	±0.5°C
1	3mL	Syringe	±0.15mL
6	1000mL	Beaker	±5mL
5	Silicone	Oxygen-tubes	-
26	50mL	Test-tube	-
26	3mL	Screw-top glass test-tubes	±0.15mL
1	5x5	Styrofoam test-tube rack	-
1	150W	Heat-lamp	-
1	-	Grow-light	-
5	>10g per species	Macroalgae	-
1	NH_3 Solution #1; NH_3 Solution #2; NO_2 Reagent #1	API Testing-Kit	-
-	1000mL	Artificial-seawater (35g seawater-salt-mix / 8mg/L NH_4Cl / 80mg/L $NaNO_3$)	-
-	100mL	HCO_3 Indicator-Solution	-
26	-	Cuvettes	-

To stabilise 50mL test-tubes in water-bath

METHOD[5]

1 Artificial-Seawater Preparation and Macroalgae Acclimitisation

1.1 Prepare artificial-seawater, repeat as required.
- 1.1.1 Dissolve 8mg of NH_4Cl and 80mg of $NaNO_3$ in 1000mL of tap water.
- 1.1.2 Increase salinity to 35ppt[6] using seawater-salt-mix.

1.2 Sort macroalgae by species.

[5] Always rinse all equipment with distilled water between and after uses to prevent contamination, and follow all lab guidelines including personal protective equipment.
[6] Average salinity of ocean water (Webb, P. n.d.).

1.3 Rinse with distilled water.
1.4 Submerge in artificial-seawater in 1000mL beakers under grow-light with oxygen-tubes and plastic cover.
1.5 Allow 48h to acclimate[7].

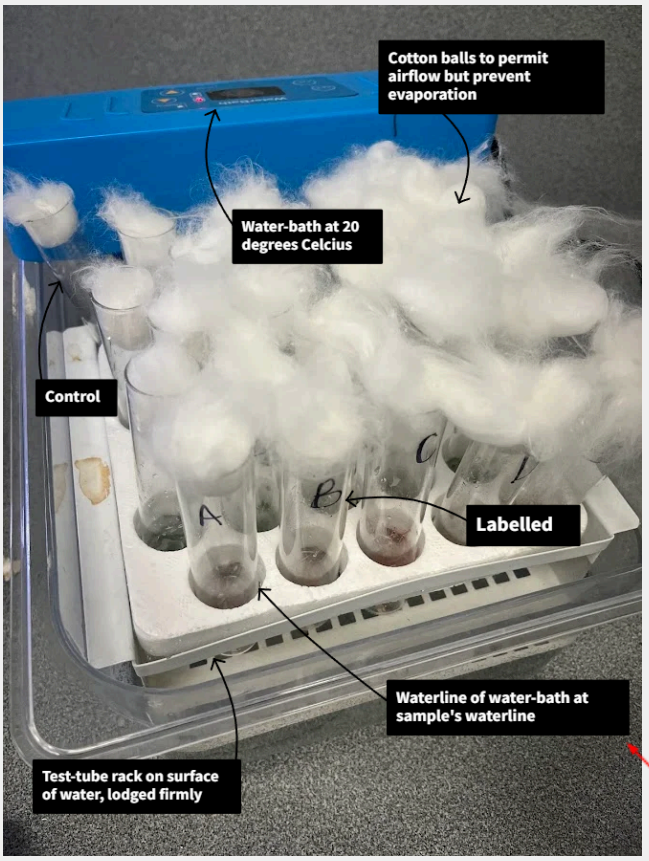
Cover prevents excess evaporation

2 Preparation of Macroalgae Samples

Water will contribute to mass of sample and reduce accuracy

2.1 Dry[8] and prepare five 0.5g samples[9] / species on electronic scale[10].
2.2 Submerge macroalgae in 20mL of artificial-seawater in 50mL test-tube.
2.3 Set-up as per Figure 3, including a control without macroalgae.

Figure 3: Labelled samples in Water-bath



Means temperature of sample is uninfluenced by air temperature and held at Water-bath's temperature

2.4 Measure initial NH_3 and NO_2 concentrations of artificial-seawater[11].
2.5 After 17h remove macroalgae from test-tubes.
2.6 NH_3 and NO_2 Indicators.
2.7 Follow API testing-kit method.
2.8 Put samples through colorimeter.

To follow scientific precedents in acknowledging methods from direct sources

[7] See Table 1: Variables.
[8] See Evaluation.
[9] As close to 0.5g as possible given propagating considerations: some species have larger blades and will need more biomass to survive (see Evaluation).
[10] When 'propagating' the macroalgae, snip as close as possible to the stipe rather than the tips of the blades (gives the samples the highest chance of survival).
[11] See *API Testing-Kit: NH_3 and NO_2 Indicators* method.

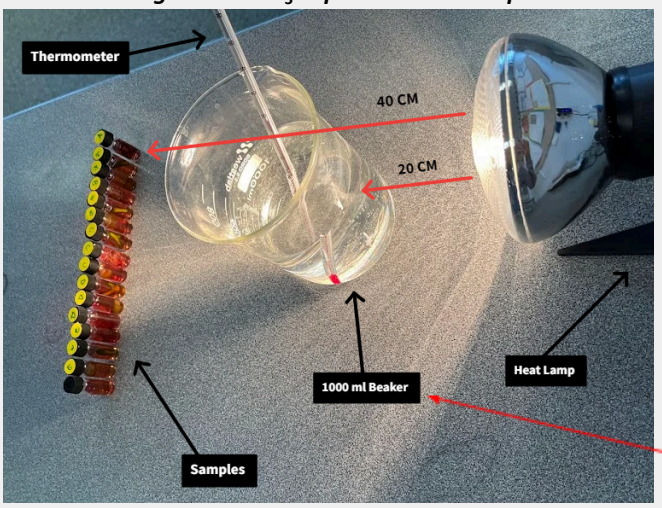
3 HCO_3 Indicator

3.1 Prepare five 0.3g samples / species in labelled screw-top test-tubes with 3mL of HCO_3 indicator-solution and a control without macroalgae.

3.2 Line up half of the samples as outlined in Figure 4[12]. Randomise order using random-number-generator (Wheel of Names, 2019).

Beam of light has unequal strength (more concentrated in centre) → variation in light received by samples in middle compared to ones on end. Randomising order eliminates bias from this error to minimise its significance.

Figure 4: HCO_3 Experimental Set-up



Heat-sink to minimise the effect of the heat lamp on the samples; thermometer to monitor temperature

3.3 After 30 minutes, note colour change compared to standard concentration colour range[13]. After 1h there should be significant colour change. If not, leave until one is observed.[14]

3.4 Indicator changes from red to yellow if CO_2 increases, and from red to purple if it decreases.

4 Colorimeter

4.1 Open Vernier-Graphical-Analysis app and connect colorimeter to laptop with USB cord.

4.2 Select correct wavelength for solution (NH_3 635 nm; NO_2 565 nm; HCO_3 565).

4.3 Prepare a cuvette with 3mL of solution to be tested and a blank with 3mL of the original artificial-seawater-solution.

4.4 Calibrate colorimeter following manufacturer's instructions.

4.5 Place sample cuvette in slot and close lid.

4.6 When reading stabilises record AU.

4.7 Repeat for each sample.

To follow scientific precedents in acknowledging methods from direct sources

[12] Unable to test all samples simultaneously, as the beam of light does not extend to reach all samples when lined up.
[13] If there is no access to a standard range of concentrations at regular increments, there are colour charts available online, although this step is not vital since quantitative measurements are collected.
[14] As this test measures the pH of the water, which changes as CO_2 is absorbed during photosynthesis, the photosynthetic process will need time to occur and trigger a colour change.

RISK ASSESSMENT

Table 10: Risk Assessment

Hazard	*Risk*	*Alterations to Minimise Risk*
Heat lamp	Contact with surface → severe burns (moderate-high likelihood and severity)	PPE. Avoid contact.
Broken glass	Glass equipment may break → sharp fragments (cuts) (moderate-high likelihood and severity)	Sweep up broken glass. Dispose of safely.
Field work	Sun exposure → sunburn, dehydration. Beach debris → slips, trips. (moderate-high likelihood and severity)	Appropriate gear. Bring water. Gloves when handling samples.
Chemical Hazard	*Risk*	*Alterations to Minimise Risk*
NH_4Cl	Irritant (high-likelihood; low-severity). Harmful if swallowed (low-likelihood; moderate-severity)	PPE. Ventilation. Avoid ingestion.
$NaNO_3$	Oxidizer (moderate-likelihood; high-severity). Toxic if ingested (low-likelihood;-high severity)	Store separately. PPE. Avoid heat sources.
Ethical Considerations	*Risk*	*Alterations to Minimise Risk*
Macroalgae collection	Disruption of natural habitat (moderate).	Avoid oversampling. Non-destructive sampling-techniques. Return specimens to habitat when possible. Adhere to local conservation guidelines. Minimise stress during transport/analysis.
Attached organisms	Unintentional harm to other species and disruption of microecosystems (moderate).	Inspect samples before removal. Return attached organisms.
Academic	Accuracy / reliability of data and scientific integrity (high).	Use standardised collection methods. Document all procedures. Transparency about limitations.

RAW DATA

Table 4: Absorbance (AU) of NH_3 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.941	1.134	1.389	1.248	1.179	1.1782
UL	0.444	0.482	0.499	0.535	0.607	0.5134
SH	0.523	0.51	0.571	0.54	0.814	0.5916
CF	0.396	0.491	0.442	0.552	0.518	0.4798
HB	0.601	0.523	0.506	0.578	0.562	0.554
Control	0.949	-	-	-	-	0.949

Table 5: Absorbance (AU) of NO_2 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.48	0.706	0.411	0.586	0.667	0.57
UL	0.394	0.639	0.45	0.547	0.653	0.5366
SH	0.102	0.674	0.506	0.559	0.664	0.501
CF	0.082	0.118	0.106	0.179	0.219	0.1408
HB	0.169	0.175	0.195	0.312	0.579	0.286
Control	0.1	-	-	-	-	0.1

Table 6: Absorbance (AU) of HCO_3 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.859	0.747	0.555	1.011	0.881	0.8106
UL	1.005	0.937	1.05	0.999	0.953	0.9888
SH	0.46	0.654	0.641	0.399	0.451	0.521
CF	0.724	0.791	0.797	0.718	0.669	0.7398
HB	0.757	0.846	0.674	0.044	0.737	0.6116
Control	0.553	-	-	-	-	0.553

Table 7: Absorbance of Known NH_3 Standard Concentrations for Calibration Curve

Concentration (ppm)	1	2	4	6	8	10
Absorbance	0.43	0.835	1.794	2.119	2.432	2.482

Table 8: Absorbance of Known Standards of HCO_3 (pH) Test for Calibration Curve

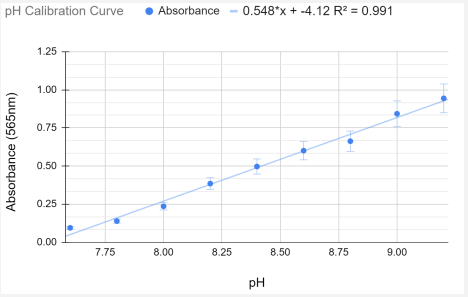
pH	9.2	9.0	8.8	8.6	8.4	8.2	8.0	7.8	7.6
Absorbance	0.944	0.843	0.663	0.601	0.497	0.385	0.236	0.139	0.095

PROCESSED DATA

Table 9: Mean Absorbance (AU) of NH_3, NO_2 and HCO_3 per Macroalgae Species

Macroalgae by Colour	NH_3	NO_2	HCO_3
G	1.1782	0.57	0.8106
UL	0.5134	0.5366	0.9888
SH	0.5916	0.501	0.521
CF	0.4798	0.1408	0.7398
HB	0.554	0.286	0.6116
Control	0.949	0.1	0.553

Table 11: Calculations

Formula	*Sample calculation*
Mean $\bar{x} = \frac{\Sigma x}{n}$ n = total number of samples	$\bar{x} = \frac{(0.941 + \ldots + 1.179)}{5} = 1.782$
Standard Deviation $\sigma^2 = \frac{\Sigma(xi - \mu)^2}{N}$ N = size of population μ = population mean xi = each value from the population	Online standard deviation calculator used (Calculator.net, 2024)
Standard Error $SE = \frac{\sigma}{\sqrt{n}}$ σ = standard deviation of samples n = number of samples	$SE = \frac{0.147}{\sqrt{5}} = 0.066$
Calibration Curves Known concentration of standards plotted against their absorbance to create a calibration curve on Excel. The equation of the line of best fit was used to calculate the concentrations of experimental values (x) from absorbance (y).	***Figure 5: pH Calibration Curve***  $0.8106 = 0.548x - 4.12$
Analysis of Variance (ANOVA) Determines between the null hypothesis (H_0) and alternative hypothesis (H_1) The two values significant to this analysis are the *F* and *p* values. *F* = denotes a difference among the species' means (higher value = greater difference). Indicates whether the variations between the species are significant: if $p < 0.05$ the null hypothesis can be rejected (SurveySparrow, 2024)	H_0: *There is no difference in the NH_3 absorbance based on macroalgae species.* H_1: *There is a significant difference in NH_3 absorbance based on macroalgae species.* Online calculator used (statpages.info, n.d.) ***Figure 6: NH_3 ANOVA Calculation*** Source of Variation | Sum of Squares | d.f. | Variance | F | p Between Groups: | 1.6918 | 4 | 0.4229 | 50.8243 | 0.0000 Within Groups: | 0.1664 | 20 | 0.0083 Total: | 1.8582 | 24
Post-hoc tests: Tukey's HSD $Diff$ = mean difference between each pair of groups CI = confidence interval (95%) p = whether difference is statistically significant	Online calculator used (statpages.info, n.d.) ***Figure 7: NH_3 Tukey's HSD*** Red vs L.Green: Diff=-0.6648, 95%CI=-0.8374 to -0.4922, p=0.0000 Red vs D.Brown: Diff=-0.5866, 95%CI=-0.7592 to -0.4140, p=0.0000 Red vs D.Green: Diff=-0.6984, 95%CI=-0.8710 to -0.5258, p=0.0000 Red vs L.Brown: Diff=-0.6242, 95%CI=-0.7968 to -0.4516, p=0.0000 L.Green vs D.Brown: Diff=0.0782, 95%CI=-0.0944 to 0.2508, p=0.6614 L.Green vs D.Green: Diff=-0.0336, 95%CI=-0.2062 to 0.1390, p=0.9762 L.Green vs L.Brown: Diff=0.0406, 95%CI=-0.1320 to 0.2132, p=0.9532 D.Brown vs D.Green: Diff=-0.1118, 95%CI=-0.2844 to 0.0608, p=0.3307 D.Brown vs L.Brown: Diff=-0.0376, 95%CI=-0.2102 to 0.1350, p=0.9643 D.Green vs L.Brown: Diff=0.0742, 95%CI=-0.0984 to 0.2468, p=0.7024
Difference $\Delta = F - I$ F = final concentration I = initial concentration	$\Delta NH_3 = 2.6487 - 2.1102 = 0.5385$

CALIBRATION CURVES

Figure 8: Calibration Curve for NH_3

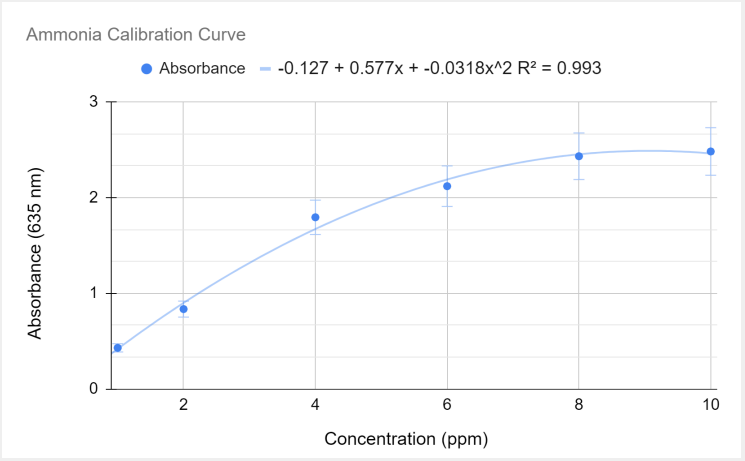


Figure 9: Calibration Curve for NO_2 (Nery, N. and Putra, E.D.L. 2018)

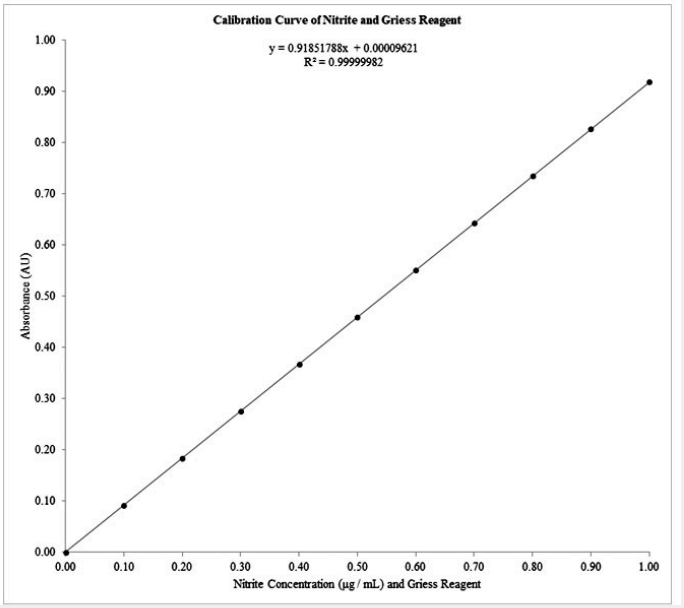
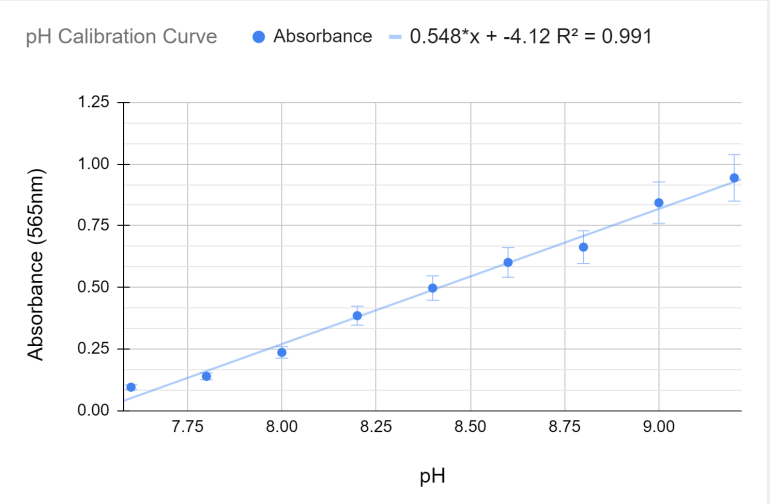


Figure 10: Calibration Curve for pH



GRAPHS

Figure 11: NH_3 Concentration at 0 Hours vs 17 Hours

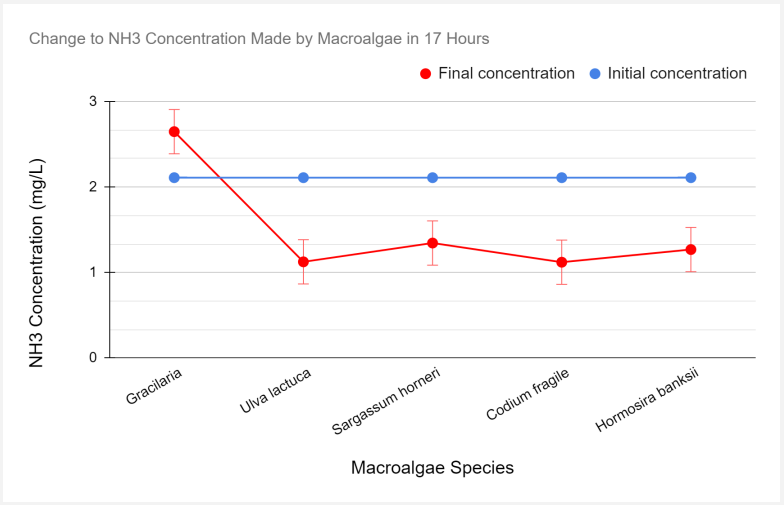


Figure 12: NO_2 Concentration at 0 Hours vs 17 Hours

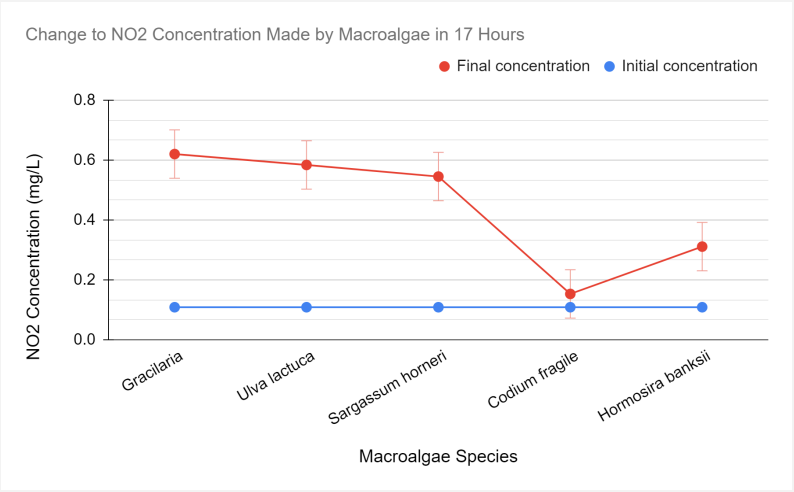
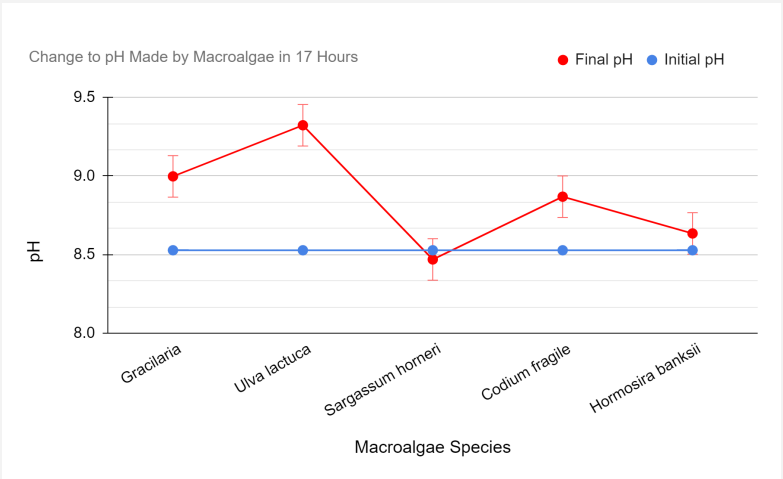


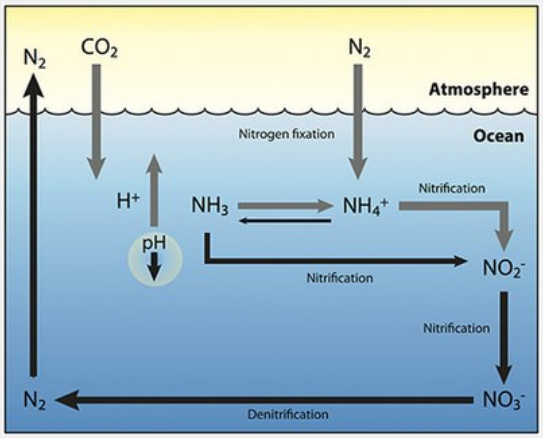
Figure 13: pH at 0 Hours vs 17 Hours



ANALYSIS

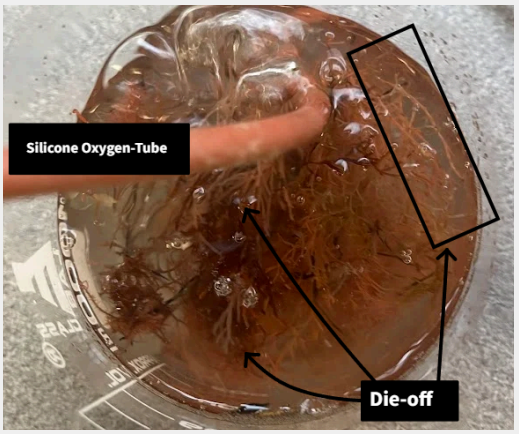
Results revealed variations in NH_3 and NO_2 absorption between species, albeit no consistent pattern, suggesting species-specific nutrient preferences. NH_3 concentration was reduced by four of the five species (Figure 11) and NO_2 concentration increased across all species (Figure 12). This suggests that the nitrification process is occurring (Figure 14), but denitrification was not completed due to insufficient experiment duration[15]. ANOVA confirmed statistically significant variation between species for NH_3, NO_2 and pH tests.

Figure 14: Nitrogen Cycle (O'Brian P.A. et al., 2016)



G is an anomaly - substantiated by the post-hoc test ($p < 0.0001$), because, unlike other species, it caused NH_3 concentration to increase. It was observed that parts of the macroalgae were translucent which indicates die-off [16]. Partial decay would cause NH_3 concentration to increase. *G*'s higher standard deviation indicates potential inconsistent measurements and random error. *G* showed a high pH, second only to *UL*, likely due to the phycobiliproteins it contains[17]. No significant variation was found between other species (ANOVA).

Figure 15: Photo of Gracilaria



[15] See Evaluation.
[16] For more delicate species such as *Gracilaria.*
[17] See Hypothesis.

NO_2 increased across all species. The post-hoc test established significant variation between *CF* and most other species. *CF* had a minimal increase of 0.04mg/L, whereas other species increased NO_2 by as much as 0.5mg/L (Figure 12). Research on short-term uptake of fixed inorganic N claims that the competitive advantage *CF* has over other algae and its invasive success (Roleda, M.Y., 2019) is due to its ability to uptake NO_2 (DeBoer, J.A., 1978) where other species can not. This uptake is however dependant on ammonium (NH_4)[18], where concentrations of 0.1mg/L can completely inhibit NO_3 and NO_2 uptake by *CF* (Hanisak & Harlin, 1978). This explains why *CF* by comparison released no significant amount of NO_2, and was unable to absorb any. Post-hoc test shows significant differences between *CF* and all species except *HB*.

UL had the highest photosynthetic activity with a pH of 9.3. It is reasonable to assume this is due to physiological adaptations for good light conditions in its natural habitat; including high concentrations of pigments optimal for photosynthesis (chlorophyll), and thin, sheet-like thalluses that increase surface area-to-volume ratio, enhancing both light and nutrient capture, and enabling rapid growth strategy. The pH of the *SH* solution decreased by 0.1, indicating it was respiring (producing CO_2). Although *CF* demonstrated moderate photosynthetic ability, it showed high NH_3 absorption and the lowest increase in NO_2, suggesting highly efficient N assimilation mechanisms.

CONCLUSION

In regards to the research question, the five macroalgae species from West Beach, SA demonstrated varying abilities to absorb NH_3 and NO_2, and photosynthesise. Most demonstrated partial denitrification; decreasing NH_3 and increasing NO_2 concentrations, with the exception of *G* which caused NH_3 to increase, likely due to decay. pH showed *UL* and *G* had the highest rate of photosynthesis, and *SH* to be respiring. These trends can be explained by physiological adaptations, such as growth rate, species and structure. From this species-specific information, conclusions can be drawn around these macroalgae as bioremediators. *UL* and *G* are optimal for increasing the oxygen-content of water due to high photosynthetic capabilities, and most species are able to significantly reduce NH_3 concentrations. Further research needs to be conducted to determine long-term and specific bioremediation patterns.

EVALUATION

This study has revealed nutrient absorption and photosynthetic capabilities of aforementioned macroalgae species to some extent, providing insights into how different macroalgae species might influence nutrient cycles in coastal ecosystems - informing species-specific approaches to efficient water-quality management. Such information is becoming increasingly pertinent in research surrounding predicting ecosystem responses to, and resilience-building against, climate-change.

Given time and resource constraints in a secondary school, there are errors and limitations:
Random error: Uneven light distribution of heat lamp despite heat-sink.

a) Photosynthesising capability fluctuated depending on proximity to the light beam's centre. Samples on the ends had an absorbance approximately 0.05AU lower than their corresponding sample in the centre, due to

[18] In this case the function of NH4 is comparable to the NH_3 used in this experiment, the only difference being the pH of the water.

lower light-exposure. Ideally, although time consuming, samples would be tested individually, or using a strip-light for even light distribution.

Systematic error: Inconsistent samples.

b) *CF* sample mass was around 0.5g higher than other species during NH_3 and NO_2 testing[19] due to propagation requirements, proportionally affecting nutrient absorbance. The standard sample mass should be set to the highest propagation requirement out of the different macroalgae species.

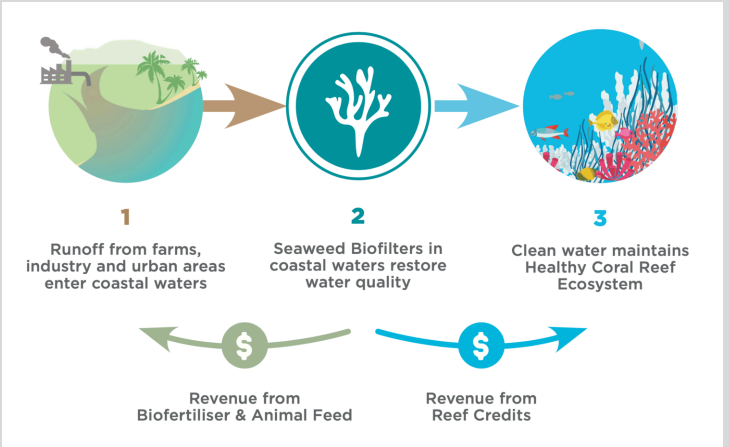
c) Species had uneven water-to-biomass ratios, such that the water-filled-beads made up the majority of *HB*'s sample mass, meaning its nutrient absorption would be proportionally affected. Dry-biomass of each species should be used to find equivalent masses, that would mean water-weight could be accounted for.

A tissue analysis of the carbon:nitrogen ratio (C:N) of the macroalgae would provide an additional metric to complement concentration measurements, providing a more holistic insight into the macroalgae's nutrient cycling. Data-collection at smaller intervals over a longer time-period would have accounted for both rapid "surge-uptake" (Roleda, M.Y., 2019) and long-term patterns, including determining whether the NO_2 increase was part of denitrification processes. Additionally, an experimental design measuring the effect of temperature on nutrient uptake of various macroalgae species, including seasonal variations, would better inform climate-change predictions.

APPLICATION

Effective management of coastal eutrophication includes cultivation of biodiverse macroalgae alongside other strategies including reduced fertiliser inputs and vegetation buffer strips. Introducing macroalgae biofilters to Australia's Great Barrier Reef (Figure 16) is planned to combat toxic waste-water run off that contributes to invasive species outbreaks, including crown-of-thorns starfish (*Acanthaster planci*)[20] (A. Marsden, 2020).

Figure 16: Great Barrier Reef Biofilter Benefits (Australian Seaweed Institute, 2024)

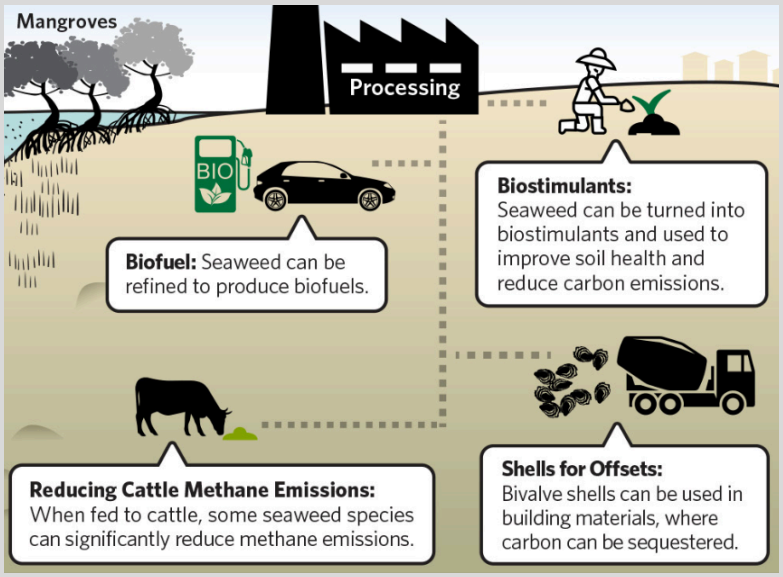


[19] Size requirements to sustain life become obsolete during HCO_3 testing due to short experiment duration (Reefcentral.com, 2007).

[20] See Glossary.

An additional benefit of this solution is the macroalgae derived bioproducts (Figure 16,17) that are more sustainable than chemical alternatives, e.g., biostimulants generate less carbon and their run-off does not contain toxic chemicals. However, return on investment is uncertain with high production costs and limited demand. Scientists are optimistic that over time the balance will tip in favour of bioproducts over their currently cheaper traditional alternatives (The Nature Conservancy, 2024).

Figure 17: Product Uses of Macroalgae (The Nature Conservancy, 2024)



This study informs a species-specific approach to local eutrophication management. In SA, River Torrens outlets, such as the West Beach estuaries, pose a pollution threat to coastal ecosystems. Results revealed *CF* is optimal for NO_2 removal and *UL* has high photosynthetic activity. Cultivating both species targets excess nutrients and depleted oxygen, combating eutrophication better than a single species. Additionally, species diversity reduces vulnerability to severe weather and disease (University of Michigan News, 2022). However, this study is geographically limited as only species native to SA were tested, making results not globally applicable.

Cultivation of coastal macroalgae can aid in remediation and protect waters before they become eutrophic, but as a stand-alone strategy is unable to eliminate a broad environmental issue such as eutrophication. Pollution-prevention strategies are needed in conjunction for effective mitigation.

GLOSSARY

Definitions

- Anoxic: waters depleted of dissolved oxygen
- Crown-of-thorns starfish: An invasive species that over the last 40 years have been one of the major contributors to coral decline (Gbrmpa.gov.au, 2019)
- Endemism: the state of a species only being found in a single defined geographic location (Iberdrola. n.d.)
- Estuary: the tidal mouth of a large river, where the tide meets the stream (Oxford Languages, 2023)
- Eutrophication: excessive richness of nutrients in a lake or other body of water, frequently due to run-off from the land, which causes a dense growth of plant life (Oxford Languages, 2023)
- Hypoxic: similar to 'anoxic'
- Stipe: a stalk or stem (Oxford Languages, 2023)

Abbreviations

- ASI: Australian Seaweed Institute
- Biostimmulants: materials that improve terrestrial crop yields and health, nutrient uptake, stress tolerance, and soil quality (The Nature Conservancy, 2024)
- CF: *Codium fragile*
- G: *Gracilaria*
- GBR: Great Barrier Reef
- HB: *Hormosira banksii*
- HCO_3: Hydrogen-Carbonate
- NH_3: Ammonia
- NH_2: Nitrite
- PPE: Personal protective equipment
- SA: South Australia
- SH: *Sargassum horneri*
- UL: *Ulva lactuca*

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Log Book

1. Ideas

Ideas for Experiments	**Status**	**Notes**
Influence of proximity to urbanisation on Magpie population	No	● Magpie population could be measured by quadrat sampling. Amount of Magpies could be counted or birdsong heard in X minutes could be used to measure population. ● Topic is quite broad ● “Proximity to urbanisation” could be hard to objectively define or control?
Impact of macroalgae species on ability to reduce nutrient concentration in seawater	Yes (topic and experimental design approved by teacher)	● Local study! Able to collect specimens at local beach and simulate conditions in school lab ● Interesting research application potential. Use of macroalgae in climate change management strategies etc ● What species are available for collection? ○ Research conducted on the species available in South Australia: ● Controlled variables include light conditions, water movement, initial biomass of samples ● At what temperature should the samples be kept? Optimal temp for growth varies between species and seasonal acclimatisation. ○ Golden Kelp was the lowest,

		thriving at temperatures from 12 degrees, while Neptune's Necklace could tolerate temperatures up to 40. ○ Also takes into consideration the normal sea temperatures in South Australia (min. 12 in winter, max 23 in summer), and how these could be impacted by climate change. ● Do macroalgae samples have to acclimatise to simulated conditions?? ○ Acclimate to experimental temperatures for a set period (e.g., 24-48 hours) before beginning measurements
Impact of the stage in a wetland's flowpath on nutrient concentration	No	● Wetlands serve as biofilter filters, nutrient sinks and transformers for nitrate percolating through them meaning it should reduce over time ● Water samples could be taken at various places along the flowpath and then compared ● Requires access to a wetland and permission to sample ● Place where the sample is taken matters. I.e. at the edge, in the middle etc

2. Risk Assessment and Assistance

- Standard lab safety measures for apparel need to be followed – wear an apron, gloves and goggles.
- Ethical considerations in using macroalgae, including disruption of the natural habitat. I will avoid oversampling and use non-destructive sampling-techniques. Additionally, unintentional harm to other species and disruption of micro ecosystems could be done to attached species. I will need to inspect samples before removal and return any attached organisms to their natural habitat.
- Electrical components of water bath may be electrocution/ignition hazards – hence, I would need to check for electrical safety, i.e. intact wires, etc., before each trial and keep it away from other electrical and flammable hazards in the science lab.
- Heat lamp will get quite hot and direct contact with its surface would cause burns. I will ensure it is secured safely during experimentation, avoid direct contact, and only handle the lamp once it has cooled down.
- Electronic balance may be knocked off laboratory bench and this may cause injuries to feet – I would be able to control this through keeping it back from the edge of the lab's benches that I put it on; if any substances are spilled, I would also have to wipe them off the balance immediately in order to preserve its cleanliness and, by extension, its precision in measuring biomasses of samples. The balance would also need to be checked for damage before each trial.
- Glass stirring rod, thermometer and watch glasses may break so they should be checked for any chipped edges and/or other damage before use and if they break, glass pieces should not be touched, especially with bare fingers, and should instead be swept up with a brush and dustpan (provided in school labs).

3. Commencement of Experimental Trials

Date	**Activities**	**Ideas/Note/Thoughts**
23/04/2025	Risk Assessment on RiskAssess approved	Use a colorimeter to quantify indicator and product quantitative values, improving accuracy by reducing human error. These values can then be converted to concentration using a calibration curve, which can be generated using known standards. Will use API testing kit (for measuring NH_3 and NO_2), as it is accessible in a school environment and provides quick results with minimal margin for error.
30/04/2025	Macroalgae samples collected from West Beach.	5 different species selected for a greater range of data. I tried to include species with varying degrees of pigmentation types for variation.

		
01/05/2025	Macroalgae samples set up at school in simulated conditions.	Will let them acclimatise over the weekend and commence testing on Monday. Currently in beakers, with oxygen tubes, grow-light, and lids to retain moisture.
05/05/2025	Trial 1 for NO_2, NO_3 and HCO_3 completed	Everything went well but setup took too long - I need to acquire all equipment and glassware necessitated and collect them in a personal plastic box prior to the next experimental session. Realised I need to ensure I dry biomass samples as much as possible before using them for testing to reduce contamination and influencing change in biomass measurements.
07/05/2025	Trial 2 for NO_2, NO_3 completed	**Qualitative observations** ● Over the days, some of the macroalgae species experienced die-off, specifically the *Ulva lactuca* and *Gracilaria*, where the dead biomass showed a loss of pigmentation. ● There was condensation on the side of the glass containers where the macroalgae was stored, and in test tubes in the water bath.
	Trial 3 for NO_2, NO_3 completed	
	Trial 4 for NO_2, NO_3 completed	
08/05/2025	Trial 5 for NO_2, NO_3 completed	
	Trial 2 for HCO_3 completed	
	Trial 3 for HCO_3 completed	
09/05/2025	Trial 4 for HCO_3 completed	
	Trial 5 for HCO_3 completed	
	Calibration curve data completed	

4. Research

Hypothesis

Dark green seaweed: Siphonous structure: These algae are coenocytic which means they undergo repeated nuclear division without the accompanying formation of cell walls

- Classify macroalgae in three taxonomic groups: brown, green, red
- Colour in macroalgae from photosynthetic pigments
    - Different pigments absorb different wavelengths of light
- All algae have chlorophyll pigments
    - Red algae have phycobiliproteins to absorb blue and green light, which penetrates deeper into water
    - Brown algae also has orange pigments called carotenoids, Fucoxanthin to absorb green light
    - All have chlorophylls, red and brown have additional pigments (phycobiliproteins and carotenoids)
- Ability to absorb different wavelengths of light → suited for different depths
    - Green is for shallow water
    - Brown intermediate
    - Red for deeper water

Different depths = different nutrient availability and affects absorption strategies

- "The rate of algal productivity (photosynthesis) reflects the rate of nitrogen assimilation of algae (g N m–2 day–1)."

Hence, the different macroalgae species will have varying abilities to absorb nitrate out of seawater, possibly related to:

- Physiological adaptations
    - Nutrient Transport Systems
    - Enzyme Systems
    - Nutrient storage
    - Energy Allocation
    - Ability to regulate internal and surface pH
- Habitat and environmental adaptations
    - Depth range
    - Tolerance to environmental fluctuations (e.g., intertidal vs. subtidal species)

    - Morphology
- Photosynthetic Capabilities:
    - Photosynthetic efficiency will correlate with pigment composition and typical habitat depth.

Understanding species-specific nitrogen physiologies and nitrogen source preferences will enable polyculture of different seaweed species and the use of seaweeds as biofilters in integrated multi-trophic aquaculture systems. (Jabr, F. 2023).

Environmental Issue

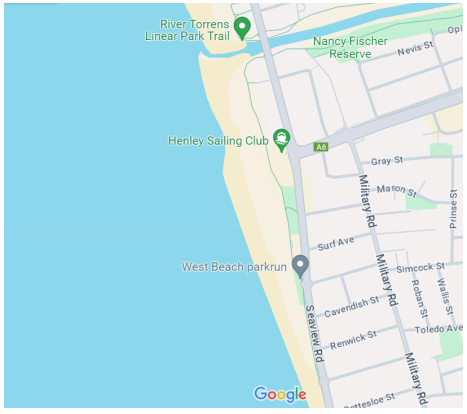
- “Denitrification is a crucial part of the nitrogen cycle, in whichnitrate nitrogen is transformed to gaseous forms, which pre-vents dynamically balanced ecosystems from undergoing eutrophication” (Liu, R.-R. et. al., 2020).
- The toxicity of NH_3 is related to dissolved carbon dioxide (CO_2) concentration and pH of the water. As dissolved CO_2 decreases, the pH increases and increases the toxicity of NH_3 (Timmons and Ebeling, 2007).

Site description

- Macroalgae in South Australia (SA) globally significant levels of species diversity and endemism (approx 60% of macroalgae species are endemic) (Womersley 1981a, 1990; Huisman 2000)
    - warm Leeuwin Current and the presence of localised coldwater coastal upwellings
- Despite being historically used by Indigenous Australians, Australia's commercial seaweed production currently lags behind other countries like Asia, Europe, and America.

- SA has been a major centre of macroalgal taxonomic and ecological research since the 1940s (Womersley 1947, 1948, 1956, 1959; Womersley & Edmonds 1958). (Edyvane, K.S. 2008).
- The Gulf of St Vincent (GSV) is a large (~7 150 km2), relatively shallow (generally <30 m), sheltered, tidal estuary (or embayment) (Shepherd & Sprigg 1976).
    - Inverse estuary → minimal freshwater input and high summer evaporation rates
    - Sea surface temperature range of 12-26°C
    - Kangaroo Island protects GSV from water exchange and high wave energies of the Southern Ocean
    - Rocky headlands; sand (15 845 ha, 62.2%) and seagrass (7 645 ha, 30.0%), some reefs (1 966 ha, 7.7%). (Edyvane, K.S. 2008).
- Prominent ecological features that affect ecology
    - Adelaide airport 5 km East of the beach
    - Two estuaries:
        - Breakout creek Wetlands at the end of the River Torrens, which outlets into ocean north end of West Beach

        - (Brown Hill and Keswick creek) join into Patawalonga creek, which in merged with Sturt river (goes through Oaklands Wetland) just before it turns into Holdfast bay and outlets in Glenelg, 2 km south of West Beach
        - (Similar to the Gulf of Mexico but on a smaller scale. Application?)
    - Lots of anthropogenic activities (many people go there, walk dogs, swim in ocean, use boats)
        - West beach boat ramp
        - Henly sailing club
        - Henly and Grange jetties nearby
    - Seawall (made of massive pile of granite? rocks) to protect the inland area against wave action and prevent coastal erosion
- Sand erosion:
    - “already resulted in the degradation of the seawall at West Beach, which was reconstructed with a rock wall by the City of Charles Sturt in 2021”. (Sturt, C of C. 2023).
- Delivery of quarry sand to West Beach from land-based quarries commenced in August 2021. Approx 350, 000 m^3 of sand delivered to West Beach August 2021 - November 2023. (Department for Environment and Water., n.d.)
    - Why is this needed?: rising sea levels, buildings on top of the natural dune systems have ‘locked up’ natural sand
    - Dune Restoration



5. Results – Raw Data

Table 4: Absorbance (AU) of NH_3 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.941	1.134	1.389	1.248	1.179	1.1782
UL	0.444	0.482	0.499	0.535	0.607	0.5134
SH	0.523	0.51	0.571	0.54	0.814	0.5916
CF	0.396	0.491	0.442	0.552	0.518	0.4798
HB	0.601	0.523	0.506	0.578	0.562	0.554
Control	0.949	-	-	-	-	0.949

Table 5: Absorbance (AU) of NO_2 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.48	0.706	0.411	0.586	0.667	0.57
UL	0.394	0.639	0.45	0.547	0.653	0.5366
SH	0.102	0.674	0.506	0.559	0.664	0.501
CF	0.082	0.118	0.106	0.179	0.219	0.1408
HB	0.169	0.175	0.195	0.312	0.579	0.286
Control	0.1	-	-	-	-	0.1

Table 6: Absorbance (AU) of HCO_3 Samples

Macroalgae	Sample A	Sample B	Sample C	Sample D	Sample E	Mean
G	0.859	0.747	0.555	1.011	0.881	0.8106
UL	1.005	0.937	1.05	0.999	0.953	0.9888
SH	0.46	0.654	0.641	0.399	0.451	0.521
CF	0.724	0.791	0.797	0.718	0.669	0.7398
HB	0.757	0.846	0.674	0.044	0.737	0.6116
Control	0.553	-	-	-	-	0.553

Table 7: Absorbance of Known NH_3 Standard Concentrations for Calibration Curve

Concentration (ppm)	1	2	4	6	8	10
Absorbance	0.43	0.835	1.794	2.119	2.432	2.482

Table 8: Absorbance of Known Standards of HCO_3 (pH) Test for Calibration Curve

pH	9.2	9.0	8.8	8.6	8.4	8.2	8.0	7.8	7.6
Absorbance	0.944	0.843	0.663	0.601	0.497	0.385	0.236	0.139	0.095

6. References

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OSA RISK ASSESSMENT FORM

for all entries in (✓) ☑ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, logbook or entry. One form per entry.

STUDENT(S) NAME: Maike Enderling ID: 0206-067

SCHOOL: Glenunga Internation High School

Activity: Give a brief outline of what you are planning to do.

Investigate the bioremediation potential of various macroalgae species through their ability to reduce nutrients (NH3, NO2) in seawater.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead? **Only batteries can be used for Models & Inventions entries*
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control / manage the risk
1. Burn from contact with heat lamp. 2. Injury (sunburn, slips) from fieldwork. 3. Chemicals associated (NH4Cl, NaNO3). Toxic if ingested. Ethical considerations 4. Disruption of natural habitat during macroalgae collection.	1. Wear PPE. Avoid contact. 2. Wear appropriate gear. Bring water. Gloves when handling samples. 3. Wear PPE. Ventilation. Avoid ingestion. Store seperately. Ethical considerations 4. Avoid oversampling. Non-destructive sampling-techniques. Return specimens to habitat when possible. Adhere to local conservation guidelines. Minimise stress during transport/analysis.

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)):

Maike Enderling

SIGNATURE(S): M. Enderling.

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Ian Lau

SIGNATURE: Ian Lau DATE: 10/06/25