

2.2.4 Biogeochemical processes in sea grass sediment

Investigator / Institution

Kieryn Kilminster

School of Plant Biology, University of Western Australia

Supervisors

Professor Di Walker

School of Plant Biology, UWA

Professor John Raven FRS FRSE

University of Dundee, Scotland

Dr. Peter Thompson

CSIRO, Hobart

Executive Summary

Early work on organic matter enrichment of coastal ecosystems suggested that seagrass growth may be constrained by biogeochemical parameters, possibly sulphide stress. Sulphide concentrations in aquatic sediments are likely to increase with increased organic matter fluxes (eutrophication) and increased temperatures (global warming). *Halophila ovalis* was chosen as a model to investigate the role of biogeochemistry in seagrass sediments both on growth and nutrient dynamics. *H. ovalis* cultured in controlled conditions subject to either organic matter enrichment or light reduction showed reduced growth and increase in leaf phosphorus concentration. Exposure of *H. ovalis* to sediment sulphide in the field caused significant reduction in growth, average leaf weight and internode-distance of *H. ovalis*. Reduced growth rate was correlated with an increase in leaf P. The response of *H. ovalis* to unfavourable sediment conditions may enable its use as a bio-indicator of estuary or coastal marine health, allowing observation of unhealthy sediment conditions before die-back of the major meadow forming seagrasses occurs.

General Background

Seagrass meadows are dynamic and productive environments. Interactions within seagrass habitats, between seagrasses, epiphytic algae, benthic microalgae, sedimentary bacteria, and a large diversity of protists and invertebrates, are complex and often not well understood. Seagrasses manipulate sediment characteristics by detritus accumulation, root oxygen loss (Connell et al., 1999), dissolved organic matter exuded from roots during photosynthesis (Holmer et al., 2001) and demand for nutrients (Short and McRoy, 1984). These processes may influence the fluxes of nitrogen (N) and phosphorus (P), and the subsequent availability of nutrients to the seagrass. Western Australia has some of the most extensive seagrass meadows in the world, with a large diversity of species represented. Many of these species are unique to Australia (Kirkman and Walker, 1989). Understanding the processes maintaining seagrass nutrition is particularly pertinent for research in coastal waters of Western Australia where residual nutrient concentrations in water columns are low.

General aims and study site

This work focused on a small species of seagrass, *Halophila ovalis*, from the Swan River Estuary. Research was undertaken both in the laboratory (with collected samples) and in the field. *H. ovalis* was chosen as a model to investigate the role of biogeochemistry in seagrass sediments both on growth and nutrient dynamics. An advantage of *H. ovalis* is that response time to perturbations is quick, since growth rate is fast, so this makes it an ideal seagrass to investigate parameters affecting growth and nutrition.

Manipulations affecting growth of *Halophila ovalis*

Organic matter addition to *H. ovalis*, in culture

It was hypothesised that seagrass may benefit nutritionally from organic matter additions stimulating heterotrophic mineralization. Cores of *Halophila ovalis* were grown under controlled conditions (18 °C, 12 hr light / 12 hr dark, ~ 300 μmoles of photons $\text{m}^{-2}\text{s}^{-1}$ PAR) for ~ 4 weeks. Unvegetated sediment was also incubated in the same manner. Cores was enriched with either particulate organic matter (POM) added as ground wrack to top surface (0, 1, 5, 12 g core^{-1}), or dissolved organic matter (DOM) added with sucrose diffusion tubes (0, 0.8, 2.4, 5.8 g core^{-1}).

Following enrichment with POM, growth rate and average leaf mass reduced (Table 2.1), total ammonium and phosphate pools in porewater increased, seagrass utilized phosphorus (P) preferentially to nitrogen (N) from the porewater, average leaf N concentration increased by 15 % and average leaf P concentration increased by 30 %. Following enrichment with DOM, growth rate reduced but average leaf mass increased (Table 2.1), proportion of root biomass decreased but the proportion of leaf biomass increased, porewater concentrations showed little change except for an increase in phosphate in porewater for highest DOM enrichment, average leaf N increased by 13 % and average leaf P increased by 28 %. Patterns of N and P distribution in plant parts were very similar for POM and DOM enriched *H. ovalis* (Fig. 2.12), with nutrient concentrations of leaves approximately twice that observed in roots or rhizomes.

Light reduction of *H. ovalis*, in culture

H. ovalis was grown under controlled conditions (18 °C, 12 hr light / 12 hr dark, ~ 300 μmoles of photons $\text{m}^{-2}\text{s}^{-1}$ PAR unless otherwise stated) for 2 - 7 weeks. Aquaria were covered with Hessian to reduce light levels for treatments to 100 and 40 μmoles of photons $\text{m}^{-2}\text{s}^{-1}$ PAR.

Growth rate and average leaf weight reduced (Table 2.1), average leaf N increased by 18 % and average leaf P increased by 25 % following light reduction of *H. ovalis*. Patterns of nutrient distribution (N and P) within seagrass tissues followed a similar pattern to that observed following organic matter enrichment (Fig. 2.12).

Table 2.1 Average growth rate and leaf mass for *Halophila ovalis* grown under perturbations of either organic matter addition, or light reduction. (mean \pm SE)

Experiment	Growth rate (mg DW apex ⁻¹ day ⁻¹)		Leaf mass (mg)	
	Control	Treatment	Control	Treatment
POM addition	1.33 \pm 0.15	0.82 \pm 0.10	3.9 \pm 0.3	3.2 \pm 0.1
DOM addition	1.22 \pm 0.27	0.58 \pm 0.15	3.7 \pm 0.2	5.1 \pm 0.6
Light reduction	1.32 \pm 0.26	0.98 \pm 0.21	3.3 \pm 0.6	2.4 \pm 0.5

* Treatment for POM = 12 g wrack core^{-1} , DOM = 5.2 g sucrose core^{-1} , and LIGHT = 40 μmoles of photons $\text{m}^{-2}\text{s}^{-1}$ PAR

Conclusions of growth manipulation experiments

Seagrass grown in culture showed very similar responses for either addition of organic matter (POM or DOM) or light reduction. Although seagrass may acquire nutrients from organic matter enrichment, growth may be constrained by sediment conditions. The strongest indicator of this stress appeared to be increase in leaf P. Similarly, an increase in leaf P was demonstrated for plants subject to reduced light. These common responses led me to consider sulphide as an underlying cause of negative plant response to either organic matter enrichment (stimulating microbial sulphate reduction) or light reduction (reducing photosynthesis and thus the ability of roots to oxygenate sediment).

Comparison to other seagrasses

Data on nitrogen and phosphorus concentrations in above-ground and below-ground plant parts of 19 species of seagrass world-wide was compiled from published sources. Nutrient concentration of *Halophila ovalis* shows a central tendency when compared to data on seagrass nutrient concentrations for both above-ground and below-ground plant parts collated from published sources (Fig. 2.13). Concentration of nutrient within leaf tissue is higher than that of below-ground plant parts. Variability in seagrass nutrient concentrations has been attributed to differences arising within and among species with most variability due to within-species differences (Duarte, 1990). Although *Halophila* is an oddity in the spectrum of seagrass species (it is small, fast growing, has oval leaves and plant tissue which is not recalcitrant in ecosystems), its nutrient concentrations show a central tendency compared with the range of concentrations observed for all seagrasses. I propose the use of *H. ovalis* as a model to explore processes which determine nutrient concentration variability within seagrasses.

Nitrogen and phosphorus concentrations within *H. ovalis* tissues showed similar trends despite being grown in culture under three different experimental manipulations (POM and DOM enrichment or light reduction). Nitrogen and phosphorus concentrations were not strongly correlated for above-ground or below-ground plant parts of seagrass world-wide (Fig. 2.13). The ratio of nitrogen and phosphorus is more tightly constrained for the roots and rhizomes of *H. ovalis* than the leaves (linear relationships in Fig. 2.12). N:P observed for rhizomes is lower than for roots, and indicates that rhizomes contain more phosphorus relative to nitrogen than roots (Fig. 2.12). When roots and rhizomes are combined into one below-ground compartment the linear relationship between N and P is no longer observed (Fig. 2.13). Most data on nutrient concentrations for other seagrass species has combined roots and rhizomes to below-ground parts, so I can only speculate that different N:P constraints for roots and rhizomes may exist for other seagrass species.

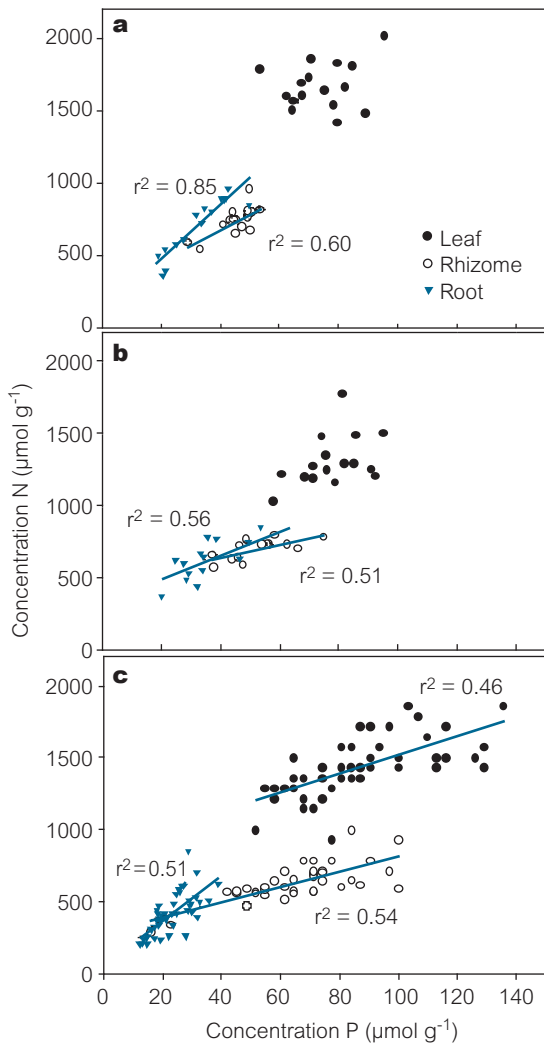


Figure 2.12: Nitrogen vs. phosphorus concentrations in leaves, rhizomes and roots of *Halophila ovalis* grown in culture with particulate organic matter addition (A), dissolved organic matter addition (B) and light reduction (C).

Leaf nitrogen and phosphorus were shown to increase significantly following either organic matter enrichment or light reduction (Fig. 2.14), with phosphorus increasing almost twice as much as nitrogen. These natural tendencies for seagrass to retain or acquire N and P under non-optimal growth conditions may lead to the over-estimation by researchers of minimal requirements of nitrogen and phosphorus for growth.

Field response to sulphide

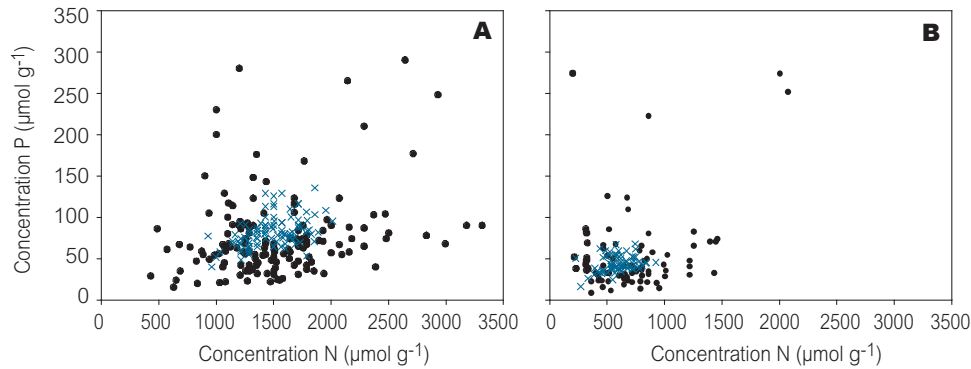


Figure 2.13: Concentration of nitrogen and phosphorus in seagrass tissue for A. leaf and B. below-ground plant parts, for published data from 19 species of seagrass (\bullet) and *Halophila ovalis* from the Swan River Estuary (\times). Leaf concentrations of N and P are typically 2-3 times higher than for below-ground parts, and *Halophila ovalis* shows a central tendency within the global data set.

Exposure of below-ground roots and rhizomes of seagrass to “free” sulphides depends on the rate of sulphate reduction (SRR), porewater pH, potential of plant produced oxygen to re-oxidize sulphides and availability of transition metals in sediment capable of sequestering sulphides.

The short-term effect of sediment sulphide on the growth, nutrition and morphology of *H. ovalis* was investigated at Pelican Point, in the Swan River Estuary. Sediments were enriched with Na_2S (equivalent to 0, 1.1 and 4.2 g m^{-2}) and plant growth was monitored over ~ 3 weeks. Biomass, internode-distance and growth rate were determined, and plant material analysed for soluble carbohydrate, carbon, nitrogen and phosphorus.

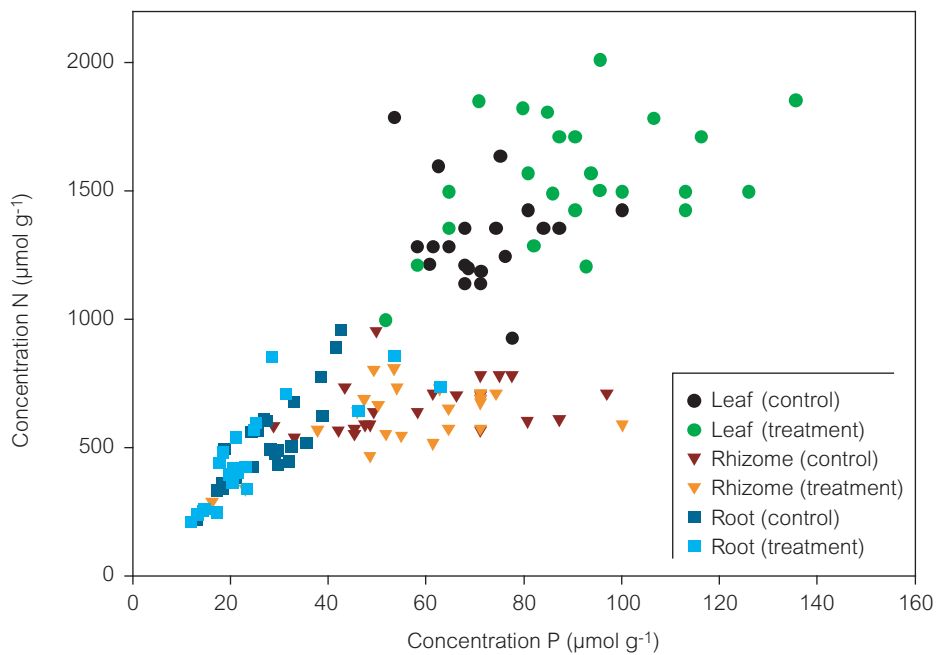


Figure 2.14: Nitrogen vs phosphorus concentrations in cultured *Halophila ovalis* tissue for control and treatment (highest organic matter enrichment or strongest light reduction). Leaf P increases with exposure to organic matter, or light reduction, relative to the control.

Following sulphide enrichment of sediments, growth rate, internode-distance and average leaf mass was lower than in control ($p < 0.05$) as determined by one-way ANOVA with treatment as a fixed factor. There was no significant effect of sulphide treatment on the nutrient constituents (C, N, P or soluble carbohydrates), however sulphide did negatively influence growth rate. Growth rate was negatively correlated with leaf P ($r^2 = 0.373$, $p < 0.001$) and positively correlated with internode-distance ($r^2 = 0.414$, $p < 0.01$). Together internode-distance and concentration of phosphorus in leaves accounted for 63 % of the variability in the growth rate of *H. ovalis* (by multiple linear regression).

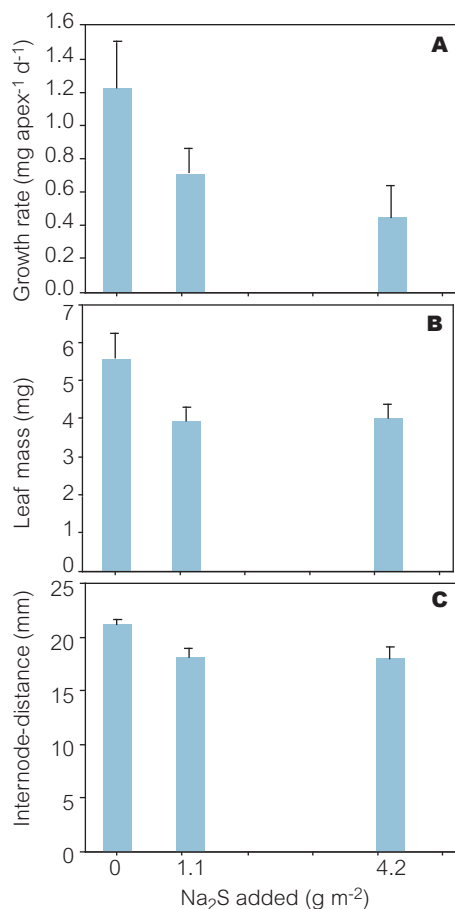


Figure 2.15: A. Growth rate of *H. ovalis*, B. average leaf and C. internode–distance reduces following sediment enrichment with sodium sulphide

H. ovalis had reduced growth rate and reduced areal production but showed morphological acclimation to sulphide exposure, producing smaller, lighter leaves and reduced internode-distance. The plasticity demonstrated by *H. ovalis* of adjusting internode-distance in response to sulphide loading may provide a survival advantage. Reduced internode-distance would increase root and shoot density, since *H. ovalis* has a predominantly linear growth pattern with one root for every leaf pair. Since oxygen is lost diffusively through the roots of *H. ovalis* with photosynthesis, reduced internode distance and subsequent increase in root density may be an acclimation to counteract increased sulphide concentration. Response time to sulphide stress would be expected to be faster for *H. ovalis* relative to other larger, slower growing seagrasses. The response of *H. ovalis* (increased leaf P, reduced internode-distance and reduced average leaf weight) may enable its use as a bio-indicator of estuary or coastal marine health, allowing observation of unhealthy sediment conditions before dieback of the major meadow forming seagrasses occurs.

General discussion

Biogeochemical constraints on the growth of *Halophila ovalis* were hypothesised following laboratory experiments (organic matter enrichment and light reduction) where reduced growth rate was observed. Increased sulphide exposure was suggested as the mechanism of growth constraint, and this was tested in the field with a direct addition of sodium sulphide to the sediment. Sediment sulphide clearly inhibits plant growth rate, and results in smaller internode-distances and leaves - responses similar to those observed in the laboratory experiments. The effect on leaf nutrient (N and P) concentrations was less pronounced than in the laboratory experiments and may indicate that changes in sediment chemistry (not just presence of sulphide) may also determine nutrient concentrations within seagrass.

Acknowledgements

This work was funded by the Strategic Research Fund for the Marine Environment (SRFME), the School of Plant Biology and the Graduate Research School, University of Western Australia.

Conferences attended and presentations

Strategic Research Fund for the Marine Environment Symposia, *Floreat 2003, 2004, 2005*

Plant Biology Postgraduate Summer School, *Rottneest 2003, 2004, 2005, 2006*

GRDC Root and Soil Biology Workshop, *Canberra, 2003*

International Society for Plant Anaerobiosis (ISPA) *Perth, 2004*

SEAGRASS 2004 *Townsville, 2004*

Thesis submitted July 2006 and PhD subsequently awarded.

Publications and awards

Kilminster K.L., D.I. Walker and J.A. Raven (submitted). Changes in growth, internode-distance and nutrient concentration of the seagrass *Halophila ovalis* with exposure to sediment sulphide. (Submitted to *Marine Ecology Progress Series*)

Kilminster K.L., D.I. Walker, P.A. Thompson and J.A. Raven J.A. (2006). Limited nutritional benefit of the seagrass *Halophila ovalis*, in culture, following sediment organic matter enrichment. *Estuarine, Coastal and Shelf Science* 68: 675-685.

Travel award towards travel to attend GRDC Workshop in Canberra 2003

References

Connell, E.L., Colmer, T.D., Walker, D.I., (1999). *Aquatic Botany* 63, 219-228.

Duarte, C.M., (1990). *Marine Ecology Progress Series* 67, 201-207.

Holmer, M., Andersen, F.Ø., Neilsen, S.L., Boschker, H.T.S., (2001). *Aquatic Botany* 71, 1-17.

Kirkman, H., Walker, D.I., (1989). Regional Studies - Western Australian Seagrass. In: Larkum, A.W.D., McComb, A.J., Shepherd, S.A. (Eds.), *Biology of Seagrasses*. Elsevier, Amsterdam, pp. 157-181.

Short, F.T., McRoy, C.P., (1984). *Botanica Marina* XXVII, 547-555.