

Negative effects of stress-resistant drift algae and high temperature on a small ephemeral seagrass species

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Abstract Seagrasses are threatened by multiple anthropogenic stressors, such as accumulating drift algae and increasing temperatures (associated with eutrophication and global warming, respectively). However, few seagrass experiments have examined whether exposure to multiple stressors causes antagonistic, additive, or synergistic effects, and this has limited our ability to predict the future health status of seagrass beds. We conducted a laboratory experiment to test whether abundance of *Gracilaria comosa* (3 levels; 0, 1.2, and 3.4 kg WW m⁻²), an algae that is resistant to wide environmental fluctuations (e.g. light, temperature, salinity, and oxygen levels), has negative effects on the small ephemeral seagrass, *Halophila ovalis* and whether the effects are exacerbated by high temperature (3 levels; 20, 25, and 30°C). We found an additive negative effect of the two stressors when tested simultaneously on 14 seagrass performance measures, with most data variability explained by the drift algae. For the individual plant performance measures (above- and below-ground growth and mortality, leaf area, internode distance, and root

length and root volume), we found 5 additive effects, 4 synergistic effects, and 5 effects that were significant only for drift algae. We also documented a significant additive effect of drift algae and temperature on dissolved porewater sulphide (DS). A follow-up correlation analysis between DS and the 14 plant performance measures revealed significant or near-significant linear correlations on 9 of these responses (above- and below-ground growth, leaf area and weight, leaf mortality, and internode distance). In summary, we showed (a) that a stress-resistant drift algae can have strong negative effects on a small ephemeral seagrass, (b) this negative effect can increase both additively and synergistically with increasing temperature depending on performance measure, and (c) the negative effects may be mediated by a build-up of porewater DS. An implication of our findings is that resource managers aiming to preserve healthy seagrass beds in an almost certain future warmer world should increase efforts to keep drift algae populations low.

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Introduction

Seagrasses are important benthic marine plants that create physical structure for sessile epibiota, reduce wave energy, stabilize sediments, act as nursery and feeding grounds for herbivores and predators, and contribute significantly to primary production. The provision of these ecosystem services makes seagrasses highly valued marine ecosystems (Duarte 1999; Williams and Heck 2001). However, seagrass habitats are increasingly threatened by anthropogenic activity; for example, from stress associated with eutrophication (nutrient pollution) and climate change (Orth et al. 2006). Importantly, Orth et al. (2006) emphasized that multiple anthropogenic stressors act in concert but noted that

few experimental seagrass studies have investigated more than one stress factor at a time. Recent evidence also suggests that stressors not only occur simultaneously but can act interactively in ways that cannot be predicted based on results from single stress experiments, i.e. effects can be antagonistic ($2 + 2 < 4$) or synergistic ($2 + 2 > 4$) (Koch and Erskine 2001; Crain et al. 2008; Darling and Cote 2008). If such ‘ecological surprises’ (Paine et al. 1998) associated with multiple stressors are common in seagrass habitats, it will present managers with a major challenge both to predict stress impacts and to come up with solutions to conserve and maintain healthy seagrass beds.

Two major anthropogenic stressors that threaten seagrass habitats are eutrophication and global warming (Short et al. 1995; Short and Neckles 1999; Orth et al. 2006). Micro- and macroalgae are favoured over seagrasses in eutrophication scenarios, due to their efficient uptake of nutrients from the water column and lack of non-photosynthetic tissue, resulting in rapid growth (McGlathery et al. 1997). For example, under high nutrient levels, shallow water unattached macroalgae (hereafter drift algae) often grow into dense mats that can become entangled and retained in seagrass beds (Cowper 1978; Cecere et al. 1992; Fox et al. 2008; Tweedley et al. 2008). These algal mats can reduce the light levels reaching newly developing seagrass shoots and reduce oxygen levels in the water column through respiration during night hours (Hauxwell et al. 2001; McGlathery 2001; Holmer and Nielsen 2007; Huntington and Boyer 2008). Algal mats may also add organic matter to the sediment which, when decomposing, further reduce oxygen levels and potentially increase concentrations of phyto-toxins such as sulphur and ammonia (Van Katwijk et al. 1997; Hauxwell et al. 2001; Holmer and Bondgaard 2001). Drift algae characterized by high surface–volume ratio, rapid growth, and high respiration ratio (e.g. *Ceramium*, *Ulva/Ulvaria*, *Enteromorpha*) (Pedersen and Borum 1996) can reduce the performance of both large and persistent (McGlathery 2001; Nelson and Lee 2001; Holmer and Nielsen 2007) and small and ephemeral (Cummins 2004) seagrass species. However, only a single experiment demonstrated that other drift algal species characterized by lower surface–volume ratios, slower growth, and higher stress tolerance (e.g. species within the Gracilariales order, Thomsen and McGlathery 2007) can also decrease the performance of large seagrasses (with a 50% reduction in *Zostera marina* shoot density when exposed to 1,700 g WW (wet weight) m^{-2} *Gracilariopsis* sp. over 3 months, Huntington and Boyer 2008). Still, it remains unknown whether and how much stress-resistant and slow-growing drift algae may impact other seagrass species, such as the small and ephemeral seagrasses such as *Halophila*, *Halodule*, or *Ruppia* sp. that typically co-exist with these more persistent algae.

A second important seagrass stressor relates to increasing temperatures associated with climate changes (Short and Neckles 1999; Orth et al. 2006; Solomon et al. 2007). Elevated temperature influences metabolic processes directly, generally increasing the rates of photosynthesis and respiration differentially and thus potentially decreasing P/R ratios. Most seagrasses typically have their photosynthetic optimum at temperatures below the seasonal maximum (e.g. Biebl and McRoy 1971) and may therefore be sensitive to elevated temperatures, particularly in summer months (Moore and Jarvis 2008). Thus, although seagrasses are generally well adapted to local fluctuations in summer temperature (Diaz-Almela et al. 2009), extreme highs have been shown to cause mortality in part due to induced negative oxygen balance within the plants (Greve et al. 2003). Also, although temperature tolerance levels are relatively well known for many seagrass species (e.g. Bulthuis 1987; Campbell et al. 2006; Lee et al. 2007; Diaz-Almela et al. 2009; Massa et al. 2009), much less is known about how additional stressors (e.g. drift algae) interact with temperature above the optimum temperature regime of the seagrasses. For example, drift algae may, via respiration processes and breakdown of deposited organic matter, indirectly enhance sulphide concentrations in the sediment porewater and thereby reduce seagrass performance (Holmer and Bondgaard 2001; Greve et al. 2003; Koch et al. 2007a, b; Kilminster et al. 2008). In addition, it is possible that seagrass resistance to sulphide toxicity is inhibited when the temperature increases above the seagrass’ optimal temperature tolerance, thereby raising the possibility for negative synergistic effects of multiple stressors (Koch and Erskine 2001; Koch et al. 2007a, b).

We here test whether mats of the drift algae *Gracilaria comosa* have a negative impact on the ecological performance of the seagrass *Halophila ovalis* and whether algal impacts are antagonistic, additive, or synergistic in combination with temperature stress above *H. ovalis*’ optima. *Gracilaria comosa* and *H. ovalis* were used to represent a stress-resistant, slow-growing drift algae and a small ephemeral seagrass species (Hillman et al. 1995; Terrados et al. 1999; Thomsen and McGlathery 2007; Thomsen and Wernberg 2009). These species are also the most abundant seagrasses and seaweeds within the Swan River study system (Hillman et al. 1995; Astill and Lavery 2001; Astill and Lavery 2004), where *H. ovalis* forms extensive meadows at shallow depths. *H. ovalis* is typically <5 cm high being amongst the smallest existing seagrasses, it is highly productive ($500 \text{ gC m}^{-2} \text{ year}^{-1}$) but also sensitive to shading (Longstaff et al. 1999), and drift algae may be a particular strong stress factor to both new and old shoots. *Gracilaria comosa* is omnipresent in seagrass beds in Swan River with consistently high densities on both site and plot scales (as billions of holdfast structures survive attached to

the shells of the ubiquitous invasive snail *Batillaria australis*; Thomsen et al. 2010a, b). However, areal-based biomass is typically low, until environmental conditions in late summer facilitate the rapid growth of this population of ‘sleeper propagules’ (Thomsen and Wernberg 2009). Thus, mean biomass values in seagrass beds for individual sites are typically between 5 and 500 gWW m⁻² (Thomsen and Wernberg 2009), whereas plot-based values (in 0.25 m² quadrats) can exceed 2 kg WW m⁻² (Thomsen, unpublished data).

Materials and methods

Experimental design and test factors

We tested for the effects of *G. comosa* and temperature on the ecological performance of *H. ovalis* in a two-factorial laboratory experiment. *H. ovalis* was exposed to three levels of algal stress (0 (Control), 1.2 (Low), and 3.4 (High) kg WW m⁻², representing common and extreme densities encountered in the Swan River estuary (hereafter Swan River), a large estuary situated in the heart of Perth, the capital of Western Australia (Astill and Lavery 2004), under three different temperature regimes (20, 25, and 30°C) over 5 weeks. The highest temperature treatment was chosen in between the optimal (ca. 25°C, Hillman et al. 1995) and maximum (ca. 35°C, Ralph 1998) tolerance levels of *H. ovalis*. Five ‘pot’ replicates were used for each of the nine algae × temperature treatment combinations, and each pot had an inner diameter of 15 cm and was filled with 5-cm sieved sediment from the seagrass sampling site (<1,000 μm, 5.0% organic carbon, and 0.17% organic nitrogen). Five pots with randomly selected levels of algae were placed in each of 9 aquaria with 25 l of 25 psu salinity seawater, which corresponds to the salinity at the seagrass collection site. The algae mat remained in the pots without need for attachment, and the water column was aerated and circulated, and one-third of the water was exchanged each week for 5 weeks. Temperature was controlled by thermostats in three individual climate rooms (±1°C), with three aquaria in each room. Saturating light of ~300 μmol photons m⁻² s⁻¹ (Hillman et al. 1995) was provided by daylight fluorescent tubes in a 12:12 h light:dark cycle. We acknowledge the potential problems associated with having all replicates of a temperature treatment in the same room, but great effort was made to keep all conditions (e.g. light) and manipulations (e.g. water exchanges and algal additions) as similar as possible between test rooms.

Shoots of *H. ovalis* and fronds of *G. comosa* were sampled in Swan River near Pelican Point at ~0.5 m depth (31°59' S/115°49' E, Western Australia). *Halophila ovalis* were pruned to two nodes with 2 leaf pairs to standardize

the experiment, and 10 shoots were planted in each pot, corresponding to typical field densities of 566 shoots m⁻² (Hillman et al. 1995). This manipulation reduces clonal integration, but integration has previously been documented to be relatively weak in *H. ovalis*, and this species has been shown to perform well from only 2 leaf pairs (Benjamin et al. 1999). Algal fronds were cleaned for epiphytes by gently whipping the leaves with paper tissue and added to the pots in 0, 25, or 60 g WW (=0, 1.2, and 3.4 kg WW m⁻²), corresponding to a Control, a Low, and a High algal stress treatment. These *Gracilaria* levels are typical for estuaries worldwide and have been observed in Swan River (Cecere et al. 1992; Peckol et al. 1994; Astill and Lavery 2004; Thomsen et al. 2006; Thomsen and Wernberg 2009; Thomsen, unpublished data). Algae were added as a single pulse treatment, and the added biomass increased over the 5-week experiment by 50 and 18% in the Low and High treatments, respectively ($n = 15$). The duration of the experiment was well above the plastochrone intervals (time for producing new shoots or rhizomes) of *H. ovalis* of 2.2 days for shoots and leaves and 3.3 days for rhizomes (Short and Duarte 2001). Water column oxygen was measured using the Winkler method (Parsons et al. 1984) three times during the experiment by sampling over the algae mat, in the mat, and at the sediment surface with a syringe.

Seagrass performance

At the termination of the experiment, 14 seagrass performance characteristics were quantified. Net leaf, rhizome, and root growth rates (mg DW apex⁻¹ d⁻¹) were calculated as the difference between initial (averaged for 20 standardized shoots) and final biomass and divided by time and the number of apices in each pot ($n = 5$). Biomass was measured separately for leaves, rhizomes, and roots after drying overnight at 60°C. The dry masses of the leaves, rhizomes, and roots (DW apex⁻¹) were calculated from the final biomass and given per apex. Each shoot was photographed, and the area of leaf (mm² apex⁻¹), length of internodes (mm apex⁻¹), root length (mm pot⁻¹), and root diameter (mm) were measured with the program WinRHIZO (Bouma et al. 2000). Root volume (mm³ pot⁻¹) was calculated from the root length and root diameter. We interpreted long internode distance as characteristic of unstressed plants, and short internode distance indicating growth stress. Side branching was quantified by counting the number of apices per apex, and similarly the number of internodes for each apex was counted. Finally, net mortality rates given as percentage loss per day were calculated from changes in leaf and root biomass between initial and end conditions (assuming a linear loss rate).

Sulphur in seagrasses and sediments

Plant leaves were analysed for elemental sulphur isotopic composition ($\delta^{34}\text{S}$) and total sulphur (TS) content. Ground leaf material was weighed into tin capsules with vanadium pentoxide and analysed as described for the sediments. Sediment sulphide levels were quantified as DS (porewater dissolved sulphide), SRR (sulphate reduction rates), and TRS (total reducible sulphides). Sediment samples were collected before seagrasses and drift algae were removed. DS was extracted at 4 cm sediment depth, in three replicates per pot with needles and pooled for each pot (Berg and McGlathery 2001). The porewater was preserved in zinc acetate and frozen until analysis according to Cline (1969). Additionally, silver sticks were incubated for 6 h to determine the depth (in mm) of the sulphide front in the sediment as a black coating of Ag_2S on the sticks. Sediment was sampled with 10 ml cut-off syringes and incubated for the measurement of SRR and TRS. One sample from each pot was injected with radioactive tracer ($^{35}\text{S}\text{-SO}_4^{2-}$), fixed in zinc acetate after 2 h of incubation, and kept frozen until analysis. The sediment was distilled according to the one-step procedure of Fossing and Jorgensen (1989), and radioactivity was determined on a scintillation counter. TRS was measured as described earlier. One sediment sample from each treatment was sampled for determination of $\delta^{34}\text{S}$ and distilled as described previously, but in the 2-step procedure to obtain the acid volatile sulphides (AVS, porewater sulphides, and iron-monosulphides) and chromium reducible sulphur (CRS, pyrite). The extracted sulphide was precipitated as Ag_2S and analysed for $\delta^{34}\text{S}$ by weighing the precipitate into tin capsules together with vanadium pentoxide, which was analysed by elemental analyser combustion continuous flow isotope ratio mass spectroscopy (EA-C-CF-IRMS) at Iso-analytical, United Kingdom. Only CRS results are presented here due to lack of sufficient material for AVS analysis.

Statistical procedures

First, we tested whether drift algae and temperature affected the overall ecological performance of *H. ovalis* by using a 2-way permutation-based multivariate analysis of variance on all 14 performance characteristics (PERMANOVA; based on normalized variables, Euclidian distances, and 999 permutations) (Anderson 2000). We then tested each of the 14 performance characteristics and 6 sulphur variables using 2-way ANOVAs (Sigma Stat vers. 3.5). Levene's test was used to test whether variances were homogeneous. Only leaf mortality had strong variance heterogeneity, but this performance measure could not be transformed to homogeneity, and significance should therefore be interpreted with caution. Some remaining tests had low variance

heterogeneity ($P < 0.05$ but > 0.01 , Levine's test, Table 2), but balanced and factorial ANOVA is relatively robust to deviations from this assumption. We searched for antagonistic and synergistic effects, and the analysis was conducted on untransformed data, despite weak variance heterogeneity, to keep the interpretations of interactions as simple as possible (Quinn and Keough 2002). Post-hoc analyses of significant results were conducted with SNK tests. Rather than applying Bonferroni corrections to multiple univariate tests (which is highly conservative), we emphasize that 1 out of the 20 tests performed may be wrong simply due to chance (Anderson 2000). Finally, because DS is argued to be the single most important sulphur stress agent (Holmer and Nielsen 2007; Calleja et al. 2007, and here showed significant responses to drift algae and temperature treatments), we performed Pearson's linear correlation analysis between DS and each seagrass performance measure using the individual pots as replicates ($n = 45$). Note that we did not manipulate and control DS, but that it is derived from individual algae \times temperature treatment combinations. All results were evaluated for significance using $\alpha = 0.05$.

Results

Seagrass performance

There were significant effects of both algae and temperature on all *H. ovalis* performance characteristics, but only a near-significant interaction (PERMANOVA, Table 1). Importantly, drift algae manipulations accounted for ca. 9 times more data variability in terms of sum of squares, compared with temperature manipulations, suggesting the former to be much more ecologically important.

The above pattern was reflected in individual ANOVA analyses (Table 2). Four performance characteristics—leaf growth, DW of roots, apical growth, and leaf mortality—had significant interaction effects of drift algae and temperature. For leaf growth and leaf mortality, effects associated with increasing drift algal cover became increasingly

Table 1 PERMANOVA of drift algae and temperature effect on 14 plant performance characteristics

Source	Df	SS	Pseudo-F	P(perm)
Alga	2	350.220	33.903	0.001
Temp	2	39.500	3.824	0.001
Alga \times Temp	4	31.818	1.540	(0.051)
Residual	36	185.940		

PERMANOVA was performed on normalized variables, using Euclidian distances and 999 permutations. Significant values ($P < 0.05$) are in bold and near-significant values ($P < 0.10$) in brackets

Table 2 Summary table of *P* values from ANOVA analyses of drift alga (Alga) e and temperature (Temp) effect on performance characteristics and sulphur levels in seagrass leaves and sediments

General response	Specific response	Figure	Alga (A)	Temp (T)	Alga × Temp	Impact
Seagrass performance	Leaf growth*	1a	<0.001	0.003	0.034	Synergistic
	Rhizome growth	1b	<0.001	0.003	0.833	Additive
	Root growth*	1c	<0.001	<0.001	0.418	Additive
	DW leaves	1d	<0.001	0.020	0.676	Additive
	DW rhizomes*	1e	<0.001	(0.067)	0.293	Single (A)
	DW roots	1f	<0.001	<0.001	0.012	Synergistic
	Leaf area	2a	<0.001	0.021	(0.084)	Additive
	Growing apical	2b	<0.001	0.004	0.029	Synergistic
	Internode distance	2c	<0.001	0.116	0.937	Single (A)
	Number of internodes*	4d	<0.001	0.005	0.130	Additive
	Leaf mortality**	3a	<0.001	0.011	0.002	Synergistic
	Root mortality*	3b	<0.001	0.501	0.677	Single (A)
	Root length	3c	0.009	0.689	0.598	Single (A)
	Root vol	3d	<0.001	0.622	0.107	Single (A)
Sulphur in seagrass	TS in leaves	4a	0.467	0.217	0.305	None
	$\delta^{34}\text{S}$ in leaves	4b	0.011	0.000	0.909	Additive
Sulphur in sediments	SSR*	5a	0.248	(0.078)	0.003	Complex
	DS (PW H ₂ S)*	5b	0.042	0.007	0.738	Additive
	TRS	5c	0.198	0.798	(0.061)	None
	HS stick	5d	0.001	0.720	0.105	Single (A)

See online table for details on F- and sum of squares values. Significant values ($P < 0.05$) are in bold and near-significant values ($P < 0.10$) in brackets

$\delta^{34}\text{S}$ sulphur isotopic composition, TS total sulphur in leaves, SSR sulphate reduction rates in sediment, TRS total reducible sulphides in sediment, DS dissolved H₂S in porewater, HS stick depth of the sulphide front in the sediment as a black coating of Ag₂S on the sticks

** and * correspond to strong ($P < 0.01$) and weak variance ($P > 0.01$) heterogeneity, respectively

severe with increasing temperature (Figs. 1a, 3a), where for DW of roots and apical growth the reversed pattern was found (Figs. 1fc, 2b). The latter finding was caused by a strong reduction in DW of roots and apical growth at high temperatures also without drift algal stress (thereby reducing the absolute stress difference between drift algae and temperature stress). We found highly significant single factor effects of drift algae cover on every response variable, with the poorest seagrass performances associated with high drift algal cover compared with control treatments (Figs. 1, 2, 3; consistent pattern = Control \geq Low \geq High, based on SNK test). Results were generally similar, but slightly less convincing, for temperature treatments. Here, we found significant effects on 9 out of the 14 performance characteristics (not significant for DW roots, internode distance, root mortality, root length, and root volume), with poorest seagrass performance at 30°C (Figs. 1, 2, 3; most common pattern = 20 \geq 25 \geq 30°C, based on SNK-test) (performance at 25°C was grouped with either 20°C, in between, or the 30°C treatments). The univariate tests also attributed most data variability to drift algal treatments (5–10 times more sum of square variability associated with

algae than temperature manipulations, online appendix). Thus, of the 14 individual performance responses, 5 (rhizome and root growth, DW leaves, leaf area, and number of internodes) were classified as additive effects of the two stressors, 4 (leaf growth and mortality, DW roots, and growing apical) as synergistic, and 5 performance responses (DW rhizomes, internode distance, root mortality, length, and volume) were only significant for a single test factor.

Sulphur in seagrasses and sediments and water column oxygen

There was no effect of the two stressors on total leaf sulphur content (Fig. 4a), but $\delta^{34}\text{S}$ in leaves varied significantly with both temperature (lowest at 25°C) and drift algae manipulations (highest at high drift and lowest in control treatments, Fig. 4b). We found significant but complex algae \times temperature interaction effects on SRR in the rhizosphere sediment and the sulphide front. SRR increased with increasing algal cover at 20 and 25°C, but with an opposite pattern at 30°C (Fig. 5a). The sulphide

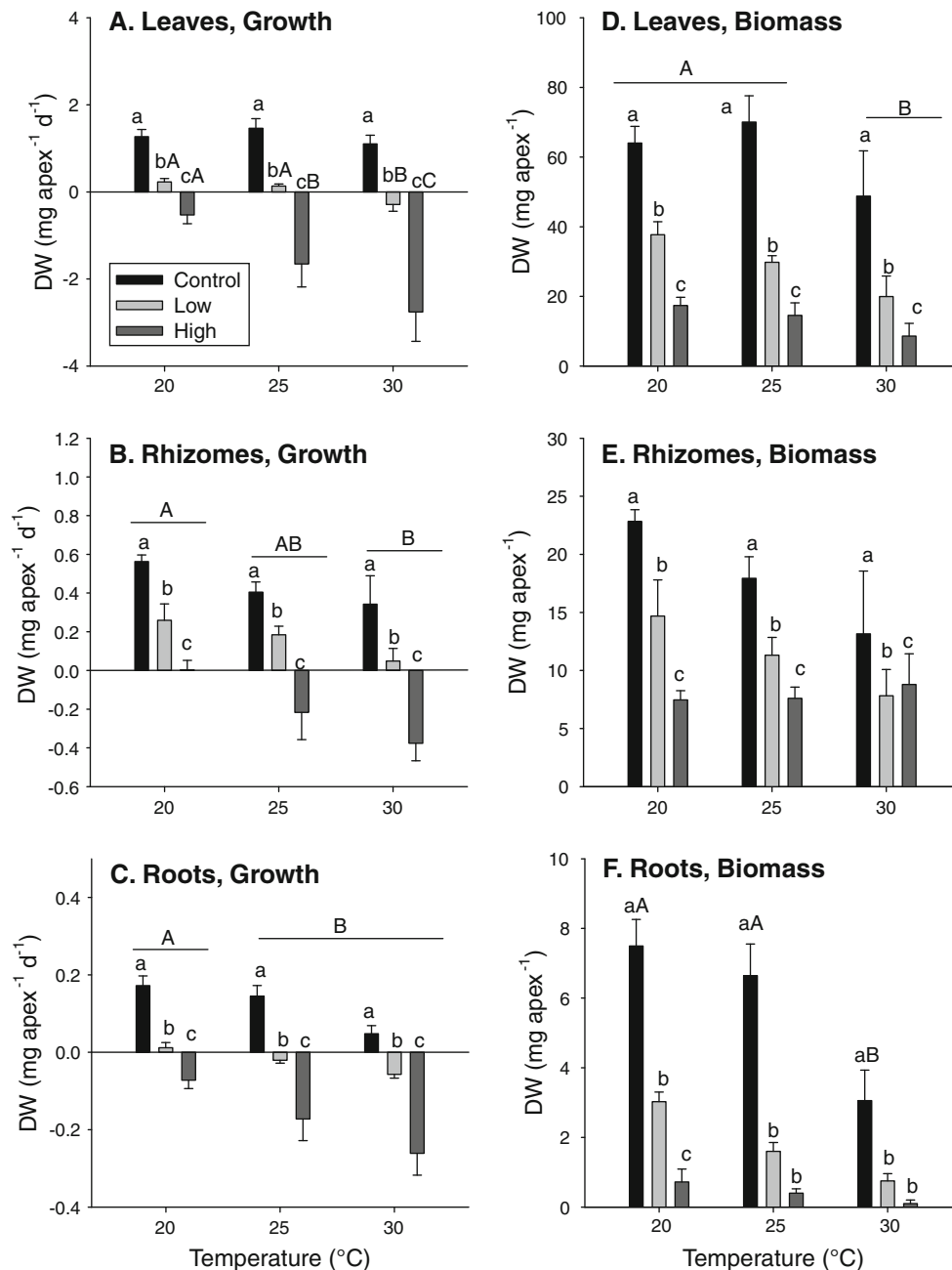


Fig. 1 Effects of drift algae (Control, Low, and High) and temperature (20, 25, and 30°C) on seagrass performances, here growth (left) and biomass (right) of leaves, rhizomes and roots, respectively. Letters

above bars correspond to significantly different treatments based on post-hoc SNK tests (small letters drift algae effects, capital letters temperature effects, see also Table 2)

front was deepest without algal stress, but only at the two lowest temperatures (Fig. 5d). The dissolved porewater sulphide (DS) was affected significantly by both drift algae and temperature (but no significant interaction, Table 2; Fig. 5b), with highest DS concentrations at high algal cover and high temperature levels. Finally, there were no significant effects on TRS pools (Fig. 5c). The oxygen saturation in the water column was generally high both during the day (228–268 μM) and night (175–215 μM),

and we found with no statistical differences between treatments (data not shown).

Correlation of DS vs. seagrass performances

We found significant linear negative correlations between DS and leaf growth, rhizome growth, and root growth, and near-significant negative correlations between DS and leaf weight, leaf area, root weight, internode distance, and

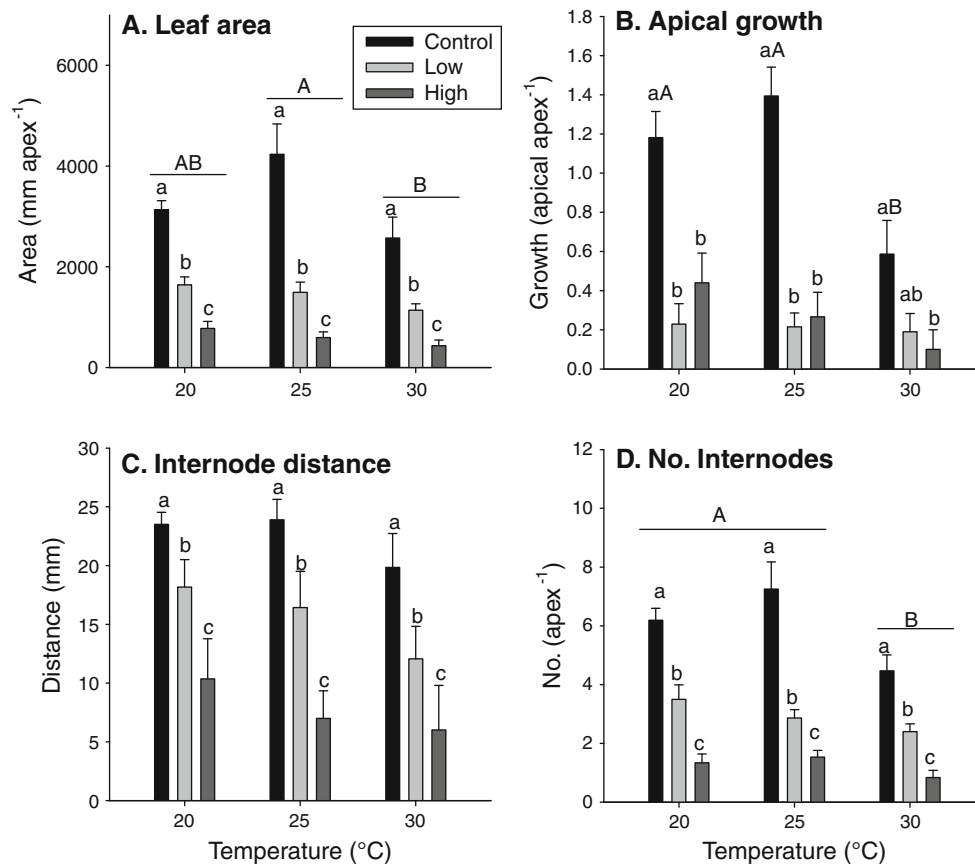


Fig. 2 Effects of drift algae (Control, Low, and High) and temperature (20, 25, and 30°C) on seagrass performances, here leaf area, apical growth, internode distance, and number of internodes, respectively.

number of internodes (Table 3). There was also a near-significant positive correlation between DS and leaf mortality. In contrast, there were no correlations between DS and rhizome weight, number of growing apices, or root mortality, root length or root volume.

Discussion

The presence of macroalgae in seagrass meadows can lead to shading of the seagrasses and hypoxia near the sediment surface (Hauxwell et al. 2001; McGlathery 2001; Holmer and Nielsen 2007; Huntington and Boyer 2008), and seagrasses growing at their upper thermal tolerance can experience physiological stress (Koch et al. 2007b). In addition, respiration of macroalgae during night (Krause-Jensen et al. 1999), burial of macroalgae organic matter (Holmer et al. 2004), and increasing temperatures can stimulate sulphide concentrations in the sediments through stimulated microbial sulphate reduction, increasing the exposure of seagrasses to sulphide, a known phytotoxin. Sulphate reducing bacteria in marine sediments have their optimum around 35–40°C (Hancke and Glud 2004), and rates gener-

ally increase with a factor of 2–3 for each 10°C increase. SRR in seagrass sediments can be modified in response to plant stress, e.g. inhibited due to reduced exudation of root exudates or increased due to less oxidation of the sediments (Holmer and Laursen 2002; Calleja et al. 2007). Thus, seagrass tolerance to algae mats and temperature needs to be considered in the context of plant–sediment interactions. Algae mat coverage and maximum temperatures in our experiments are similar to observations from field surveys in the Swan River, although the dynamics of the mats are not well studied, and the duration of coverage may be less than the 5 weeks deployed in this experiment (Astill and Lavery 2001).

In contrast to mats of ephemeral drift algae (Krause-Jensen et al. 1999; Holmer and Nielsen 2007), *G. comosa* showed no signs of deterioration during the experiment, and oxygen concentrations in the water column in the *H. ovalis* pots remained well above anoxic and hypoxic conditions both during day and night (>68% saturation). The shading by *G. comosa* was, however, extensive and light reaching *H. ovalis* was reduced below saturation (<200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, Hillman et al. 1995) in Low and below the compensation point in High treatments

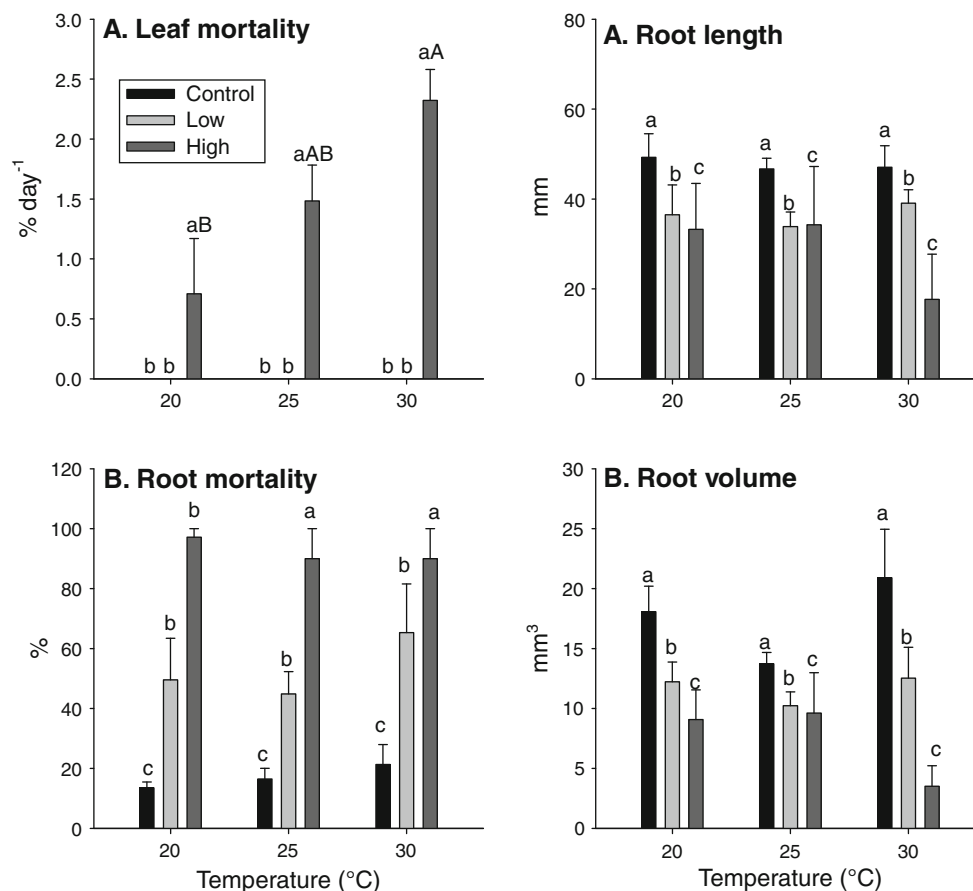


Fig. 3 Effects of drift algae (Control, Low, and High) and temperature (20, 25, and 30°C) on seagrass performances, here leaf and root mortality (left), and root length and volume (right), respectively. Letters

above bars correspond to significantly different treatments based on post-hoc SNK tests (small letters drift algae effects, capital letters temperature effects, see also Table 2)

(<40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, Hillman et al. 1995). *H. ovalis* is able to photoacclimate and adapt to short-term reductions in light (Bite et al. 2007) but cannot survive long periods in darkness (Longstaff et al. 1999). As our experiment lasted 5 weeks, shading most likely is the ultimate cause of the observed mortality, in particular for the High treatment. The proximate causes of reduced performance could be either changes in photosynthetic capacity (e.g. C balance) or reduced oxygenation of the sediments (Connell et al. 1999) leading to increased sulphide concentrations in the sediments (Holmer and Laursen 2002; Calleja et al. 2007) and sulphide toxicity (Holmer and Nielsen 2007). That sulphide toxicity is contributing to performance, which is indicated by the negative correlations between *H. ovalis* growth and morphological changes and porewater sulphide concentrations. Porewater sulphide concentrations responded to the presence of *G. comosa* and to temperature with concentrations up to 30 times higher at 30°C compared with 20°C in the algae treatments. In the control treatment, porewater sulphide concentrations also increased, but only with a factor of 10 from 5 to 47 μM . Furthermore, sulphide was

present almost to the sediment surface in the algae treatments, potentially exposing the plant meristems to sulphide. This could be due to reduced sulphide oxidation capacity in the algae treatments, or by a combination of reduced supply of oxygen from the water column and from the seagrasses (Connell et al. 1999; Borum et al. 2005). Similar increased sulphide concentrations have been found in *Z. marina* sediments covered with filamentous algae (Holmer and Nielsen 2007) or exposed to night time anoxia (Mascaro et al. 2009) and in seagrass sediments amended with organic matter (Terrados et al. 1999; Koch et al. 2007b). Competition for nutrients in the water column could potentially contribute to reduced growth of *H. ovalis* due to the small size and limited storage capacity (Marba et al. 2006), but as *H. ovalis* was growing in organic-rich sediments (5% organic carbon) and the nutrient availability was likely high, root uptake could compensate for reduced leaf uptake, as has been found in the field (Connell and Walker 2001).

The negative effects of algae cover were accelerated at increasing temperatures. A high mortality rate (ca. 2% d^{-1})

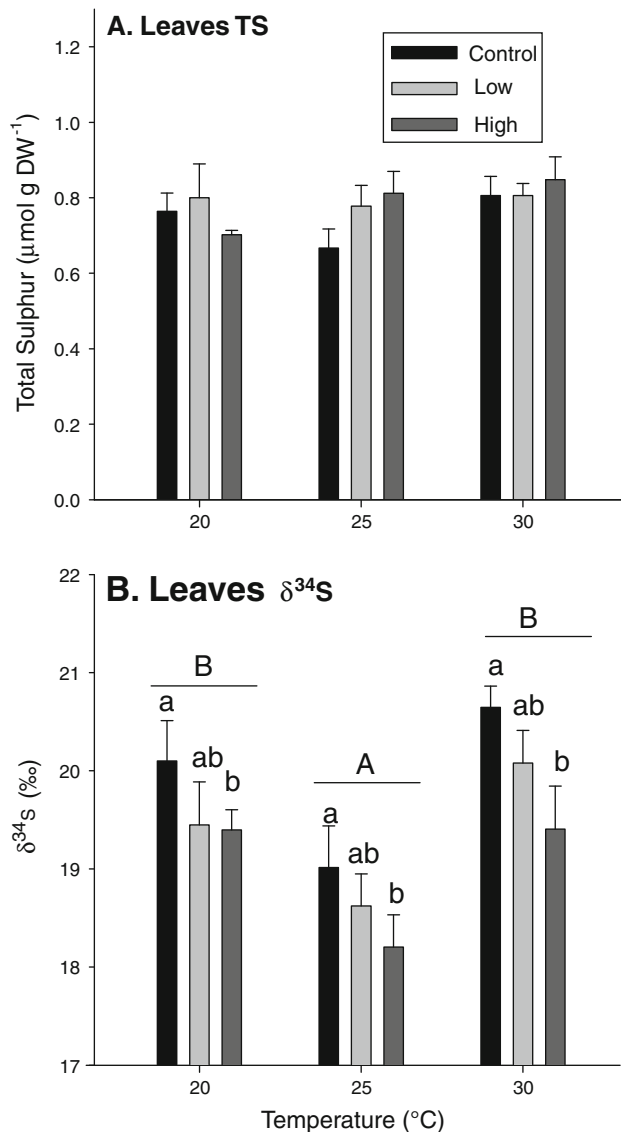


Fig. 4 Effects of drift algae (Control, Low, and High) and temperature (20, 25, and 30°C) on elemental sulphide and $\delta^{34}\text{S}$ in seagrass leaves. Letters above bars correspond to significantly different treatments based on post-hoc SNK tests (*small letters* drift algae effects, *capital letters* temperature effects, see also Table 2)

was observed in the High temperature treatment, indicating that the meadows would likely experience major loss at temperatures near 30°C. Temperatures above 30°C have already been observed in the Swan River (Wernberg, Hobo temperature loggers, unpublished data), and the presence of macroalgae may increase the temperatures even further during the day with high activity in the mats. In another laboratory experiment, synergistic effects of sulphide and temperature have been shown for *Thalassia hemprichii* (Koch et al. 2007a, b), whereas no thermal effects were observed for *Halodule wrightii* (Koch et al. 2007b). Koch et al. (2007b) suggested reduced reoxidation capacity of intruding sulphides into the seagrasses as a possible mecha-

nism for seagrass loss upon synergistic stressors. In contrast, our study indicated little sulphide intrusion (<3% in leaves and <14% in roots based on stable sulphur isotopic composition, data not shown) for *H. ovalis*. Also the S^0 accumulation, an indicator of sulphide reoxidation capacity, was low (<1 $\mu\text{g g DW}^{-1}$) with no significant difference between treatments, suggesting limited capacity for sulphide reoxidation. *Halophila ovalis* may instead be exposed to high levels of sulphides and thus more sensitive to sulphide exposure compared to *Z. marina* and *T. hemprichii*. Similar low accumulation of S^0 has been found in *H. ovalis* growing under tropical conditions in Thailand (Holmer et al. 2006), suggesting a general low accumulation of S^0 in *H. ovalis*, but the mechanism for sulphur dynamics in seagrasses needs to be explored in more detail.

The negative effects of *G. comosa* and temperature on above-ground production of *H. ovalis* resulted in reduced leaf biomass and leaf area, similar to findings for *H. ovalis* in response to direct exposure to sulphide injections (Kilminster et al. 2008). This is consistent with loss of photosynthetic activity and reduced growth for a range of seagrass species upon sulphide exposure (see references in Kilminster et al. 2008). Several studies have reported observations of reduced side branching and growth of apices (Holmer and Nielsen 2007; Kilminster et al. 2008), but our study is the first to document significantly reduced number of growing apices. This was observed for both algae treatments, where no apices were found in the High treatment at all, and the number of apices was reduced by up to 85% in the Low treatment, indicating reduced vegetative growth. Furthermore, reduced growth of apices was observed in the control sediment at 30°C, which would affect the capacity of *H. ovalis* to expand vegetatively under increasing temperatures. Reduced growth of apices could be due to reduced meristematic activity, which has been correlated directly with sulphide concentrations in *Posidonia oceanica* meadows and has been attributed to this active dividing tissue suffering from inhibition of enzymes and anoxia upon sulphide exposure (Garcias-Bonet et al. 2008). Vegetative growth is an important route for expansion of *H. ovalis* meadows, in particular during the growth season (Hillman et al. 1995) and cover by *G. comosa* may significantly affect this ability.

The effects of drift algae on below-ground *H. ovalis* biomass were pronounced, with reduction in all measured parameters including rhizome and root growth, biomass, internode distance, number of internodes, root length, and root volume. Shortening of internode distance and stunted root development have been found in *H. ovalis* upon exposure to sulphides (Kilminster et al. 2008) and drift algae (H Höffle personnel communication), probably due to some of the same mechanisms as observed for the meristems. Kilminster et al. (2008) suggested that reduced internode

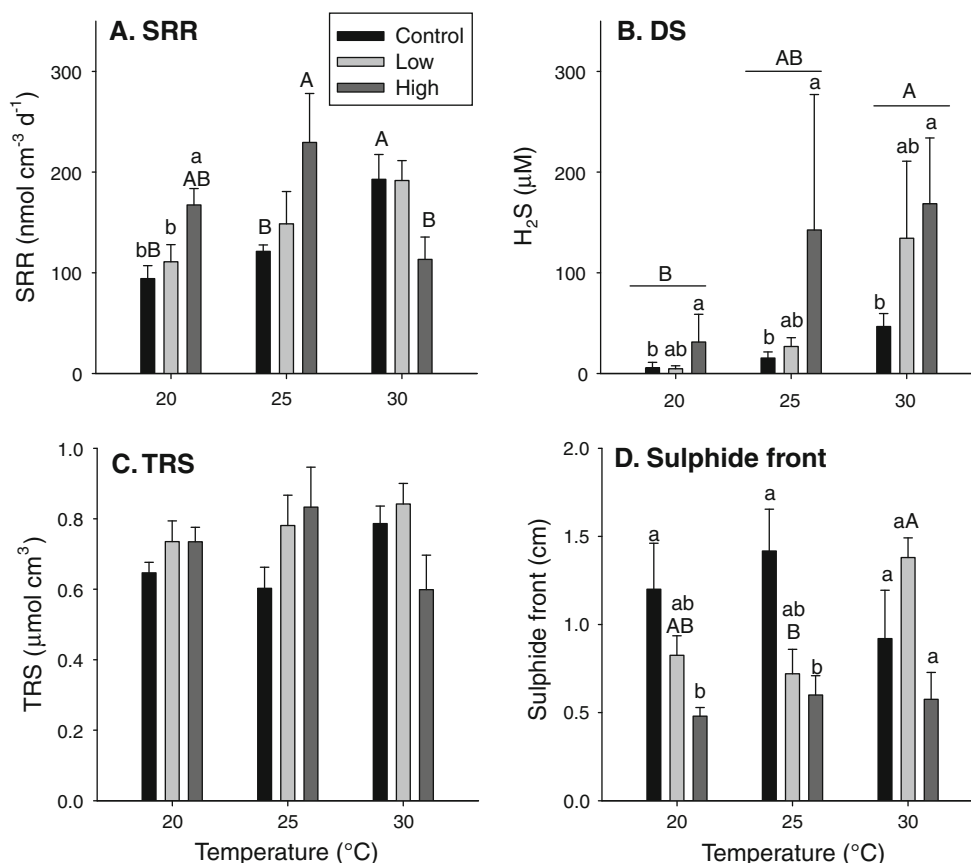


Fig. 5 Effects of drift algae (Control, Low, and High) and temperature (20, 25, and 30°C) on sulphide levels in sediments. *SSR* sulphate reduction rates, *DS* dissolved H_2S in porewater, *TRS* total reducible sulphides, *Sulphide front* depth of the sulphide front as a black coating of

Ag_2S on silver sticks. Letters above bars correspond to significantly different treatments based on post-hoc SNK tests (*small letters* drift algae effects, *capital letters* temperature effects, see also Table 2)

Table 3 Correlation coefficients and significance level between sulphides in porewater (DS) and 14 plant performance characteristics

Plant characteristics	r_{Pearson}	<i>P</i>
Leaf growth	-0.347	0.019
Rhizome growth	-0.297	0.048
Root growth	-0.356	0.017
Leaf weight	-0.260	(0.084)
Rhizome weight	-0.139	0.364
Leaf area	-0.282	(0.060)
Root weight	-0.270	(0.073)
No. of growing apex	-0.155	0.310
Internode distance	-0.278	(0.065)
No. of internodes	-0.264	(0.080)
Leaf mortality	0.249	(0.099)
Root mortality	0.169	0.267
Root length	-0.205	0.177
Root volume	-0.247	0.101

Significant values ($P < 0.05$) are in bold and near-significant values ($P < 0.10$) in brackets

distance could increase root density and thereby enhance sediment oxidation and reduce the sulphide pressure on the seagrass. This was not the case in our study where both shoot and root density declined in the Low and High treatments and sulphide pools increased, suggesting less sediment oxidation despite reduced internode distance. As the morphological changes in below-ground biomass follow closely to changes in the above-ground biomass (Table 3), our results suggest a general reduced performance of *H. ovalis* when under stress from *G. comosa* and increasing temperatures. Morphological acclimations by seagrasses are commonly observed in response to environmental pressures, e.g. physical forcing (Peralta et al. 2005), fresh water run-off (Irlandi et al. 2002), and turbidity (Miller et al. 2005), but only few studies have included studies of the below-ground biomass. Halun et al. (2002) found similar results of reduced root production and rhizome elongation as well as reduced shoot production for *Cymodocea rotundata* exposed to in situ injections of sulphides, suggesting that sulphide toxicity affects both above- and below-ground biomass and production.

We conclude that biomass of *G. comosa* and high temperatures affect the ecological performance of *H. ovalis*, with strong negative effects on the measured plant performance responses, in particular for the leaf, rhizome and root growth and mortality. The most important impacts were associated with drift algal stress, indicating that the selected drift algal stress levels were more important than the temperature treatments. We emphasize that the negative effects associated with the two stressors can be both synergistic and additive, providing a warning that impact of multiple stressors may be difficult to predict from single-factorial stress experiments (Crain et al. 2008; Darling and Cote 2008). We also found indirect evidence of sulphide toxicity as a possible mechanism, in concert with light limitation, for drift algae reducing the ecological performance of seagrasses (Holmer and Bondgaard 2001; Holmer et al. 2005). We finally note that *H. ovalis* beds in the Swan River likely will become more patchy and less dense in the future as the abundance of *G. comosa* and temperatures are expected to increase in the near future due to eutrophication, urbanization, and global warming.

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