FUNCTIONING OF PHILIPPINE SEAGRASS SPECIES UNDER DETERIORATING LIGHT CONDITIONS

Functioning of Philippine Seagrass Species under Deteriorating Light Conditions

DISSERTATION

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by

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Theses Wilfredo Uy

1. Reduction of light availability in the seagrass meadow to 10-20% of incident light is critical for the survival of tropical seagrasses (Duarte, 1991; Vermaat et al., 1997; this thesis).

   Duarte C.M. 1991. Seagrass depth limits. Aquat. Bot. 40, 363-377.

   Vermaat J.E., Agawin N.S.R., Fortes M.D., Uri J.S., Duarte C.M., Marba N., Enriquez S., Van Vierssen W. 1997. The capacity of seagrasses to survive increased turbidity and siltation: the significance of growth form and light use. Ambio 26, 499-504.

2. Seagrasses often inhabit reduced or slightly anoxic sediments (Terrados et al., 1999). Reduced sediment conditions are stressful to submerged plants. Prolonged or severe shading adds stress beyond the survival capacity of the plants when living on reduced sediment.

   Terrados J., Duarte C.M., Kamp-Nielsen L., Agawin N.S.R., Gacia E., Lacap D., Fortes M.D., Borum J., Lubanski M., Greve T. 1999. Are seagrass growth and survival constrained by the reducing conditions of the sediments? Aquat. Bot. 65, 17-197.

3. Although direct quantitative evidence is largely absent, the ongoing siltation and eutrophication of Philippine nearshore waters and the relations between these processes and seagrass abundance established elsewhere, imply that seagrasses are declining throughout the region (Fortes, 1988).

   Fortes M.D. 1988. Mangroves and seagrass beds of East Asia: habitats under stress. Ambio 17, 207-213.

4. Photosynthetic capacities and leaf relative growth rates of the seagrass are poor predictors of the effects of light deterioration in coastal waters (this thesis).

5. Being clonal plants, seagrasses show the same repertoire as their terrestrial counterparts, including contrasts in clonal integration between species (De Kroon and Van Groenendael, 1990; this thesis).

   De Kroon H, Van Groenendael J. 1990. Regulation and function of clonal growth in plants: an evaluation. In: Van Groenendael J., De Kroon H. (eds) Clonal growth in plants: regulation and function. SPB Academic Publishing,The Hague, pp 177-186.

6. Stable isotopes, particularly where combinations of chemical species are used, have demonstrated their usefulness in ecophysiology and foodweb studies (Hemminga and Mateo, 1996; Mateo et al., 2001; this thesis).

   Hemminga M.A., Mateo M.A. 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Mar. Ecol. Progr. Ser. 140, 285-298.

   Mateo M.A., Renom P., Hemminga M.A., Peene J. 2001. Measurements of seagrass production using the stable isotope ^{13}C: a comparison with classical ^{14}C and O_2 methods. Mar. Ecol. Progr. Ser. 223, 157-165.

7. An economic valuation of coastal natural resources should not ignore the spatial interactions among marine habitats (i.e. coral reefs, seagrass beds and mangrove stands; Nagelkerken, 2000).
   Nagelkerken I. 2000. Importance of shallow-water bay isotopes as nurseries for Caribbean reef fishes. PhD-thesis, University of Nijmegen, The Netherlands, 168 pp.

8. "When things are bad, we take comfort in the thought that they could always be worse. And when they are, we find hope in the thought that things are so bad they have to get better" (Anonymous).

THESES

belonging to dissertation

Functioning of Philippine Seagrass Species under Deteriorating Light Conditions

Wilfredo Hojilla Uy

19 December 2001

Wageningen University /IHE Delft

The Netherlands

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Summary

Enhanced eutrophication as well as siltation are important environmental issues in Southeast Asian coastal waters. Both lead to increased turbidity and hence to lower light availability for the originally extensive seagrass beds that fringe most of the Philippine coastlines. Consequent large-scale declines of seagrass beds have been reported from around the world. Since these seagrass meadows have important functions both for local human populations as well as for larger-scale economics, their decline may have unexpected societal consequences. Seagrass beds have well-established importance as highly productive carbon-fixing systems, as nursery and foraging grounds for commercial fish and shrimps, and act as sediment trap and breakwater.

Longer-term, consistent reduction in light availability to plants may lead to acclimation in surviving species or succession to more shade-adapted communities. Long-term shade effects on tropical seagrass systems have remained unstudied so far, despite the obvious relation to increased eutrophication and siltation. This study looked in detail to the possible responses and capacity to acclimate to substantial and longer-term light deprivation in two common and contrasting Philippine seagrass species: *Thalassia hemprichii* and *Halodule uninervis*. The former species is often dominant, comparatively large, long-lived and late successional, whereas the latter is shorter-lived, smaller-sized and has a less clear late successional status. A logistically demanding long-term *in situ* shading experiment was set-up and monitored for one experimental shading year and one subsequent year of recovery at two sites in Northern Mindanao, Philippines (Naawan and Sulawan, both in Misamis Oriental). Shade responses were quantified at several levels: morphometry, shoot dynamics and clonal integration, photosynthetic capacity, carbohydrate and nutrient allocation. Experimental shade levels were approximately 0%, 60% and 85% reduction of full light reaching the seagrass stands. The interaction with increasingly reduced sediment conditions often observed along eutrophication gradients was incorporated in this study.

First and foremost, the highest shading level led to pronounced shoot mortality, with little or no new shoot recruitment. *T. hemprichii* survived better than *H. uninervis* (18% versus 5% of the original shoots remained after one year), probably due to the more robust rhizome network of the former, providing a tighter clonal integration and containing substantial quantities of reserve carbohydrates that could be mobilized. In those few surviving shoots, relative leaf growth rates were maintained across the three shading levels. After removal of the experimental shades, recovery of *T. hemprichii* was faster than that of *H. uninervis*, but full recovery to pre-experimental densities in the plots that had received the highest shading was estimated to exceed two years in the former and even longer in the latter species.

Both species displayed considerable plasticity in shoot morphology as a response to shading: leaf surface area and specific chlorophyll content increased, and also leaf turnover was reduced. No clear response to shading was observed in the photosynthesis-light curve parameters. Photosynthetically fixed carbon was substantially less in the shade, and allocation to rhizomes and new shoots was strongly reduced. This is similar to a general plant response to shading: fixed carbon is used for the maintenance of the leaves at the expense of belowground tissue. The longer-lived *T. hemprichii* allocated a comparatively larger proportion to its rhizomes than *H. uninervis,* in line with its tighter clonal integration. The tracking of allocation of stable isotopes ^{15}N and ^{13}C confirmed the difference in clonal integration between the two species. Shade effects were more pronounced when the plants were grown on more reducing sediments: whole-plant diel oxygen and carbon balances remained negative confirming the substantial shoot mortality in these conditions.

This dissertation has shown that photosynthetic capacities and relative growth rates are comparatively poor predictors of the long-term effects of light deterioration in our coastal waters. Rather, more conspicuous responses were observed in shoot morphology and shoot dynamics as a consequence of shading, shoot density decreased, leaf length increased, shoot recruitment dropped and chlorophyll content of the leaves of surviving shoots increased. Increased shoot mortality leading to reduced density was due to a substantial reduction in physiological integration, i.e. the translocation of carbohydrates and nutrients among shoots sharing the same horizontal rhizomes was significantly reduced.

Chapter 1

General Introduction

Seagrasses are fully submerged vascular plants inhabiting shallow coastal seas. In the tropics, particularly in the Philippines, seagrasses reach their highest species diversity and abundance, forming extensive, dense meadows that are a major component of the coastal zone (Fortes, 1988; 1989; Phillips and Meñez, 1988). A total of 50 species has been reported worldwide (Den Hartog, 1970) and 16 species are reported in the Philippines (Meñez et al., 1983; Fortes, 1989). The luxuriant growth of the plants is reflected in the often very high primary productivity of these seagrass beds (McRoy and McMillan, 1977; Zieman and Wetzel, 1980; Hillman et al., 1989; Erftemeijer et al., 1993, Vermaat et al., 1995), ranking these plant-dominated systems higher than many other ecosystems.

The abundant occurrence of seagrass meadows in tropical coastal waters is directly relevant to human populations in the coastal zone, as seagrass meadows are nursery and foraging areas for numerous valuable fish and crustacean species (Fortes, 1989; Phillips and Meñez, 1988; Dawes, 1998). By acting as sediment traps and natural breakwaters, they function as natural barriers against marine erosion (e.g. Ward et al., 1984; Fortes, 1988; Bell and Pollard, 1989; Hemminga and Nieuwenhuize, 1990).

As the human population increases, people to live closer to the sea. Over the past decades, this has resulted in greater anthropogenic impacts on the coastal zone worldwide (Holligan and de Boois, 1993). The well known effects of such socio-economic developments, such as increased sediment loading on coastal waters and eutrophication, presently already impose deteriorating habitat conditions on many tropical seagrass communities, endangering their persistence (Fortes, 1988; Shepherd et al., 1989; Lundin and Lindén, 1993). Apart from the loss this may be for a pure conservationist, the loss of seagrass communities is of direct concern to the human coastal population, for reasons already mentioned above.

The presence of seagrasses in the shallow coastal waters critically depends on the availability of light to allow photosynthetic processes and growth (Duarte, 1991a; Kirk, 1983; Vermaat et al., 1997; Hemminga and Duarte, 2000). Reduced light availability can be considered a major stress (sensu Grime, 1989) for these angiosperms. In some well-documented cases, decline of seagrasses revealed to have been caused by deteriorating light conditions (Shepherd et al., 1989); whereas, it has been suspected to be a causal factor in many other, less investigated cases. Both, increased sediment loads in the water (e.g. as a result of increased erosion due to poor land use, coastal engineering works and dredging activities) and increased dissolved nutrient inputs in coastal waters may result to a decreased light penetration in the water. Low light conditions will force the seagrasses to lower levels of photosynthetic activity, or to put

it in other words, to lower levels of carbon fixation (Alcoverro et al., 1999). It is known that the light level at which growth finally reduced to zero, differs among species (Hillman et al., 1989; Vermaat et al., 1997). The effect of dissolved nutrients is probably indirect, mainly via an increase in phytoplankton and epiphytic biomass (Phillips et al., 1978; Sand-Jensen and Borum, 1983).

In this thesis, it is hypothesized that different seagrass species have, in their various growth situations, different capabilities for acclimation and persistence under such deteriorated light conditions. Long- and short-term shading experiments were carried out to simulate light climate deterioration, and quantified photosynthetic acclimation, carbon and nutrient allocation, clonal integration and survival. The project focused on two species with contrasting lifespan and strategies: a short-lived, early successional species, *Halodule uninervis* (Forsskal) Ascherson, and a long-lived, late successional species, *Thalassia hemprichii* (Ehrenberg) Ascherson (cf. Duarte, 1991b; Vermaat et al., 1995).

Fig. 1. Monospecific stand of *Thalassia hemprichii* at the Sulawan site. Note the extensive calcareous epiphytic algae and the loosely attached forams on the leaves.

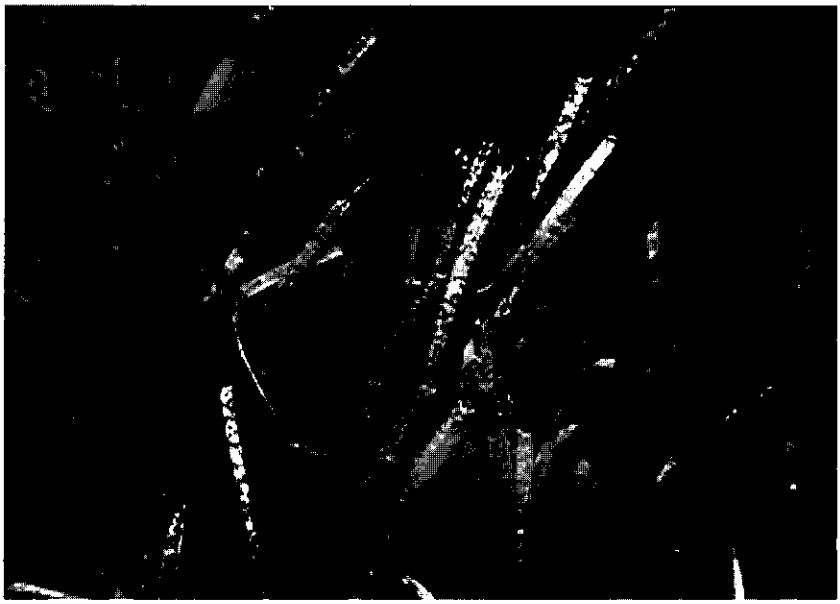

Fig. 2. Monospecific stand of *Halodule uninervis* (wide-leaved variety) at Naawan, Misamis Oriental. Note the relatively long stems generally covered with filamentous and fleshy algae.

The dugong grass, *Thalassia hemprichii,* is a common species in tropical SE Asian, particulalry in Philippine seagrass beds which often dominates mixed meadows (Fig. 1, Erftemeijer and Herman, 1994; Vermaat et al., 1995; Arriesgado, 2000). *Halodule uninervis*, on the other hand, may occur in most seagrass beds but is reported to be abundant on less stable, muddy to sandy sediments (Fig. 2, Brouns, 1987; Inglis, 2000). Two varieties are reported for this species: wide-leaved and narrow-leaved (Phillips and Meñez, 1988).

Study sites

Two study sites were established in northern Mindanao, southern Philippines (see Fig. 1, Chapter 2). Site 1 is in Naawan, Misamis Oriental (8° 38'N; 124° 27'E). This site is situated in front of the Mindanao State University at Naawan campus. The intertidal area is generally narrow and extends approximately 100 m from the shoreline to the reef edge. The upper reef, particularly in the sandy area which is exposed during low tide, is covered with narrow-leaved *H. uninervis*. Extensive seagrass meadows are generally located in the subtidal area after the reef edge along sandy to sandy muddy areas. Depth ranges from 1- 7 meter. The wide-leaved *H. uninervis* and *Cymodocea serrulata* (R.Br.) Aschers. & Magnus generally dominate the subtidal seagrass meadow. The site is situated near the Talabaan river that contributes silt loading to the

site during rainy days. The area is also exposed to relatively strong wave action during the southwest monsoon season from April to August.

The second site is in Punta Sulawan, Tubajon, Laguindingan, Misamis Oriental (8° 38.25' N, 124° 27.6' E). The site is situated in a relatively remote area, approximately 10 km from the national highway. The site is also a focus of societal attention, due to the proposed construction of an international airport within the direct vicinity of the extensive seagrass meadow. This site is one of the few extensive mixed seagrass meadow in this part of the country, with an estimated area of 116 ha. It is a frequent visiting site for most students from neighbouring towns and cities because of its still relatively pristine condition. Human impact in the seagrass bed includes boat tracks during low tide, and harvesting of bivalves by digging. The area is also the source of assorted shells for the local shellcraft industry promoted by the local council. Other invertebrates collected for food in the area include gastropods, sea urchins, sea cucumbers and sea hare egg cases. A total of 83 species of fish were identified in the area (Deocadez, 2000), making it a relatively species-rich seagrass bed (cf.. Nagelkerken et al., 2000). The area has an extensive mangrove reforestation project by the local townspeople who work in coordination with the local government offices. A total of 34 hectares of mangrove were planted with ages ranging from 1 to 12 yrs old. These mangroves, *Rhizophora* spp., were planted along seagrass beds forming a band approximately 100-300 meters in width and more than a kilometer across designed to protect the shoreline.

Overview of the different chapters

The different studies conducted focused on the two common species, *T. hemprichii* and *H. uninervis*. These species were compared as to their responses or possible adaptations to shading (Chapters 2-5) and sediment conditions (Chapter 4) including their periphyton load (Chapter 6). Assimilation and allocation of stable isotopes ^{13}C (Chapter 3) and ^{15}N (Chapter 5) were also used to quantify the effects of shading. These chapters compose the different studies conducted to understand how seagrass respond to conditions of light deterioration: Chapter 2 describes the long-term effect of light reduction on the growth and morphology of the two species of seagrass, including their photosynthetic capacities. Three shade treatments were installed *in situ* to simulate conditions of light deterioration. After one year of experimental shading, the shades were removed and the plots were subsequently followed to assess the capacity for recovery. The re-allocation of photosynthetically assimilated carbon along different parts of the plant tissues in response to long-term shading is covered in chapter 3, using the stable isotope ^{13}C. Chapter 4 describes the interactive effects of shading and sediment conditions. This experiment was performed under laboratory conditions. An oxygen balance was constructed to explain plant response. Chapter 5 covers allocation of absorbed nutrients among ramets, that share the same horizontal rhizomes, under normal and shaded conditions. In this chapter, a quantitative approach was employed using the stable isotope ^{15}N. Chapter 6 investigates the possible effect of

experimentally reduced vitality of the seagrass on leaf periphyton loads. Experiments were done in both the individual shoot and community levels. Vitality was reduced by cutting the belowground rhizome connections. Finally, Chapter 7 summarizes and integrates the different studies conducted.

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Chapter 2

Growth, morphology and photosynthetic responses of *Thalassia hemprichii* and *Halodule uninervis* to long-term *in situ* light reduction

Uy, W.H., Hemminga, M.A., Vermaat, J.E. Fortes, M.D.

Abstract

Two different seagrass meadows in the southern Philippines, dominated by *Thalassia hemprichii* (Ehrenberg) Ascherson and *Halodule uninervis* (Forsskal) Ascherson respectively, were subjected to one year *in situ* shading, arranged in randomized block design with 3 shading levels (control = 0%; medium = 50-67% and high = 81-89%) in three replicate plots. Post-recruitment recovery was followed for another year in both species. Shoot density of both species decreased with increasing shading. After one year in the high shading level, only 6% and 20% of the original stocks remained for *T. hemprichii* and *H. uninervis*, respectively. Consequently, above and belowground biomass decreased in both species with a tendency to reduced proportion of the belowground to the above in *T. hemprichii*, while in *H. uninervis*, relative proportions remained the same. Shoot lengths (leaves + stem) of the remaining plants increased to as much as 1.7 and 2.2x for *T. hemprichii* and *H. uninervis*, respectively. Neither leaf width nor the number of leaves per shoot differed among shading treatments but temporal variation was strong. Plastochron interval and leaf elongation rate increased with shading in *T. hemprichii* but was not significant for *H. uninervis* ($p>0.05$). Percent nitrogen content increased with shading in both species while C:N ratio decreased. Photosynthetic capacities were generally higher in the shaded plants in both species, but this only explained 3% of the variation (3-way ANOVA, $p<0.01$), whilst seasonal variation and the species effect contributed much more (explaining 21% and 13% of the variation respectively). Complete recovery was achieved in the medium shading plots after 8-9 months for both species. The highly shaded plots had reached approximately half (*T. hemprichii*) and or 1/3 (*H. uninervis*) of control densities after 12 months post treatment. Apparently recruitment of *T. hemprichii* at the experimental sites was more successful than that of *H. uninervis*. This is attributed to the higher exposure to waves in the *H. uninervis* site and the colonization of the plots by other species, notably *Syringodium isoetifolium*.

Keywords: tropical seagrass, growth dynamics, photosynthesis, shading, recovery

Introduction

Deterioration of light conditions brought about by increased sediment loading or eutrophication is considered a major threat to tropical seagrass meadows (Fortes, 1988; Shepherd et al., 1989). Light availability generally governs depth distribution and abundance of seagrasses (Dennison, 1987; Duarte, 1991). Reduction of light availability caused by siltation has been shown to affect growth and dynamics of seagrasses (Duarte et al., 1997; Vermaat et al., 1997; Terrados et al., 1998). Hemminga (1998) reviewed the possible cascading effects of light reduction and argued that the heterotrophic root/rhizome system offers a competitive advantage in nutrient poor waters, but that it makes the plants vulnerable when light conditions deteriorate and respiratory demands exceeded photosynthetic oxygen production.

A number of shading experiments, both in situ and in the laboratory, have shown negative effects on seagrasses, i.e. a reduction of shoot density (Dennison and Alberte, 1982; Bulthuis, 1983; Abal et al., 1994; Czerny and Dunton, 1995; Fitzpatrick and Kirkman, 1995; Rollon, 1998; Bach et al., 1998) and shoot length (Bach et al., 1998; Longstaff and Dennison 1999). Increases in shoot length also have been reported (Abal et al., 1994; Grice et al., 1996). Moreover, relative growth rates (RGR) were sometimes found constant with depth (Rollon, 1998) and shading (Gordon et al., 1994, Bach et al., 1998) but more often significantly reduced with shading (Backman and Barilotti, 1976; Dennison and Alberte, 1982; Bulthuis, 1983; Abal et al., 1994; Grice et al., 1996; Lee and Dunton, 1997).

Most of the experimental work described above was short term (≤ 3 months) and on temperate species, e.g. *Heterozostera tasmanica* (Bulthuis, 1983), *Posidonia australis* (Fitzpatrick and Kirkman, 1995), *Posidonia sinuosa* (Gordon et al., 1994), *Zostera capricorni* (Abal et al., 1994), *Zostera marina* (Backman and Barilotti, 1976; Dennison and Alberte, 1982), or western hemisphere tropical species *Thalassia testudinum* (Czerny and Dunton, 1995; Grice et al., 1996; Lee and Dunton, 1997) and *Halodule wrightii* (Czerny and Dunton, 1995). Studies on tropical South-east Asian species are few (Bach et al., 1998; Rollon, 1998; Longstaff and Dennison, 1999).

Here we report on a long term *in situ* shading experiment with a long-lived species, *Thalassia hemprichii* and a common short-lived species, *Halodule uninervis* (cf. Vermaat et al., 1995). Our aims were (1) to assess the effects of, and possible responses to prolonged shading in these two contrasting species in terms of density, growth, morphology, photosynthetic capacities, biomass and nutrient allocation in the above- and belowground compartments; and (2) to quantify recovery over a one year period.

Materials and Methods

The study was conducted in Mindanao, Southern Philippines located specifically at Sulawan (8° 26' N; 124° 17' E) and Naawan, (8° 38' N; 124° 27' E), both in Misamis

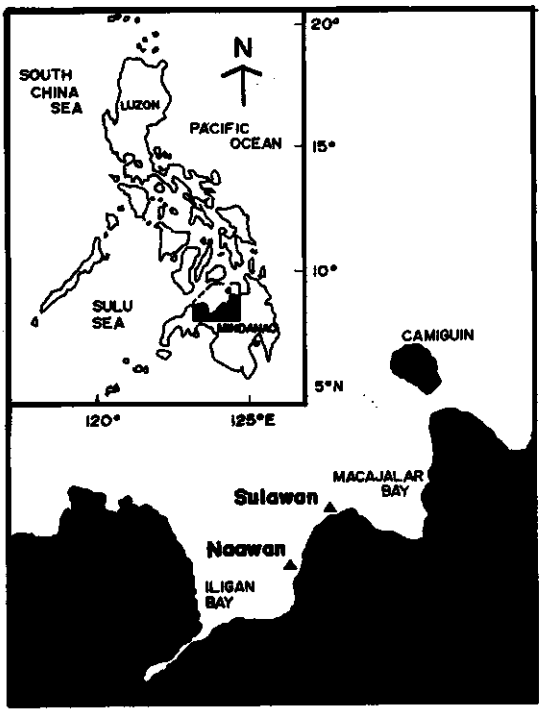

Fig. 1. Location of the two study sites in Northern Mindanao marked with triangles. Inset gives location in the Philippines

Oriental (Fig. 1). Sulawan is located in a cove with relatively extensive mixed seagrass beds with an estimated area of 110 ha. A large area is exposed to air during low low tide and a mangrove transplantation site is located in the area near the coastline. Seven seagrass species are reported in the area and *T. hemprichii* is dominant (Arriesgado, 1999). The Naawan site in front of the Mindanao State University at Naawan campus is characterized by a narrow and relatively shallow reef flat. Along the sandy areas at the margins of the fringing reef are patches of monospecific beds of *H. uninervis* and *Cymodocea serrulata* and occasional *Syringodium isoetifolium*. The area is exposed to strong wave action, particularly during the southwest monsoon season from April to August. The study site, however, is located at the inner margin of the reef and relatively protected. The site is more turbid than Sulawan due to a nearby river.

In situ shading started July 14, 1997 for *H. uninervis* and September 5, 1997 for *T. hemprichii* and lasted for one year. During a one year post-treatment period, we continued to monitor the plots. We used three shading levels i.e. 0, 40 and 75% reduction of incident light referred to here as control, medium and high shade levels, respectively. Daily irradiance ranged from 30 to 50 E m^{-2} d^{-1}, in the rainy and dry seasons, respectively. Black screens, of different mesh sizes, measuring 1.6 x 1.6 m were used to achieve the desired shade level. The maximum shade level used in the study was designed to be just above the reported light compensation points of the two species to allow growth to occur (Agawin et al., 1996, Vermaat et al., 1997). The control, without the shade was provided with a frame minus the shade screens. The actual shading levels achieved in the field, however, were higher because of epiphyte growth and suspended sediments settling on the screens. Thus, the average shade levels were different for the two sites. The *H. uninervis* site in Naawan had 0%, 67±5% (±SE) and 89±2% for control, medium and high shade levels respectively. The *T. hemprichii* site in Sulawan had 0%, 50±4% and 81±4%. A total of nine plots (3 shading levels x 3 replicates), arranged in randomized complete block design, was installed at each site. The height

of the shade screens was approximately 10 cm above the tallest plant. A permanent (1x1 m) quadrat was fixed in the center of each shade frame with a peg in every corner for monitoring purposes. The size of the permanent quadrat allowed a 30 cm peripheral shade/lighted or "buffer" zone inside the 1.6x1.6 m shade frame. The shade screens were cleaned every other day or daily whenever necessary and were replaced every month. We have not carried out any 'trenching' or cutting of rhizome connections at the outer edge of the shaded perimeter, since we considered this to be an undesired additional disturbance that might interfere with the plant's response to shading (e.g. Fitzpatrick and Kirkman, 1995)

Shoot density was measured monthly in the same 4 subquadrats (20 x 20 cm) within the 1x1 m permanent quadrat. Ten shoots inside the permanent quadrat were randomly chosen for measurements of the following: shoot height = total length of the shoot measured from the apparent base of the stem to the longest tip of the leaves; leaf width = average width of the leaves; leaf number = total number of leaves per shoot.

Core samples for above and belowground biomass (15 cm diameter and 20 cm deep) were harvested in all the experimental plots at an interval of 1, 3, 6 and 12 months shading period. About 5 to 6 days before the coring, the shoots in pre-selected areas were punched with a syringe needle (gauge 22) a little above the sheath-leaf junction for growth rate measurements. All harvested core samples were placed in pre-labelled plastic bags and were kept cool during the transport. At the laboratory, each sample core was sorted by species and cleaned for measurements. Shoots with punched holes were separated first for growth measurements as follows: total leaf length (mm) = total of all leaf length per shoot measured from the junction of the leaf sheath and leaf, up to the tip of each leaf; length of new leaves = measured from the leaf junction to the marked syringe hole; 'virgin' leaf length = length of the new leaf without the marked syringe hole; leaf width (mm) = average width of the leaves per shoot; sheath length (mm) = measured from the leaf base to the visible tip of the sheath or remnants of the dead leaves; vertical rhizome length (stem) = measured from the point of insertion of the stem on the horizontal rhizome to the point of insertion of the youngest leaf; number of leaf scars = counted from the point of insertion of the youngest leaf to the base of the stem. Growth rates and leaf plastochron intervals or leaf turnover were calculated according to Hunt (1982) and Duarte et al. (1994), respectively. In both species, leaf width was not significantly different among the shade levels in both species thus leaf lengths were used instead of leaf area.

$$\text{RGR (\% per day)} = [\ \ln(\ LL_f / LL_i\ ) / t\ ] * 100 \qquad (1)$$

$$\text{LER (mm d}^{-1}) = \frac{\text{total length of new leaf tissues}}{\text{marking period}} \qquad (2)$$

$$\text{LPI (days)} = \frac{\text{number of punched shoots x marking period}}{\text{total number of new unmarked leaves}} \qquad (3)$$

where: RGR = leaf relative growth rate, LL_f = total leaf length of a shoot minus newly formed primary leaves without marks, LL_i = LL_f minus new leaf tissues formed on marked leaves, t = time duration between leaf marking and harvest, LER = leaf elongation rates, LPI = leaf plastochron interval or the time interval between the formation of each successive leaves. LPI is expressed as number of days which, when multiplied with the number of leaf scars of each shoot, will give the estimated shoot age. Vertical rhizome elongation rate was calculated by regressing the length of vertical rhizomes against the shoot age or number of leaf scars (Duarte et al., 1994). After morphometric measurements were completed, the samples were sorted and weighed separately for the following: leaves, leaf sheath or remnants of dead leaves, stem or vertical rhizomes, horizontal rhizomes, roots. Samples were oven-dried for at least 48 hours at 50-60° Celsius before weighing. After weighing, all samples were stored dry for later analyses. The nitrogen and organic carbon content of leaves, stem, rhizomes and roots were analyzed with a Carlo Erba NA 1500 CN-analyzer.

To determine photosynthetic responses to reduced light, photosynthesis-irradiance (P-I) curves for both species were determined intermittently for one year (before the shading, then 2nd week, 1st month and every 3 months thereafter). A number of shoots was collected randomly from each plot. The shoots were cleaned carefully in filtered seawater and epiphytes on the leaves were removed by gentle scraping, using the edge of a glass slide. For *T. hemprichii*, only the first two youngest leaves were used in the experiment. The third leaves were generally coated with calcareous epiphytes, and thus were not included. To be consistent, we collected only shoots with the youngest leaf at least half the length of the second leaf. For *H. uninervis*, the whole shoot with the first three leaves intact was used in the experiment. A flow-through system was used in the determination of P-I curves through evolution of oxygen at different light regimes. Shoots weighing approximately one gram were placed in clear perspex tubes (2.6 cm x 20 cm), sealed with rubber stoppers with glass tubing inserted for connections. A total of 4 chambers were used to represent 3 replicates and a control (without plants). Flexible tygon tubing was used to connect the chambers to a storage vessel with seawater and a downstream oxygen electrode. A peristaltic pump was used to regulate the flow (ca. 78 ml per minute). The D.O. meter (model WTW Oxi325) was then connected to a computer for automated recording at an interval of 10 seconds. Prior to measurements, oxygen concentration of the medium was reduced to 4-5mg L^{-1} by bubbling with nitrogen gas. Then the plants were incubated for 20 minutes per light level. Seven to eight light levels were applied at an increasing intensity up to a maximum of 1800 µmol PAR $m^{-2}s^{-1}$. Light was provided by a halogen projector lamp (Sylvania ENX 360W 82V). All experiments were conducted during the daylight period from 0800 to 1800hrs at the Mindanao State University at Naawan laboratory. Water temperature ranged between 26-29°C. pH was not measured but was assumed to be within the normal seawater range (i.e. 7.9-8.8 at Naawan). Light levels were measured with a LICOR (L1-189) cosine-corrected underwater quantum sensor. After P-I measurements, chlorophyll a and b was extracted from the shoots using ethanol (Wintermans and deMots, 1965).

The Michaelis-Menten rectangular hyperbola model was iteratively fitted to the photosynthesis-irradiance datasets (Lederman and Tett, 1981; Hootsmans and Vermaat, 1994)

$$P_{net} = (P_{max} * I) / (K_m + I) - R \quad (4)$$

Where: P_{net} = net photosynthetic rate (mg O_2 g DW^{-1} hr^{-1}), I = Irradiance (µmol PAR m^{-2} s^{-1}), P_{max} = gross maximal photosynthetic rate (mg O_2 g DW^{-1} hr^{-1}), K_m = the half saturation constant (µmol PAR m^{-2} s^{-1}), and R = respiration rate (mg O_2 g DW^{-1} hr^{-1}). From equation 4, derived parameters are as follows: LCP = the calculated irradiance level (µmol PAR m^{-2} s^{-1}) at zero photosynthetic rate and $\alpha = P_{max} / K_m$ = initial slope of the curve.

Average values for each plot in each shading treatment (3 plots or replicates per treatment) for all parameters were subjected to 3-way ANOVA testing for significant differences among species, shading treatments and sampling months. The contrast between species includes one between sites. Multiple comparison tests were done only on final values (12^{th} month shading) using Tukey's Honestly Significant Difference (HSD) test at 0.05 significance level. Homogeneity of variance was tested and data were log- or square-root transformed whenever necessary. A Kolmogorov-Smirnov test was used to test for differences in age structure between shade treatments and between sampling months for both species. Bonferroni corrections were applied to maintain experiment-wise error at 0.05 level of significance. All statistical tests were performed using SPSS/PC+ statistical package (Norusis, 1986).

Results

Shoot density of the two species was significantly different ($p<0.001$, Table 1) and the interaction between species and shading was significant as well, suggesting that the two species were differently affected by shading. The species effect explained about 15% of the total variation and was less important than shading levels (37%) but more than the factor month (6%). Shoot density declined strongest under the highest shading in both species (Figs. 2-3, Table 2). Significant density reductions of 82% and 95% were observed after the one year period for *T. hemprichii* and *H. uninervis*, respectively. At the intermediate shading level, the significant losses were less for *T. hemprichii* (37%) and *H. uninervis* (56%). Over the first 2-4 months of shading, shoot density in both species remained relatively unchanged and then declined rapidly.

Shoot length of the two species was affected significantly by shading ($p<0.001$, Table 1) and in the interaction between species and shading. The factor month contributed most (33%), but still shade level (22%) as well as species (20%) were significant, suggesting a length acclimation to shade in both species over and above a seasonal cycle (Figs. 2-3). A seasonal cycle in shoot length was clearly present in *T. hemprichii*. It ran parallel to leaf width and was inversely related to the annual

Table 1. Results of 3-way ANOVA examining the effects of species, shading levels, and time of sampling in month and their interactions on the morphometrics, biomass and growth of *T. hemprichii* and *H. uninervis*. Data used were those collected during the shading period. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS / total SS), and the level of significance (*p*). Factors are considered significant when $p<0.05$ written in bold numbers.

	Shoot density			Shoot length		Leaf width		leaves per shoot	
Factors	***df***	**%**	***p***	**%**	***p***	**%**	***p***	**%**	***p***
Species	1	15	**<0.001**	20	**<0.001**	32	**<0.001**	92	**<0.001**
Shade	2	37	**<0.001**	22	**<0.001**	1	**0.019**	0	0.244
Month	11	6	**<0.001**	33	**<0.001**	21	**<0.001**	4	**<0.001**
Species x Shade	2	4	**<0.001**	2	**<0.001**	1	**0.010**	0	0.136
Species x Month	11	1	0.265	3	**<0.001**	12	**<0.001**	2	**<0.001**
Shade x Month	22	21	**<0.001**	12	**<0.001**	10	**<0.001**	1	**<0.001**
Species x Shade x Month	22	3	**0.035**	2	**<0.001**	6	**<0.001**	0	0.105
Within + Residual	144	13		6		15		1	
Total	215	100		100		100		100	

	Above ground biomass			belowground biomass		Leaf plastochron interval		Leaf elongation rate		Leaf relative growth rate	
Factors		***df***		**%**	***p***	**%**	***p***	**%**	***p***	**%**	***p***
Species	1	33	**<0.001**	51	**<0.001**	19	**<0.001**	20	**<0.001**	0	0.937
Shade	2	15	**<0.001**	2	0.063	7	**0.025**	10	**<0.001**	5	0.118
Month	3	13	**<0.001**	16	**<0.001**	6	0.087	16	**<0.001**	15	**0.009**
Species x Shade	2	2	0.303	0	0.743	5	0.091	2	0.142	6	0.101
Species x Month	3	1	0.815	2	0.151	6	0.085	23	**<0.001**	6	0.150
Shade x Month	6	4	0.335	7	**0.011**	6	0.411	6	**0.033**	4	0.710
Species x Shade x Month	6	3	0.610	3	0.342	8	0.221	2	0.548	6	0.571
Within + Residual	47	29		19		43		19		58	
Total	70	100		100		100		100		100	

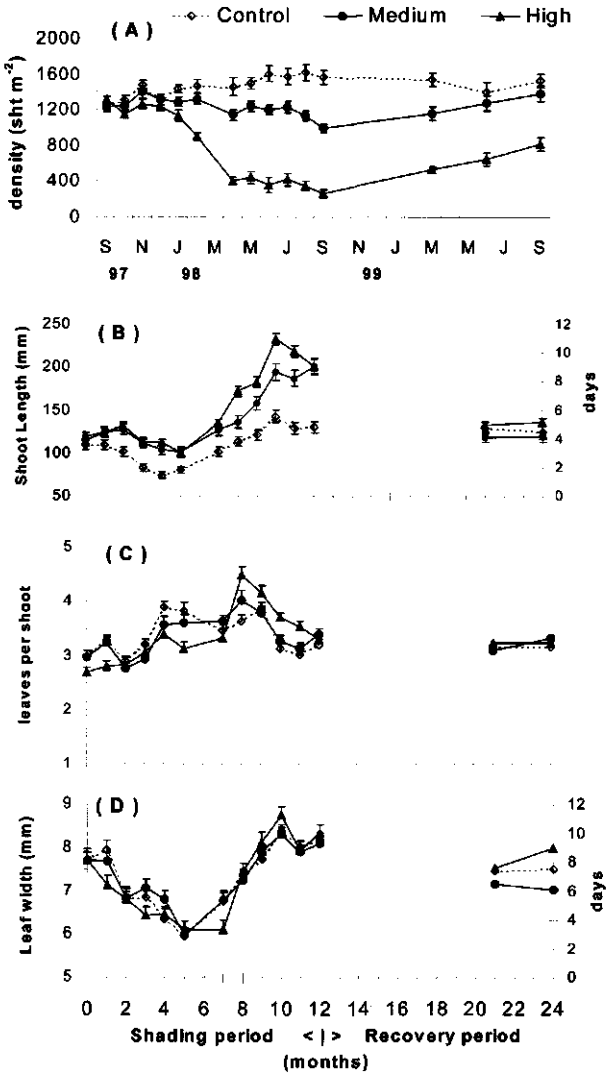

Fig. 2. Average values of (A) shoot density, (B) total shoot length (C) number of leaves per shoot, and (D) leaf width from monthly *in situ* measurements of *T. hemprichii* under different shade levels. Error bars are standard errors of mean (n=30, for density n=12). The thin smooth lines in B & D are the number of days low low tide occurred during the day.

cycle of day-time low tide exposure (Fig. 2). Shoots were smallest in January (74 ± 2.7 mm) when low tide occurred at midday and largest in July (143 ± 3.9 mm) when low low tide occurred during the night. At the end of the experimental shading, shoot length in the highly shaded plots had increased by as much as 224% for *H. uninervis* and

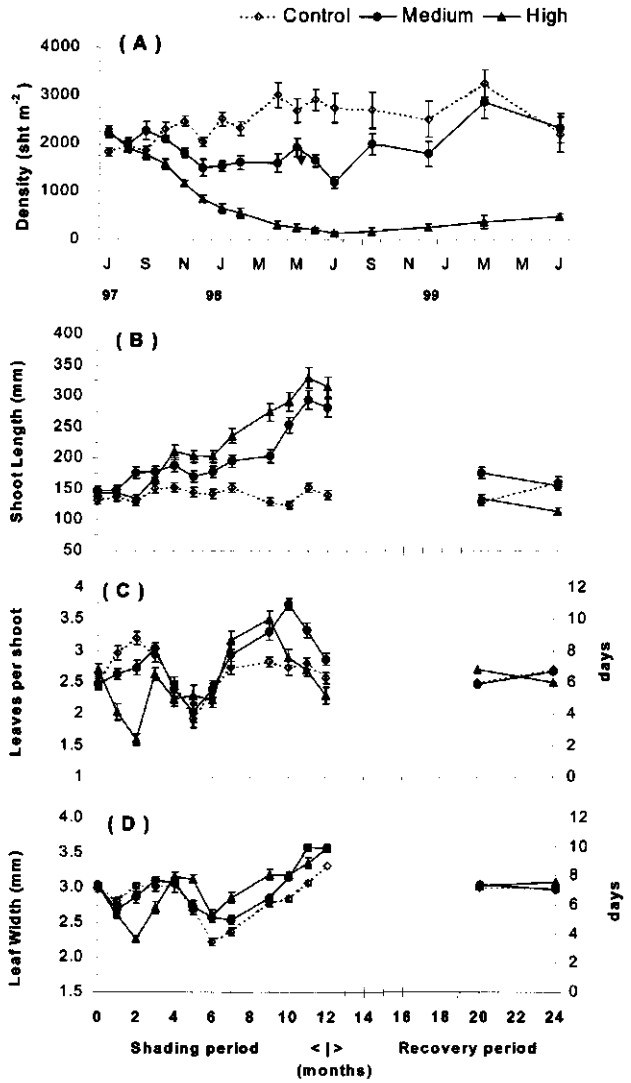

Fig. 3. Average values of (A) shoot density, (B) total shoot length (C) number of leaves per shoot, and (D) leaf width from monthly *in situ* measurements of *H. uninervis* under different shade levels. Error bars are standard errors of mean (n=30, for density n=12). The thin smooth lines in C & D are the number of days low low tide occurred during the day.

172% for *T. hemprichii.* Although seasonality in shoot length was not significant in *H. uninervis,* the leaf width and number of leaves per shoot also exhibited significant seasonality (Fig. 3). In a two-way ANOVA, seasonality explained 54% and 72% respectively for those two parameters, whilst shading was not significant (results not shown).

Table 2. Summary of morphometric, growth and biomass data of *T. hemprichii* and *H. uninervis* at the end of one year experimental shading. Data are presented as means of 3 replicates ± standard errors. Results of the one-way ANOVA are shown as *p* values. Differences among shading treatments within species are considered significant if $p<0.05$ indicated as bold letters. Multiple comparisons test using Tukey's HSD were made among shading levels within species, and different letters indicate significantly different means.

	Thalassia hemprichii				*Halodule uninervis*			
Parameters	**Control**	**Medium**	**High**	***p***	**Control**	**Medium**	**High**	***p***
Shoot density (m^{-2}) *	$1573^{a} \pm 53$	$998^{b} \pm 78$	$269^{c} \pm 60$	**<0.001**	$2752^{a} \pm 653$	$1196^{ab} \pm 197$	$127^{b} \pm 59$	**0.009**
Shoot length (mm) *	$131^{a} \pm 4$	$201^{b} \pm 1$	$225^{b} \pm 14$	**<0.001**	$141^{a} \pm 3$	$281^{b} \pm 10$	$315^{b} \pm 18$	**<0.001**
Number of leaves per shoot *	3.2 ± 0.2	3.4 ± 0.2	3.3 ± 0.1	0.631	2.6 ± 0.0	2.9 ± 0.1	2.3 ± 0.3	0.121
Leaf width (mm) *	8.2 ± 0.3	8.1 ± 0.1	8.4 ± 0.1	0.614	3.3 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	0.198
Mean leaf length/ shoot	$78^{a} \pm 4$	$111^{ab} \pm 1$	$129^{b} \pm 14$	**0.014**	56 ± 5	73 ± 2	65 ± 6	0.074
Vertical rhizome length (mm)	18 ± 4	34 ± 8	20 ± 1	0.126	60 ± 6	75 ± 22	100 ± 24	0.212
No. leaf scars	48 ± 8	62 ± 7	39 ± 4	0.127	15 ± 1	21 ± 3	24 ± 5	0.093
Internode distance (mm)	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.092	3.9 ± 0.6	3.5 ± 0.5	4.1 ± 0.1	0.696
Leaf biomass (g DW m^{-2})	70 ± 4	76 ± 6	48 ± 9	0.052	$34^{a} \pm 10$	$22^{ab} \pm 6$	$3^{b} \pm 1$	**0.039**
Vertical rhizomes biomass (g DW m^{-2})	45 ± 10	58 ± 16	14 ± 2	0.075	$89^{a} \pm 19$	$49^{ab} \pm 23$	$10^{b} \pm 1$	**0.049**
Horizontal rhizome (g DW m^{-2})	$275^{a} \pm 55$	$104^{b} \pm 16$	$73^{b} \pm 27$	**0.015**	$64^{a} \pm 9$	$34^{b} \pm 4$	$7^{c} \pm 1$	**0.002**
Roots (g DW m^{-2})	$69^{a} \pm 16$	$28^{ab} \pm 6$	$19^{b} \pm 3$	**0.024**	$72^{a} \pm 15$	$41^{ab} \pm 9$	$8^{b} \pm 2$	**0.013**
Above ground biomass (g DW m^{-2})	$115^{ab} \pm 9$	$134^{a} \pm 21$	$62^{b} \pm 10$	**0.027**	$124^{a} \pm 24$	$71^{ab} \pm 29$	$13^{b} \pm 3$	**0.044**
Below ground biomass (g DW m^{-2})	$345^{a} \pm 63$	$132^{b} \pm 21$	$91^{b} \pm 25$	**0.009**	$135^{a} \pm 24$	$76^{ab} \pm 14$	$15^{b} \pm 4$	**0.005**
Above : Below ratio	$0.35^{a} \pm 0.05$	$1.01^{c} \pm 0.01$	$0.71^{b} \pm 0.07$	**<0.001**	1.01 ± 0.37	0.87 ± 0.21	0.93 ± 0.12	0.931
Plastochron Interval (days)	$6.0^{a} \pm 0.0$	$11.3^{b} \pm 1.8$	$11.3^{b} \pm 1.2$	**0.006**	10.5 ± 3.1	10.0 ± 0.0	8.9 ± 2.0	0.862
Leaf elongation rate (mm d^{-1})	6.1 ± 0.6	8.1 ± 0.5	8.4 ± 1.4	**0.042**	7.1 ± 1.4	7.9 ± 0.4	7.4 ± 0.5	0.806
Relative leaf growth rate (% d^{-1})	4.7 ± 0.2	4.8 ± 0.3	4.2 ± 0.3	0.294	7.9 ± 0.1	7.4 ± 0.2	7.2 ± 0.7	0.937
Vertical rhizome elongation rate (mm plastochron^{-1})	0.28 ± 0.05	0.50 ± 0.11	0.40 ± 0.01	0.182	2.49 ± 0.33	3.23 ± 0.37	3.53 ± 0.47	0.229

LEGEND: * data taken from monthly *in situ* measurements

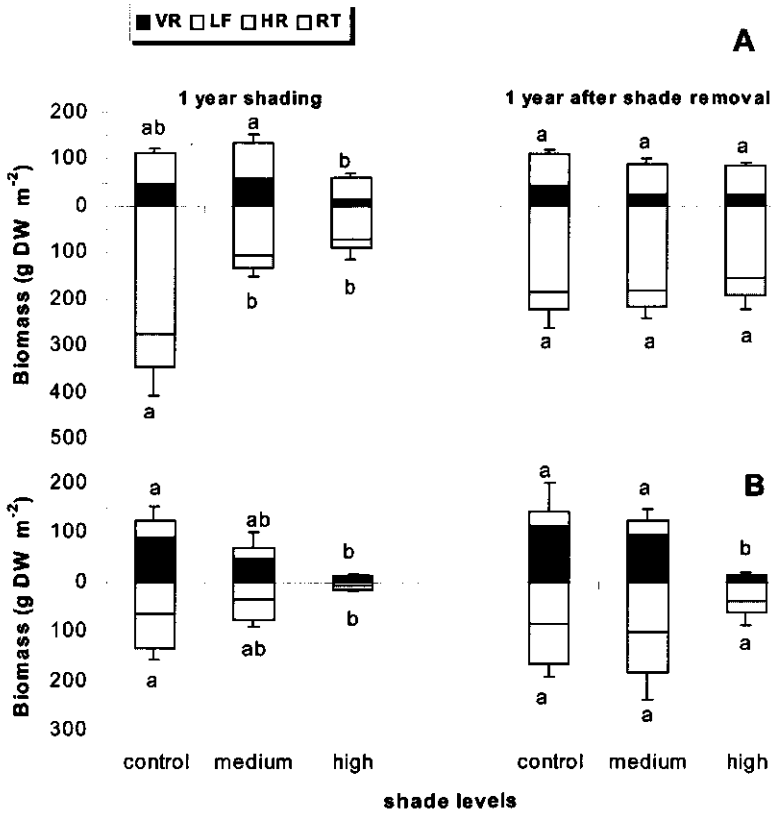

Fig. 4. Biomass allocation after one year shading and one year subsequent post-treatment year: dry weight of leaves (LF), vertical rhizomes (VR), horizontal rhizomes (HR) and roots (RT) of (A) *T. hemprichii* and (B) *H. uninervis*. Error bars are standard errors of above and below ground biomass. Values presented are means of 3 core samples (15 cm diam.) per shade levels. Bars with similar letters indicate no significant differences (Tukey's HSD, maintaining EER at 0.05).

After one year, shade screens were removed and recovery was monitored for another year. Shoot densities of both species under medium shaded had returned to control densities within 8-9 months after removal of benthic shades (Figs 2-3). The most strongly shaded beds, however, had not yet reached full recovery. *H. uninervis* recovered more slowly than *T. hemprichii*, possibly because recolonization by other species had been more extensive in the *H. uninervis* plots (Table 3). Indeed, after one year of shading, *Halophila ovalis*, *Syringodium isoetifolium* and *Cymodocea rotundata* had increased in density in the highly shaded *H. uninervis* beds. In the *T. hemprichii* site, only *H. ovalis* increased.

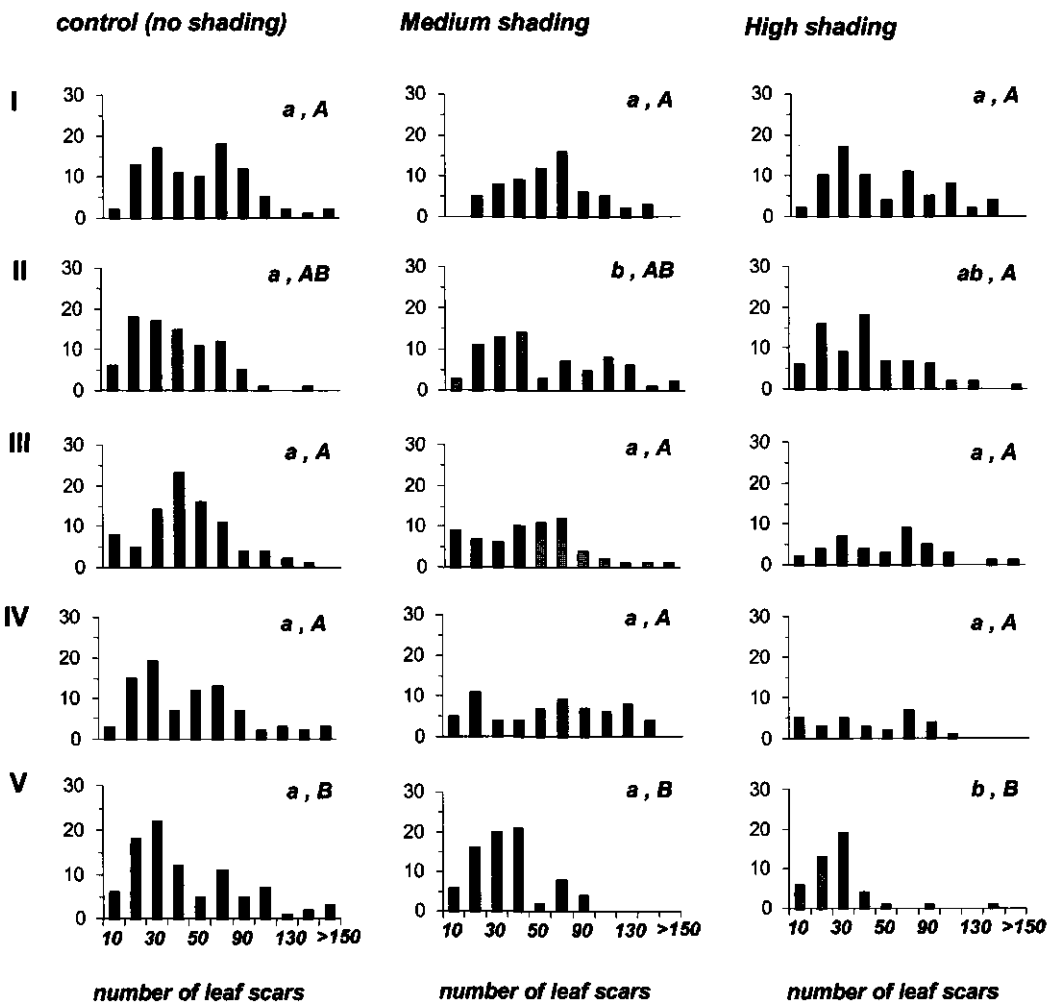

Fig. 5. Frequency distribution of *T. hemprichii* shoot age based on the number of leaf scars or plastochron intervals. Boxes with the same lowercase letters in a row are not significantly different, while boxes with the same capital letters among months (in a column) indicate no significant differences using the Kolmogorov-Smirnov test. Values are totals of 3 replicate cores (15 cm diam.). A Bonferroni correction was applied to maintain an experiment wise error of 0.05. The roman numerals correspond to the sampling months: I = Oct 97; II = Dec 97; III = Mar 98; IV = Sep 98 end of experimental shading and V = Sep 99, 12 months after shades were removed for recovery monitoring.

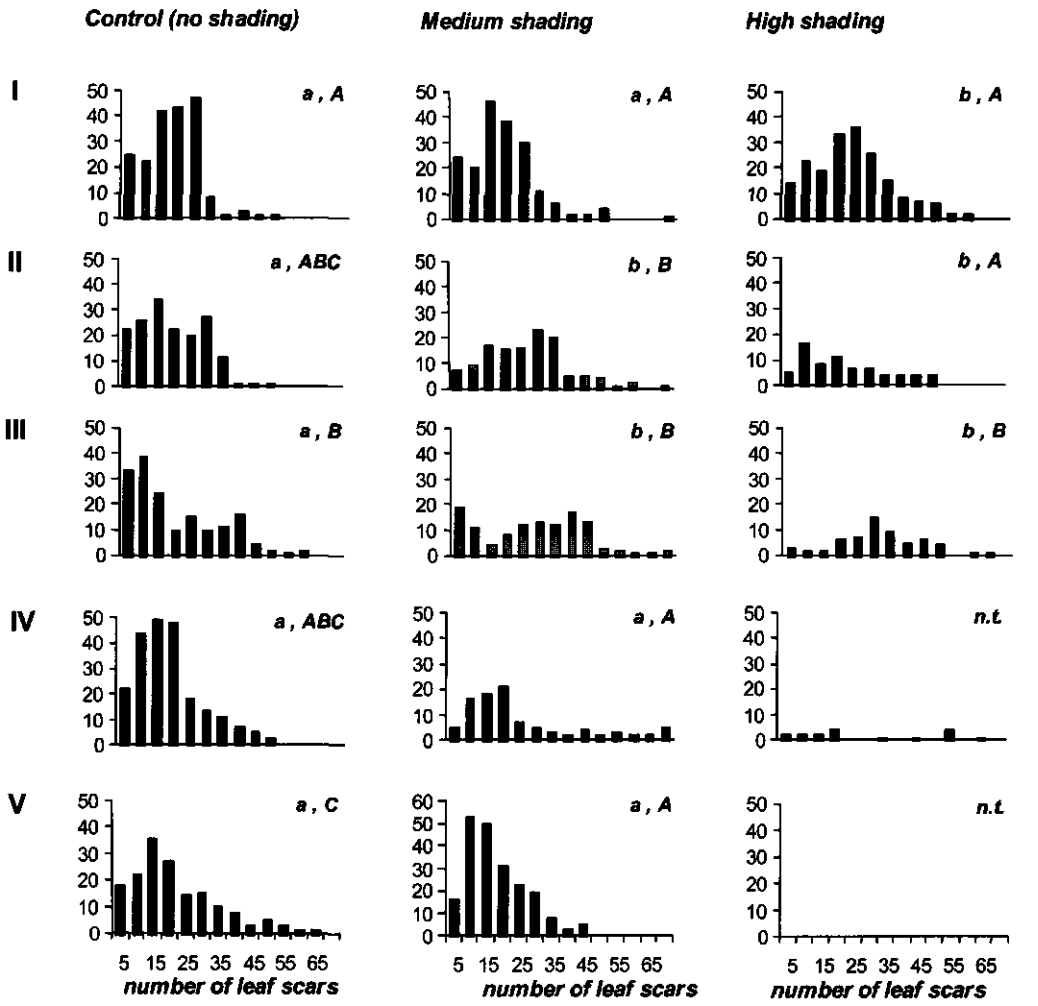

Fig. 6. Frequency distribution of *H. uninervis* shoot age based on the number of leaf scars. n.t. means not tested due to very few samples. Values are total of 3 replicate cores (15 cm diam.). The roman numerals correspond to the sampling months: I = Aug 97; II = Oct 97; III = Jan 98; IV = Jul 98 end of experimental shading and V = Jul 99, 12 months after shades were removed for recovery monitoring. Further as Fig. 5.

Biomass response to shading largely paralleled density. Shading significantly reduced above ground biomass (Tables 1 and 2, Fig. 4). Species differences explained much of the variation (33%) with *T. hemprichii* having more than twice the leaf biomass of *H. uninervis*. Similarly, belowground biomass decreased significantly ($p<0.05$, Table 2) in both species particularly during the 12th month of shading. There

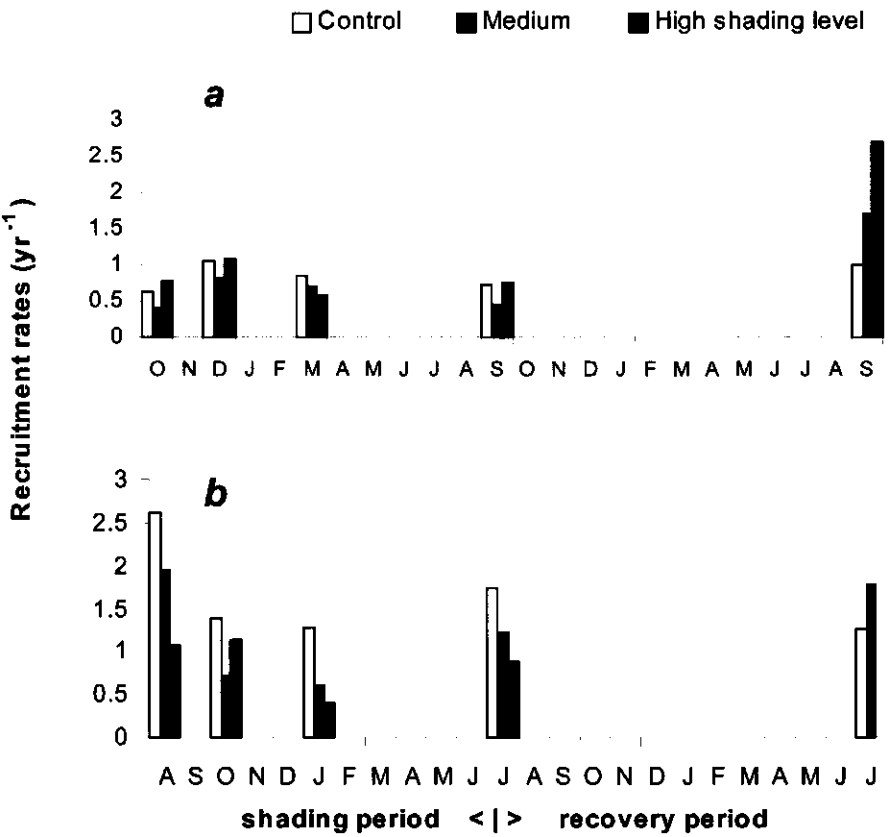

Fig. 7. Shoot recruitment rates (yr^{-1}) of (a) *T. hemprichii* and (b) *H. uninervis* during and after the shading period. Data pooled in 3 cores due to limited samples.

was, however, a continuous decline in the above and below ground biomass in both species in the shaded plots. The relative ratio of the above to belowground biomass was higher in the shaded plants ($p<0.001$, Table 2) for *T. hemprichii*, indicating less investments in the belowground materials. In contrast the above to belowground ratio of *H. uninervis* remained relatively constant throughout the shading period. Recovery after shade removal was particularly high in *T. hemprichii*: the shaded plots reached the control biomass after 12 months (Fig. 4). In contrast, only the medium shaded plots of *H. uninervis* recovered, while the highly shaded plots remained significantly less than the control.

Table 3. Average density (shoots $m^{-2} \pm$ SE) of seagrass species in the two sites, one year after shading was removed.

Species	Control	Medium shading	High shading
Site 1: Naawan (*H. uninervis* beds)			
Halodule uninervis	2187 ± 386	2329 ± 303	483 ± 62
Syringodium isoetifolium	1010 ± 228	781 ± 337	1562 ± 252
Cymodocea rotundata	110 ± 45	135 ± 43	185 ± 41
Cymodocea serrulata	162 ± 33	92 ± 31	131 ± 66
Halophila ovalis	4 ± 4	23 ±16	102 ± 37
Total	3475	3360	2465
Site 2: Sulawan (*T. hemprichii* beds)			
Thalassia hemprichii	1531±77	1385 ±92	819 ±70
Halodule uninervis	10	0	2.1
Enhalus acoroides	2.1	0	4.2
Halophila ovalis	110	452	277
Total	1653	1837	1102

Leaf elongation rates and plastochron intervals were significantly different between species ($p<0.001$) and among shade levels ($p<0.05$, Table 1). The interaction between species and shade however was not significant indicating similarities in response with a tendency to both increase with shading (Tables 1-2). Leaf growth, when expressed as RGR, differed among species but showed no response to shading, although temporal variation was significant explaining 15% of the variation ($p<0.01$, Table 1). In *T. hemprichii*, leaf elongation rates and plastochron interval were significantly higher ($p<0.05$) under the highest shade, but for *H. uninervis* shade effects were not statistically significant. Thus increased leaf elongation and longer plastochron intervals between successive leaves led to the longer leaves for both species.

Shoot age distribution of *T. hemprichii* was not affected by the shading, in contrast to *H. uninervis*, which had significantly less young shoots in shaded treatments than in controls (Figs 5-6). Also, estimated recruitment rates of *H. uninervis* were significantly lower under shade (0.9 yr^{-1}) than in the control (1.2 yr^{-1}) at the end of the shading period (Fig. 7). Under shade, older *H. uninervis* shoots persisted and elongated further, whereas in the control plots, the shoots were generally younger and shorter, and this is attributed to increased branching in the control as compared to the shaded plots. When the shades were removed, branching in *H. uninervis* started at the top: the relatively old and long vertical stems of the shaded plants started to bend over and rooted where branching occurred. This explains the generally shorter shoots in 12 months after benthic shades were removed (Fig. 2) and the apparent loss of old shoots in Figures 5-6. Moreover, there was a remarkable increase in recruitment for both species after the shades were removed, particularly for the highly shaded *T. hemprichii* (Fig. 7).

Table 4. Results of 3-way ANOVA examining the effects of species, shading levels, and time of sampling in month and their interactions on the photosynthetic parameters and chlorophyll contents of *T. hemprichii* and *H. uninervis*. Data used were those collected during the shading period. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS / total SS), and the level of significance (*p*). Factors are considered significant if $p<0.05$ written in bold numbers.

	P_{max}			K_m		Dark respiration		LCP		Slope	
Factors	*df*	%	*p*	%	*p*	%	*p*	%	*p*	%	*P*
Species	1	13	**<0.001**	<1	0.498	6	**<0.001**	<1	0.368	4	**<0.001**
Shade	2	3	**0.006**	1	0.126	2	0.104	3	**0.001**	3	**0.003**
Month	6	21	**<0.001**	9	**<0.001**	27	**<0.001**	19	**<0.001**	15	**<0.001**
Species x Shade	2	2	**0.011**	6	**<0.001**	5	**0.001**	3	**0.004**	4	**0.001**
Species x Month	6	15	**<0.001**	45	**<0.001**	3	0.119	38	**<0.001**	39	**<0.001**
Shade x Month	12	13	**<0.001**	5	0.139	20	**<0.001**	12	**<0.001**	5	0.059
Species x Shade x Month	12	12	**<0.001**	10	**0.001**	11	**0.002**	8	**0.001**	9	**0.001**
Within + Residual	77	21		23		26		17		20	
Total	118	100									

	Chlorophyll a			Chlorophyll b		Total chlorophyll	
Factors	*df*	%	*p*	%	*p*	%	*p*
Species	1	42	**<0.001**	57	**<0.001**	55	**<0.001**
Shade	2	1	**0.016**	11	**<0.001**	7	**<0.001**
Month	6	26	**<0.001**	9	**<0.001**	15	**<0.001**
Species x Shade	2	1	0.121	2	**<0.001**	2	**<0.001**
Species x Month	6	6	**<0.001**	5	**<0.001**	5	**<0.001**
Shade x Month	12	6	**<0.001**	3	**0.005**	4	**<0.001**
Species x Shade x Month	12	6	**0.001**	7	**<0.001**	5	**<0.001**
Within + Residual	604	12		7		7	
Total	645						

Table 5. Summary of maximum photosynthetic rates (Pmax), half saturation constant (Km), dark respiration rate, light compensation point (LCP) and the initial slope (α) for *T. hemprichii* and *H. uninervis* after one year of experimental shading. Data are presented as means of 3 replicates ± standard errors. Differences among shading treatments within species are considered significant if $p<0.05$ indicated as bold letters. Multiple comparisons using Tukey's HSD were made among shading levels within species, and different letters indicate significant different means.

	Thalassia hemprichii				***Halodule uninervis***			
Parameters	**Control**	**Medium**	**High**	***p***	**Control**	**Medium**	**High**	***p***
P_{max} (mg O_2 g^{-1} DW h^{-1})	12.2 ± 0.7	12.2 ± 0.5	12.3 ± 1.2	0.999	10.8 ± 0.9	12.7± 2.6	17.7 ± 1.2	0.069
K_m (µ mol PAR m^{-2} s^{-1})	121.2^{ab} ±22	182.8^{b} ±23	96.1^{a} ± 10	**0.047**	70.2 ± 10	87.5 ± 12	92.7 ± 36	0.771
Respiration (mg O_2 g^{-1} DW h^{-1})	2.1 ± 0.4	1.6 ± 0.2	1.9 ± 0.1	0.394	3.59^{a} ± 0.6	1.1^{b} ± 0.1	2.8^{ab} ± 0.4	**0.012**
LCP (µ mol PAR m^{-2} s^{-1})	24.3^{a} ± 1.3	27.4^{a} ±0.8	18.4^{b} ±1.4	**0.005**	33.6^{a} ± 2.5	8.7^{b} ± 0.3	16.4^{b} ± 5.4	**0.006**
Slope (P_{max}/ K_m)	0.10 ± 0.02	0.07 ± 0.01	0.13 ± 0.01	0.667	0.17± 0.04	0.14 ± 0.14	0.24 ± 0.07	0.335
Chlorophyll-a (mg Chl g^{-1} DW)	2.3± 0.1	2.2± 0.0	2.1 ± 0.1	0.461	1.3^{a} ± 0.1	1.8^{b} ± 0.1	1.9^{b} ± 0.2	**0.017**
Chlorophyll-b (mg Chl g^{-1} DW)	1.5^{a} ± 0.1	2.6^{b} ± 0.2	2.6^{b} ± 0.2	**<0.001**	0.7^{a} ± 0.0	1.4^{b} ± 0.1	1.8^{c} ± 0.1	**<0.001**
Total chlorophyll (mg Chl g^{-1} DW)	3.8^{a} ± 0.2	4.8^{b} ± 0.3	4.7^{b} ± 0.3	**<0.001**	2.1^{a} ± 0.1	3.2^{b} ± 0.1	3.7^{c} ± 0.1	**<0.001**
Chl a:b ratio	1.6^{b} ± 0.1	0.9^{b} ± 0.1	1.0^{a} ± 0.1	**0.007**	1.9^{b} ± 0.1	1.4^{a} ± 0.1	1.1^{a} ± 0.1	**0.002**

Table 6. Results of 3-way ANOVA examining the effects of species, shading levels, and time in month and their interactions on the nitrogen and organic carbon content of *T. hemprichii* and *H. uninervis*. Data used were those collected during the shading period of the experiment. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS / total SS), and the level of significance (*p*). Factors are considered significant when $p<0.05$ written in bold numbers.

		Leaves		**Stems**		**Rhizomes**		**Roots**	
Factors	*df*	%	*p*	%	*p*	%	*p*	%	*p*
% nitrogen content									
Species	1	42	**<0.000**	46	<0.000	15	**<0.000**	60	**<0.000**
Shade	2	18	**<0.000**	22	<0.000	31	**<0.000**	4	**0.016**
Month	3	17	**<0.000**	8	<0.000	11	**0.001**	8	**0.001**
Species x Shade	2	0	0.972	4	0.002	0	0.906	0	0.953
Species x Month	3	3	0.056	3	0.026	1	0.556	4	**0.027**
Shade x Month	6	3	0.186	3	0.159	7	0.081	2	0.439
Species x Shade x Month	6	1	0.969	2	0.229	6	0.178	3	0.377
Within + Residual	48	16		13		29		20	
Total	71	100		100		100		100	
% organic carbon content									
Species	1	25	**<0.000**	2	0.267	23	**<0.000**	3	0.123
Shade	2	1	0.670	0	0.949	9	**0.022**	5	0.185
Month	3	7	0.060	10	0.083	1	0.729	13	0.030
Species x Shade	2	2	0.236	0	0.907	4	0.175	2	0.456
Species x Month	3	7	0.065	4	0.452	1	0.881	5	0.310
Shade x Month	6	12	**0.041**	8	0.489	4	0.724	7	0.490
Species x Shade x Month	6	4	0.643	9	0.393	6	0.433	3	0.873
Within + Residual	48	42		67		52		62	
Total	71	100		100		100		100	

Maximum photosynthetic rates of the two species were significantly different with the species effect explaining 13% of the variation ($p<0.001$, Tables 4-5) . The shade effect (3%, $p<0.01$) was also significant but temporal variation was relatively strong explaining at least 21% of the variation ($p<0.001$, Tables 4-5). The interaction between species, shade treatments and months was significant ($p<0.001$) indicating both species were differently affected by shading. Similarly, dark respiration was significantly different between species (6%, $p<0.001$) but was not affected by shading, and temporal variation explained most of the differences (27%). The light compensation point was similar for both species ($p>0.05$) ranging from 8 to 33 µmol PAR m^{-2} s^{-1} with shaded plants having generally lower light compensation points ($p<0.01$). The interaction between species, shade and month was however significant suggesting differences in

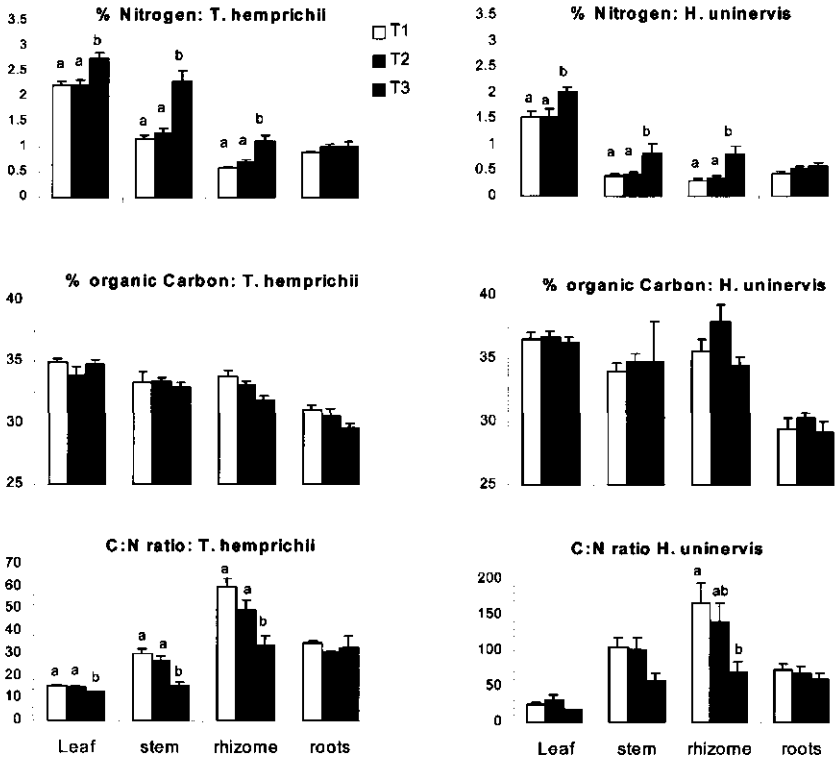

Fig. 8. Annual average of nitrogen and organic carbon content (% dry wt) of the leaves, stems, rhizomes and roots in *T. hemprichii* and *H. uninervis* during the 12 month shading period under different shade treatments: T1= control without shading, T2 = medium shading and T3 = high shading. Error bars are standard errors of means. Bar graphs having the same letters are not significantly different. Organic carbon content is not significant in all plant parts ($p>0.05$).

species response. The dark respiration rates per unit biomass were 23-68% lower (Table 5, $p<0.05$) in the shaded plots for *H. uninervis*. As a consequence, the light compensation points for the shade plants also reduced by 50-73% ($p<0.01$) and were lowest in the medium shade. In contrast, dark respiration was similar among treatments for *T. hemprichii*. The annual average in P-I curves for the two species showed a generally higher trend in the shaded plants against the control but when normalized to chlorophyll content, the P-I curves were relatively similar. Chlorophyll b content was almost 2 fold higher (2.0 and 1.7) in the shaded plants for both species (Table 5). The chlorophyll a: b ratio tends to decrease with shading in both species.

Nitrogen content of the leaves differed significantly between species ($p<0.001$, explaining at least 42% of the variation , Table 6, Fig. 8). Similar to shoot density and shoot length, shading was significant (18%) and so was the month effect (17%). The pattern in other parts of the plants e.g. stems, rhizomes and roots followed that of the leaves. The organic carbon content of the plants tissues was generally not different between species and shading levels ($p>0.05$, Fig. 8).

Discussion

In both species, the response to long-term shading were limited to increased shoot length as well as increased nitrogen and chlorophyll content, a pattern similar to findings for other seagrass species elsewhere (Abal et al., 1994; Czerny and Dunton, 1995; Lee and Dunton, 1997). Particularly in the high shading levels, shoot mortality was high. The two species differed in this respect. *Halodule uninervis* particularly missed the younger, newly recruiting shoots but *Thalassia hemprichii* densities dropped over all age classes. Under dense shade, maintenance of a proportionally large heterotrophic biomass of rhizomes and roots must have been difficult leading to large-scale mortality. In the remaining shoots, relative leaf growth remained the same and plastochron intervals increased, leading to longer leaf blades at maturity. This is a common response of most plants for increasing light interception in a low light environment, within the limits of morphological plasticity (Björkman, 1981; Bulthuis, 1983; Kirk, 1983).

The above and below ground biomass in both species decreased with shading as a consequence of the reduction in shoot density. However, the shoot : root+rhizome ratio increased in *T. hemprichii* but remained the same for *H. uninervis*. An increased investment in aboveground biomass is considered an adaptive strategy to low light (Boardman, 1977; Hemminga, 1998). In the case of *H. uninervis*, belowground biomass did not decrease, possibly because the sediment conditions in the area are sandy and not as stable as those in the *T. hemprichii* area.

The decline in the *T. hemprichii* shoot density started when the daytime low tide exposure peaked. This condition put more stress on the plants, as a consequence of desiccation, which could lead to a significant loss in biomass (Stapel et al., 1997). In *H. uninervis*, shoot density declined immediately after shading started. This is possibly because *T. hemprichii* had more reserves to endure several months of shading. Recovery of the *T. hemprichii* in the highly shaded plots, if extrapolated, would be within 2 years which is similar to the prediction of Rollon et al. (1998). Recovery could take longer for *H. uninervis*. Fitzpatrick and Kirkmann (1995) have shown in *Posidonia australis* a significant reduction in density, leaf growth rate and shoot weight after shading at 90% for 3 months but recovery was reported not to be significant even after 17 months. Similarly, Gordon et al. (1994) reported very low recovery of *Posidonia sinuosa* that were shaded for 5 months at 80-99% after 8 months post-treatment monitoring. The gaps created in the *T. hemprichii* site were not colonized by other species, which is in contrast with the burial experiment (Duarte et

al., 1997) where newly recruiting shoots of other species appeared when *T. hemprichii* densities declined.

Considerable variation was noted in all photosynthetic parameters measured in this study. Similarly, Kirk (1983) and Hootsmans and Vermaat (1994) reported that photosynthetic efficiencies of most aquatic plants also show considerable variation within species. It appears that *T. hemprichii* when grown in the shade still maintains its capacity to increase photosynthetic rates at saturating light intensity as indicated by the apparent similarity in P-I curves. This is in contrast with an obligate shade plant that have an intrinsically low maximum photosynthetic rate hence light saturation is reached early (Boardman, 1977; Björkman, 1981; Kirk, 1983). Although comparing P-I curves may indicate some photosynthetic acclimation to high light, but when normalize to chlorophyll contents of the plant, response of the plants were relatively similar. Thus the plants tend to increase chlorophyll content together with leaf surface area to compensate for the reduction of light, rather than adjusting photosynthetic rates, similar to sun and shade plants (Boardman, 1977; Björkman, 1981).

Leaf Relative Growth Rates (RGR) were not influenced by light availability in this study. Similar results were reported by Czerny and Dunton (1995) on *Thalassia testudinum* and *Halodule wrightii* when shaded at 86-90% of surface irradiance for 10 months.

The reduction in shoot length and biomass during October to January is probably due to the effect of daytime exposure that leads to large scale whitening and sloughing of the exposed leaves. Stapel et al. (1997) reported a 61% and 31% reduction in leaf and rhizome biomass during this same period in Indonesia (July to December). Shoot density was also reduced up to 48% while CNP content of leaves declined by as much as 63% and was shown to have a strong correlation with leaf biomass decline. Erftemeijer and Herman (1994) reported a 80-90% reduction of aboveground biomass during this period for *T. hemprichii* and *Enhalus acoroides* with a significant change in specific leaf growth rate and elemental composition of seagrass tissues.

Leaf Nitrogen contents found here for the unshaded shoots of *T. hemprichii* (2.09±0.02% of DW) and *H. uninervis* (1.59±0.1% of DW) are within the range reported by Terrados et al. (1999) but values obtained for the shaded plants are generally higher. Total chlorophyll (a+b) content of both species tended to increase with shading and did not show any indication of a decrease as a consequence of severe shading, as reported in most terrestrial plants (Björkman, 1981). The shaded seagrass leaves therefore were visibly greener than those of the controls. The marked increase in chlorophyll b observed, conforms to a general shade acclimation response of increased antenna chlorophyll (Björkman, 1981; Boardman, 1977), and has been reported for other seagrasses as well (Dennison and Alberte, 1982; Abal et al., 1994; Czerny and Dunton, 1995; Lee and Dunton, 1997; Longstaff and Dennison, 1999).

In conclusion, the two species investigated in this study showed similarities in several aspects but were quite different in their above vs belowground biomass allocation. *T. hemprichii* conforms with the hypothetical plant by reducing belowground in favour of aboveground tissues under low irradiance, but *H. uninervis* maintained its shoot to root ratio. Additionally, the remaining shoots of *H. uninervis* maintained growth rate and leaf turnover, In contrast, *T. hemprichii* tended to increase leaf elongation rate and plastochron interval, resulting in much longer leaves thus increasing leaf surface area for optimum light interception under the low light environment. In contrast, *H. uninervis* extended its vertical rhizome, more than 2 fold, thus placing the leaves higher and maximizing the limited light available. The age structure of *T. hemprichii* was not affected by shading considering similarities in age distribution with the control and constant recruitment. In contrast, recruitment rate was greatly reduced in *H. uninervis*, thus the shaded population had more older shoots than the control. The energy allocated for production of new shoots was probably used in the production of belowground biomass to sustain survival in a very unstable sandy substrate.

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Chapter 3

The effects of long-term light reduction on carbon allocation in selected seagrass species using the stable isotope ^{13}C

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Abstract

In a one-year *in situ* shading experiment, carbon allocation was studied in four different experimental events. Two contrasting seagrass species were selected for the study: the short-lived, early successional *Halodule uninervis* and long-lived, late successional *Thalassia hemprichii*. The stable isotope ^{13}C ($NaH^{13}CO_3$) was used to quantify carbon allocation to the different plant parts. Natural $^{13}C/^{12}C$ was lower in *H. uninervis* (-9.5 to –10.2‰) than in *T. hemprichii* (-5.9 to –6.6‰). The $\delta^{13}C$ values of the different plant parts were significantly different, in both species, the leaves being more ^{13}C-depleted than the roots and the rhizomes. In both species, tracer addition resulted in a significant enrichment compared to ^{13}C natural abundance, in *H. uninervis* the enrichment representing two fold that in *T. hemprichii*. Shading did not influence the enrichment in either species, but significant differences were observed between experimental events or sampling periods and between species. The amount of ^{13}C remaining in the plant 5 days after the 3-hour incubations was less than 5% the initial amount in the incubation bag. Most of this ^{13}C remained in the leaves (up to 80% in *H. uninervis* and 88% in *T. hemprichii*). The rest of the incorporated carbon was exported to the non-photosynthetic organs (stems, rhizomes and roots). This translocation was significantly reduced by shading from 22% to 12% in *T. hemprichii* and from 34% to 20% in *H. uninervis*. Partitioning of ^{13}C over non-photosynthetic tissue, however, was not affected by shading in *T. hemprichii*. Whilst in *H. uninervis*, a significant increase in the stem ^{13}C content was observed.

Keywords: shading, *Thalassia hemprichii*, *Halodule uninervis*, stable isotope, translocation, assimilates

Introduction

Light availability is a primary factor determining depth penetration, growth and abundance of seagrasses (Bulthuis, 1983; Duarte, 1991a; Grice et al., 1996; Vermaat et al., 1997). Deterioration of the underwater light climate, however, is widespread and probably has lead to large-scale declines in area covered by seagrasses (Fortes, 1988; Shepherd et al., 1989; Terrados et al., 1998).

The mechanism through which reduction in light availability leads to mortality is probably linked to photosynthetic capacity and respiratory needs (Dennison, 1987; Hemminga, 1998; Chapters 2 & 4). Prolonged shading may cause a keen shortage in photosynthetic supply. Different species may differ substantially in their capacity to cope with shading, similar to the sun vs. shade adaptability contrast of terrestrial species (Boardman, 1977). Here we assessed the long-term shade acclimation capacity in terms of carbon assimilation and subsequent translocation using the stable isotope ^{13}C. We did so for two tropical Philippine seagrass species of contrasting successional type and longevity: short-lived, early successional *Halodule uninervis* and long-lived late successional *Thalassia hemprichii* (cf. Duarte, 1991b; Vermaat et al., 1995).

The $^{13}C/^{12}C$ ratio is increasingly used for the unravelling of food web relations (McMillan et al., 1980; Fry et al., 1982; Herman et al., 2000), for tracing the sources and allocation of photosynthetically fixed carbon (Hemminga et al., 1996), and also for estimating production rates (Hemminga et al., 1996; Mateo et al., in press). Abel and Drew (1989) and Hemminga and Mateo (1996) reviewed the methodology, uncertainties and inherent variability regarding isotopic carbon discrimination in seagrasses. They reported that average $\delta^{13}C$ values ranged from –3.0‰ to –23.8‰ from 48 different seagrass species covering a wide geographical distribution.

In a parallel experiment (Chapter 2), we reported a significant reduction in shoot density and biomass and an increase in shoot length with shading in *H. uninervis* and *T. hemprichii.* The two species, however, differed in their response to shading in terms of the above:below ground ratio, leaf elongation rates and leaf turnover. In the present study, our aims were: 1) to quantify shading effects on the allocation of carbon to the different plant parts (leaves, stem, rhizomes, and roots) in *H. uninervis* and *T. hemprichii* using the stable isotope ^{13}C as tracer, and 2) to verify species-related differences and morphometric responses to shading.

Materials and Methods

Two sites were selected in Mindanao, Southern Philippines: the first was at Naawan, dominated by *Halodule uninervis* (Forsskal) Ascherson and the second at Sulawan, dominated by *Thalassia hemprichii* (Ehrenberg) Ascherson (see fig. 1, Chapter 2). The study was conducted in parallel with the long-term experiments on the effects of shading on growth, morphology and photosynthetic capacities of the same two species of seagrass (Chapter 2). Benthic black neutral density plastic shade screens measuring 1.6 x 1.6 m were set in a randomized block design (of three shading levels x three replicates) *in situ*. There were 3 shading levels used in the study: control (0% or full light); medium (50-67%) and high (81-89% light reduction). The control had the frame minus the shade screens. Light was measured with a Licor 185SB sensor. The shading period lasted one year starting from July 1997 for *H. uninervis* and from September 1997 for *T. hemprichii*.

To determine allocation of the incorporated carbon to the different plant parts (leaves, stem, rhizomes and roots), the stable isotope ^{13}C was used as biotracer. Incubation was done on the 1st, 3rd, 6th and 12th months of the long-term shading experiment. An incubation chamber was placed on a predetermined site within the 1x1 m quadrat inside the benthic shades. The incubation chamber was made up of a 4 cm stainless steel ring (15 cm diam) fitted with a transparent polyethylene bag (3.5 L). The bottom end of the plastic bag was tied with a thick rubber band to the steel ring. The polyethylene bag was fitted with a one-way tube at the top for insertion of the syringe needle. First the shoots were cleaned of epiphytes and other loosely attached sediments by rubbing the leaves between fingers. Then the chamber was filled with ambient seawater and was pushed sufficiently deep into the sediments to avoid cutting the rhizomes and leaking the treated water. The water inside the chamber was spiked with 20 mg L^{-1} of ^{13}C-$NaHCO_3$ (99%, Sigma) through the oneway port to reach a final concentration of 2.44 mM (total DIC including $H^{12}CO_3$, $H^{13}CO_3$, $^{12}CO_3^{2+}$ and CO_2), assuming natural HCO^-_3 concentration of 2.2 mM (Sand-Jensen and Gordon, 1984). The amount of added $NaH^{13}CO_3$ followed the higher limit (2.5 to 20 mg $NaH^{13}CO_3$ L^{-1}) used by Mateo et al. (in press) in *Zostera marina*. They demonstrated that such an addition (which represented an addition of carbon of 10 % respect to ambient DIC) did not have any fertilizing effect on seagrass carbon uptake. Incubation lasted for 3 hours and was timed around noon, i.e. between 10 and 14 hrs. In principle, this incubation length provides uptake rates close to net productivity (Mateo et al.,, in press).

The treated water inside the chamber was sucked out after the incubation. A hand pump was used to flush out the water into a receiving plastic bag. This was to ensure no leakage of the ^{13}C to the surrounding waters. Several markers were left in the incubated area immediately after removing the chamber. All incubated shoots were harvested after 5 days, using a PVC sediment corer (15 cm diameter) down to a depth of 15-20 cm. All samples in the corer were placed in plastic bags and brought to the laboratory for processing. Core samples outside of the experimental plots were also collected to determine the naturally occurring ^{13}C levels and to serve as blank samples.

In the laboratory, each sample core was rinsed with filtered seawater, cleaned and sorted as leaves (minus the dead leaf sheath), stems (vertical rhizomes), horizontal rhizomes and roots. Epiphytes were scraped clean and final rinsing was done with filtered tap water before samples were oven-dried at 60°C until reaching constant weight. Then samples were pulverized with mortar and pestle and kept dry in small glass vials in a desiccator before sending to NIOO-CEMO for analyses using the Europe Scientific Tracer mass spectrophotometer. Results are reported in the $\delta^{13}C$ notation, with the Vienna PDB limestone as the standard:

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where R is the absolute ratio (^{13}C / ^{12}C) and $R_{standard\ or\ PDB} = 0.0112372$. The fractional abundance (F) was calculated by the equation (Boutton 1991):

$$F_{sample} = {}^{13}C / ({}^{13}C + {}^{12}C) = R_{sample} / (R_{standard} + 1)$$

Enrichment with ^{13}C, or the ^{13}C excess in the plant tissues after the incubation, was calculated by subtracting the fractional abundance (F) of the blank samples (background levels) from that of the ^{13}C labelled samples. The resulting values were then normalized to biomass from corresponding organic carbon contents (cf Chapter 2). Percent allocation was determined by calculating the relative proportions of the total amount of excess ^{13}C in every plant part for each core sample.

A 3-way ANOVA was conducted to test significant differences between species, shading levels, sampling date and their interactions on the ^{13}C enrichment, as well as relative allocations for every plant part. Tukeys HSD was also done for multiple comparison tests after testing for homogeneity of variance. Data were log transformed whenever necessary to conform to the test for homogeneity of variance.

Results

The naturally occurring $\delta^{13}C$ values of *H. uninervis* (-9.5 to –10.2‰) were more negative than those of *T. hemprichii* (-5.9 to –6.6‰, Table 1). This difference between species was highly significant (3-way ANOVA, $p<0.001$, Table 2) explaining most (76%) of the variation. Differences between months and plant parts were also significant ($p<0.05$). In both species, leaves were ^{13}C-depleted compared to roots (Table 1). At the end of the experimental period, blank samples were collected 10 cm from the incubated core area to determine possible leakage or translocation of the assimilated ^{13}C. Results indicated no significant increase in the amount of ^{13}C among blank samples in *T. hemprichii* indicating no possible translocation outside of the incubated area (results not shown).

Table 1. Average values ± SE of the stable carbon isotope ratios $\delta^{13}C$ (‰) of the different plant parts (leaves, stem, rhizomes, and roots) for the blanks and the ^{13}C labelled seagrasses and under different shading levels during a period of one year. Blanks correspond to the naturally occurring $\delta^{13}C$ or the background levels.

		shading levels		
Species / plant parts	**Blanks**	**Control**	**Medium**	**High**
Halodule uninervis				
Leaves	-10.2 ± 0.6	91 ± 20	104 ± 21	102 ± 32
Stem	-10.1 ± 0.4	0.9 ± 2.3	5.4 ± 4.2	0.2 ± 3.3
Rhizomes	-9.7 ± 0.4	-5.8 ± 0.8	-5.7 ± 1.2	-8.6 ± 0.5
Roots	-9.5 ± 0.2	-2.7 ± 1.6	-5.1 ± 1.3	-8.0 ± 0.6
Thalassia hemprichii				
Leaves	-6.6 ± 0.3	59.8 ± 8	70.8 ± 9	67.3 ± 11
Stem	-6.3 ± 0.2	4.8 ± 2.1	3.8 ± 1.9	0.5 ± 1.1
Rhizomes	-5.7 ± 0.1	-2.3 ± 0.6	-3.4 ± 0.5	-3.8 ± 0.4
Roots	-5.9 ± 0.2	-2.1 ± 0.7	-3.0 ± 0.4	-4.4 ± 0.4

Table 2. 3-way ANOVA examining the effects of species, plant parts, and sampling period (Time) and their interactions on the naturally occurring $\delta^{13}C$ signatures of *H. uninervis* and *T. hemprichii*. Presented are the degrees of freedom (*df*), the sum of squares (SS), the % of total variation explained (factor SS/ total SS) and the level of significance (*p*). Bold numbers with $p < 0.05$ are considered significant. Mean values are presented in Table 1.

Factors	***df***	**SS**	**%**	***p***
Species	1	243.4	76	**<0.001**
Parts	3	7.7	2	**0.001**
Time	3	32.2	10	**<0.001**
Species x Parts	3	0.7	0	0.525
Species x Time	2	4.9	1	**0.003**
Parts x Time	9	8.8	3	**0.016**
Species x Parts x Time	6	1.8	1	0.598
Within + Residual	56	21.5	7	
Total	83	368.1	100	

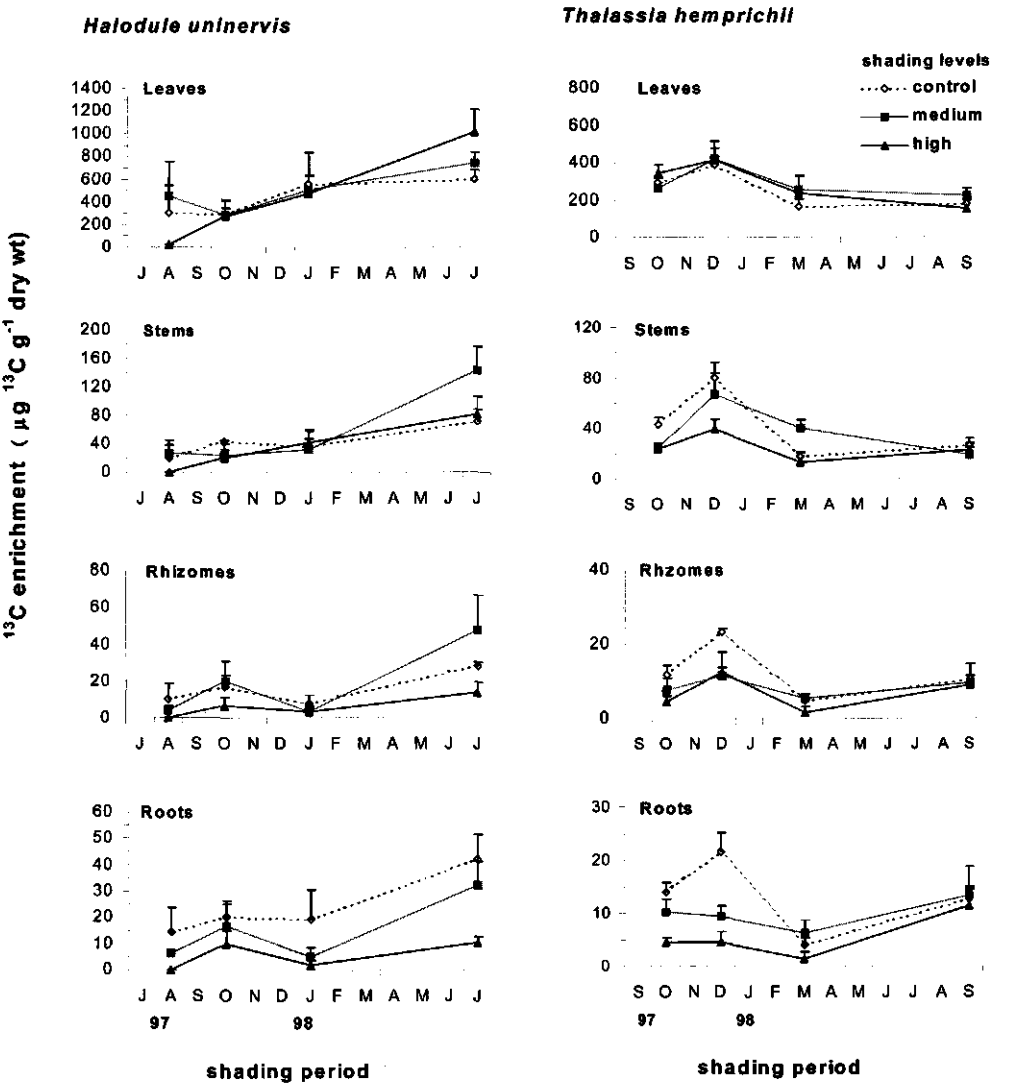

Fig. 1. ^{13}C enrichment (µg g^{-1} DW) in the different plant parts at different shading levels, after 3 hours of ^{13}C exposure and 5 days of translocation, during the one year experimental period. Error bars are standard errors of 3 replicates, except in Aug 97 for *H. uninervis* where n=2.

Table 3. Results of 3-way ANOVA examining the effects of species, shading levels, and sampling period (Time) and their interactions on ^{13}C enrichment and the percent allocation of ^{13}C in *H. uninervis* and *T. hemprichii*. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS/ total SS) and the level of significance (*p*). Bold numbers with $p < 0.05$ are considered significant. Mean values are presented in Table 4.

		Leaves		Stems		Rhizomes		Roots	
Factors	***df***	**%**	***p***	**%**	***p***	**%**	***p***	**%**	***p***
Enrichment of ^{13}C									
Species	1	11.7	**0.001**	2.1	0.085	3	0.092	5	**0.015**
Shading	2	0.5	0.740	3.8	0.069	8	**0.017**	20	**<0.001**
Time	3	7.7	**0.042**	15.9	**0.000**	24	**<0.001**	21	**<0.001**
Species x Shading	2	0.1	0.923	0.9	0.505	3	0.170	3	0.128
Species x Time	3	29.7	**<0.001**	34.2	**<0.001**	13	**0.003**	7	**0.033**
Shading x Time	6	3.6	0.653	5.5	0.249	5	0.445	1	0.947
Species x Shading x Time	6	7.4	0.228	7.4	0.110	6	0.346	7	0.196
Within + Residual	45	39.1		30.1		38		35	
Total	68	100		100		100		100	
Relative allocation of ^{13}C									
Species	1	23.0	**<0.001**	35.6	**<0.001**	13	**<0.001**	10	**0.001**
Shading	2	22.6	**<0.001**	2.6	0.151	24	**<0.001**	33	**<0.001**
Time	3	10.2	**0.002**	11.0	0.002	15	**0.001**	3	0.362
Species x Shading	2	1.8	0.213	0.3	0.810	4	0.078	10	**0.005**
Species x Time	3	6.9	**0.013**	11.2	**0.002**	1	0.792	1	0.751
Shading x Time	6	4.1	0.329	5.9	0.198	2	0.839	4	0.530
Species x Shading x Time	6	5.4	0.178	4.1	0.411	8	0.135	4	0.551
Within + Residual	45	26.0		29.4		33		36	
Total	68	100		100		100		100	

In situ labelling with $NaH^{13}CO_3$ showed a high incorporation of ^{13}C in the leaves leading to $\delta^{13}C$ values ranging from 91-104‰ and 60-71‰ for *H. uninervis* and *T. hemprichii*, respectively (Table 1). In both species, the stem $\delta^{13}C$ values were generally lower than 5‰, while those of the rhizomes and the roots were lower than 1‰. Enrichment in ^{13}C showed no significant differences between shaded levels in the leaves and stem (3-way ANOVA, $p>0.05$, Table 3). Enrichment in the rhizomes and roots was affected by shading ($p<0.05$). In all the plant parts, sampling date and the interaction between sampling date and species were highly significant indicating substantial temporal variation in *T. hemprichii*. There was a distinct seasonality in the amount of ^{13}C enrichment in the control plots. Maximum enrichment in all plant parts was observed during December, the period of daytime low tide. Shaded plants followed the same pattern with peak enrichment during this period. In contrast, *H. uninervis* showed an increasing ^{13}C enrichment with time (Fig. 1).

Table 4. Annual average ± SE of the different compartments (leaves, stem, rhizomes, roots) during the one year experimental period at different shading levels covering four sampling periods (1st, 3rd, 6th and 12th month shading) with 3 replicates each. (note) except for *H. uninervis*, n=2 for the 1st month. Values with different superscripts indicate significant differences within species (Tukey's HSD maintaining EER at 0.05)

	Halodule uninervis			*Thalassia hemprichii*		
Parameters	**Control**	**Medium**	**High**	**Control**	**Medium**	**High**
Enrichment in ^{13}C (µg ^{13}C g^{-1} DW)						
Leaves	447 ± 88	499 ± 84	482 ± 131	272 ±27	289 ± 32	286 ± 44
Stem	45 ± 9	59 ± 19	39 ± 12	45 ± 7	38 ± 7	25 ± 4
Rhizomes	16 ± 3	20 ± 8	6 ± 2	13 ± 2	9 ± 2	7 ± 2
Roots	25 ± 5^{b}	16 ± 5^{ab}	6 ± 2^{a}	12 ± 2	10 ± 2	5 ± 1
% ^{13}C allocation to different parts						
Leaves	66 ± 2^{a}	71 ± 2^{ab}	80 ± 5^{b}	78 ± 1^{a}	86 ± 1^{b}	88 ± 1^{b}
Stem	20 ± 2	21 ± 3	17 ± 5	8 ± 1^{b}	7 ± 1^{ab}	$5 \pm <1^{a}$
Rhizomes	5 ± 1^{b}	4 ± 2^{b}	1 ± 1^{a}	10 ± 1^{b}	5 ± 1^{a}	5 ± 1^{a}
Roots	9 ± 1^{b}	4 ± 1^{a}	2 ± 1^{a}	4 ± 1	2 ± <1	2 ± 1
Total ^{13}C retrieved (mg $core^{-1}$)	0.30 ± 0.1^{b}	0.28 ± 0.1^{b}	$0.09 \pm <0.1^{a}$	0.47 ± 0.1	0.46 ± 0.1	0.28 ± 0.1
% ^{13}C assimilated	2.2 ± 0.6^{b}	2.7 ± 0.6^{b}	0.8 ± 0.2^{a}	4.4 ± 0.5	4.3 ± 0.6	2.6 ± 0.5
Uptake rates (mg ^{13}C g^{-1} DW h^{-1})	0.22 ± 0.04	0.25 ± 0.04	0.22 ± 0.06	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.02

The relative allocation of ^{13}C was significantly influenced by shading: an increased amount of ^{13}C remained in the leaves (Table 3). As a consequence, shaded *H. uninervis*, exported only 20% to belowground tissues while the control was able to export as much as 33% (Table 4). Similarly, shaded plants of *T. hemprichii* exported less than the control (12% vs 22%). The species effect was significant in all plant parts ($p \leq 0.001$), as well as the time effect ($p<0.01$), except in the roots ($p>0.05$, Table 3). From the small amount of ^{13}C exported to non-photosynthetic tissues, allocation to the stem increased significantly with shading in *H. uninervis* (Table 4). In contrast, no significant influence of shading was observed in *T. hemprichii*.

The ^{13}C-enrichment was significantly reduced with shading, as well as the % amount of ^{13}C assimilated relative to the initial concentration in the incubation medium (Table 4). *T. hemprichii* assimilated twice as much as *H. uninervis* but overall assimilation was generally less than 5% of the total amount. The calculated uptake for both species, when normalized to biomass, did not show any significant differences among shade levels, whereas *H. uninervis* had a higher uptake rates than *T. hemprichii* (Table 4).

Discussion

The $\delta^{13}C$ signatures of the naturally occurring ^{13}C in the blank samples in this study were similar to those reported elsewhere (*T. hemprichii*: -5.2 to –8.1‰, *H. uninervis*: -7.8 to -13.0‰; Beer and Waisel, 1979; McMillan et al., 1980; Yamamuro et al., 1995).

The amount of ^{13}C-$NaHCO_3$ added corresponded to only 10% of the total HCO_3^- in the seawater medium (normal concentration is 2.2 mM HCO_3^-, Sand-Jensen and Gordon 1984). Despite the relatively small amount, ^{13}C uptake was sufficient to allow quantification of translocation of ^{13}C in different plant parts. Shading significantly reduced ^{13}C allocation to the stems, rhizomes and roots since a large fraction of the photoassimilates was retained in the leaves. This increased carbon retention is supported by the observed increase in leaf length with shading. During the long-term shading experiments, leaf length of both species increased (by 1.3 and 1.7 for *H. uninervis* and *T. hemprichii*, respectively, Chapter 2).

Halodule uninervis allocated a higher proportion of carbon to non-photosynthetic parts of the plant (mainly stems) with increased shading than *T. hemprichii*. The leaves of *H. uninervis* were still able to export as much as 20% in the highest shade as compared to *T. hemprichii* with only 12%. Indeed *H. uninervis* maintained its above:belowground ratio whilst *T. hemprichii* proportionally reduced its belowground biomass (Chapter 2).

Shading in *T. hemprichii* did not influence partitioning of ^{13}C over the belowground tissues (sinks). In contrast, *H. uninervis* increased investment in the stem, and stem length also increased with shading (Chapter 2). Stem elongation may be considered an

adaptive strategy to put the leaves closer to the water surface to maximize light harvesting (Vermaat et al., 1997). In both species, a relatively large fraction of the exported assimilates from the leaves was retained in stems and rhizomes, possibly as storage carbohydrates (Vermaat and Verhagen, 1996).

During the experiment, there was an increase in ^{13}C content in *H. uninervis* indicating an accumulation with time. We have difficulty explaining this. If there was ^{13}C accumulation with time as a result of ^{13}C labelling then this signature should also appear in the nearby collected blanks. However, $\delta^{13}C$ values of the blank samples even showed a slight decrease in $\delta^{13}C$ values at the end of the 12^{th} month shading period. In *T. hemprichii*, we did not observe such an increase but rather a peak in December. This could be linked to daytime low tide exposure. During this period, water temperature could increase in the thin film of water retained during low tide by as much as 10°C (29-39°C, Troll, 1931). Photosynthetic capacities of plants are known to increase with increasing temperature within the normal physiological limits of the plant (Marsh et al., 1986; Bulthuis, 1987).

The long-term shading significantly reduced shoot density in the high shade to 20% and 6% of the unshaded plots for *H. uninervis* and *T. hemprichii*, respectively (Chapter 2). As a consequence, shoot biomass was also significantly reduced. Despite this mortality in the highly shaded plots, relative leaf growth rates remained relatively constant and photosynthetic performance was even slightly higher under shade (Chapter 2). These findings conform to our present results showing no significant response in ^{13}C uptake rates and the amount of ^{13}C enrichment with shade in the plant tissues when normalized to biomass. It appears that both species were able to maintain uptake rates. Abel (1984) estimated carbon uptake for *T. hemprichii* at 2.2 μmol ^{14}C mg^{-1} Chl h^{-1} using radioactive ^{14}C. This rate agrees quite well with our results (1.8 to 2.4 μmol ^{13}C mg^{-1} Chl h^{-1}; see Chapter 2 for chlorophyll values).

In conclusion, our data suggest that the different shading levels had no influence on carbon uptake rates and the amount of ^{13}C that was assimilated per unit biomass. However, allocation of photosynthetically assimilated ^{13}C to the above and belowground compartments was affected by shading leading to a reduction in export to the nonphotosynthetic stem, rhizomes and roots in both species. A large proportion of the photosynthetically assimilated ^{13}C was retained in the leaves, probably to support continued leaf growth and maintenance. *H. uninervis* was found to invest less in the leaves (66 – 80%) than *T. hemprichii* (78 – 88%).

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Chapter 4

The interactive effect of shading and sediment conditions on growth and photosynthesis of two seagrass species, *Thalassia hemprichii* and *Halodule uninervis*

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Abstract

The interactive effect of shading (0% and 78% light reduction) and sediment redox potential (Eh: +310 to +390 and +10 to –90mV) of both *Thalassia hemprichii* (Ehrenberg) Ascherson and *Halodule uninervis* (Forsskål) Ascherson were investigated experimentally. Leaf longevity and chlorophyll (a+b) content of *T. hemprichii* increased significantly under shade while leaf growth rates and the production of new shoots, roots and rhizome internodes, as well as leaf and root biomass all decreased. In full light and oxidized sediments, rhizome branching, root length and biomass of *T. hemprichii* were highest. In contrast, *H. uninervis* showed a very limited response to shading alone, and new shoot and rhizome production were both enhanced in full light on reduced sediments. Photosynthetic capacities were significantly lower in shaded and reduced sediments for *T. hemprichii* but remained the same across all treatments for *H. uninervis*. In both species, respiration rates of roots and rhizomes, expressed per unit dry weight, were considerably higher on reduced sediment. Shading alone was shown to have influenced the growth and morphology of these seagrasses which was further enhanced on reduced sediments, particularly for *T. hemprichii*. Reduced sediment conditions largely reduced belowground biomass and increased respiratory demand. Calculation of daily carbon balances from photosynthesis vs. irradiance curves and daily irradiance indicated a net positive carbon balance could still be maintained by both seagrass species on oxidized sediment in 78% shading, whilst reduced sediments with shading caused a net negative balance. This explains the observed high shoot mortality and low rhizome elongation rates in both species.

Keywords: tropical seagrass, photosynthesis, redox potential, carbon balance, shading

Introduction

Seagrasses often grow in high density and appear to thrive on generally anoxic sediments (Moriarty and Boon, 1989; Terrados et al., 1999) which consequently may contain potentially toxic compounds such as sulfides (DeLaune et al., 1984; Goodman et al., 1995). Seagrass and freshwater submerged macrophytes, however, are known to release oxygen from their roots thus creating aerobic conditions in their rhizosphere (Sand-Jensen et al., 1982; Flessa, 1994; Pedersen et al., 1998). The subsequent oxidation of the sediments could be important for the nutrition of plants, since it affects mobility and availability of nutrients and accelerates decomposition of organic matter (Valiela, 1984; Moriarty and Boon, 1989). Light availability has already been shown to be of primary influence for growth and development of most seagrasses (Backman and Barilotti, 1976; Bulthuis, 1983; Dennison, 1987; Abal et al., 1994; Czerny and Dunton, 1995; Kraemer and Alberte, 1995; Vermaat et al., 1997; Chapters 2 & 3). Yet, the potentially powerful interaction between light availability and reducing sediment has received attention only quite recently (Goodman et al., 1995; Hemminga, 1998; Alcoverro et al., 1999).

In this study, we tested the hypothesis that with the reduction of light, the photosynthetic production would be limited and thus the transport of oxygen to the belowground tissues would be reduced. Roots and rhizomes may be vulnerable to carbon starvation. Their prolonged functioning and survival depends on a sustained supply of sucrose to maintain energy production (Vermaat and Verhagen, 1996; Touchette and Burkholder, 2000). However, translocation of sucrose from the shoot to the rhizome as well as the mobilization of stored starched are reportedly blocked during periods of anoxia (Zimmerman and Alberte, 1996; Alcoverro et al., 1999). Consequently, due to depletion of available belowground sucrose reserves, prolonged periods of anoxia associated with reduced irradiance may result in impairment of the root-rhizome functioning and, ultimately, in plant death. We chose two contrasting meadow-forming tropical seagrass species i.e. a pioneering species, *Halodule uninervis* (Forsskål) Ascherson and the late successional species *Thalassia hemprichii* (Ehrenberg) Ascherson in order to experimentally assess the interactive effect of shading and sediment anoxia under laboratory conditions. The seagrasses were cultured separately and growth, morphology, photosynthesis and respiration of the above and belowground compartments were measured to determine the influence of reduced sediment conditions and shading.

Materials and Methods

The experiments were conducted at the Mindanao State University at Naawan outdoor laboratory. The experiment consisted of two shading levels, 0% and 78% reduction of incident light hereto referred to as unshaded and shaded, respectively; and two sediment conditions, oxidized (+310 to +390 mV) and reduced sediments (+10 to –90 mV) with three replicates each. A total number of 12 aquaria (60 x 30 x 40 cm;

LxWxH) were used in which each aquarium was considered an experimental unit. The aquaria were arranged in a randomized block design with the blocks positioned in North-South direction. The set-up was situated in a makeshift lab with translucent fiberglass roofing. The whole set-up was covered with black coarse screen that reduced surface irradiance by approximately 65%, because too much light enhances the growth of epiphytes. The resulting irradiance level in the unshaded aquaria therefore was 300-650 µmol PAR m^{-2} s^{-1} during noontime. This light level used was similar to that measured in the nearby seagrass beds in Sulawan at approximately one meter depth (Uy et al., in prep.). The shaded aquaria were covered with a fine mesh black cloth reducing incident light available to the cultured seagrass by 78% leading to approximately 60-150 µmol PAR m^{-2} s^{-1}. Since the glass aquaria were arranged in a row facing each other, styrofoam pads were provided in between the aquaria to isolate and avoid shading effect for the nonshaded aquaria. A flow-through system (~1L min^{-1}) was maintained with locally available seawater to reduce temperature fluctuation within the range 27-33° Celsius.

The reduced sediment was collected in Sulawan and the non-reduced type in Initao (5 km from Naawan) both in Misamis Oriental (see Chapter 2, Fig. 1). The reduced sediment was easily identified by its dark color and H_2S smell. Enough sediment was collected with a shovel and placed in plastic bags. The collection site was located a few meters along the periphery of an extensive but sparse seagrass bed dominated by *Enhalus acoroides*. In the laboratory, the collected sediment was allocated over the aquaria to fill up to 10 cm and left for a month to stabilize. Nothing was added within one month except that the glass aquaria were covered with black cloth and filled with seawater. No significant change in sediment redox potential was observed. The non-reduced or oxidized sediments were collected from a beach, covered with coarse coralline washed sand. Sediments were brought to the laboratory and were washed vigorously with seawater until the outflow became clear. Composition of the sediments used is shown in Table 1. Water and sediment redox potential were measured regularly using a Ag/AgCl reference electrode and a platinum electrode attached to a Corning digital pH meter. Three sediment depth levels at two points in the aquaria were measured: 0 (surface) , 3 and 8 cm depth. Values in mV were subsequently corrected against the reference electrode to reflect Eh by adding +210 (Mosey, 1985).

Clones of the wide-leaved variety *H. uninervis* were collected in Naawan adjacent to the laboratory, while *T. hemprichii* was collected in Sulawan, both in the province of Misamis Oriental, Southern Philippines (see Chapter 2, Fig. 1). Collections were made along the edges of a patch. Clonal fragments or clones (the first five shoots or ramets from the apex and the intact connecting rhizomes) were harvested carefully by shovel, cleaned to remove sediments and placed in plastic bags with seawater. They were then transported to the laboratory and placed in basins of seawater for initial measurements. A total of 60 clonal fragments were selected, taking into consideration similarities in branching pattern and arrangement of nodes with a total of five ramets in each clone for *H. uninervis* and four ramets for *T. hemprichii*. The number of ramets for *T. hemprichii*

could not be extended to five since branching of the vertical rhizomes usually occurred on the fifth ramet. Then five clones were randomly taken to represent one group to be planted in separate aquaria. Maps of each clone were drawn and the following characteristics were measured: the length of horizontal rhizome, distances between ramets, number of leaves per shoot, and height of each shoot within the clone. Then a tag, made of plastic-coated wire with a small plastic strip for the code, was placed in between the second and third ramet from the apex. After the measurement, the entire clone in each set was photographed for documentation purposes.

Table 1. Composition of sediments used in the experiment under different light (L+ = unshaded; L- = shaded) and sediment conditions (R+ = oxidized, R- = reduced). Given is the weight percent of a grain size class.

Site of origin		**Initao**		**Sulawan**	
Grain size		**L+R+**	**L-R+**	**L+R**	**L-R-**
> 1.7mm	Coarse sand	3.03	2.28	14.85	13.83
850µm – 1.7mm	Coarse sand	36.34	35.99	30.88	28.72
250µm – 850µm	Fine sand	60.49	61.43	37.68	38.42
180µm – 250µm	Fine sand	0.09	0.23	5.97	6.71
75µm – 180µm	Very fine sand	0.05	0.07	8.98	10.27
< 75µm	Very fine sand	0.00	0.00	1.64	2.05

The seagrass clones were planted randomly in each assigned aquarium, careful that the roots and horizontal rhizomes were properly inserted. A map of the arrangement of the clones in every aquarium was made to facilitate the counting of new shoots. Then a plastic number tag was provided at the apical shoot of each clone. The shoots were cleaned regularly by rubbing the leaves between fingers moderately, to remove attached sediments and periphyton. The top layer of the sediment and aquarium walls was also cleaned by scraping and siphoning off the film of filamentous algae when necessary. Moderate aeration was provided to create water movement in the aquaria. Before the start of the experiment, all the clones were conditioned and observed for the first two weeks in the light before shading commenced. No mortality occurred during the two weeks observation, hence, no clones were replaced. At the end of the conditioning period, one clone in every aquarium was collected for morphometric measurements. Results indicated no significant differences in gross morphology and photosynthetic parameters (results not shown, $p>0.05$) of the clones between sediment types during the two-week acclimation period. Shading started in the third week which lasted for another six weeks for *H. uninervis* (June 7 - July 19, 1999) and nine weeks for *T. hemprichii* (Sept. 4 – Nov. 9, 1999). The culture period was shorter for *H. uninervis*

because its shoot production was faster. The number of new shoots produced was counted every week. Shoot height and number of leaves were also measured every two weeks in the same two clones in every aquarium. Water samples were collected intermittently for analyses: pH was measured using a portable pH meter; ammonium and nitrite content of the water were measured following the procedure in Strickland and Parsons (1972).

At the end of the culture period, complete clones in each aquarium were collected and placed in separate basins filled with filtered seawater. The clones were cleaned by carefully removing sediments and other attached particles in the shoots, rhizomes and roots. Then maps of the clone were drawn to facilitate measurements of the following: total rhizome length of the clone measured from the oldest shoot to the apical tip of the growing rhizomes, number of internodes and distances between shoots, height and age of each shoot, stem length, number of leaves, leaf length and width and the number and length of the roots. In *H. uninervis*, counting of the number of roots is not possible, thus we only measured maximum root length. All measurements were done in a large shallow pan with seawater, and all the shoots were kept submerged. Then all the shoots (leaves plus the intact leaf sheaths) were separated and cleaned in preparation for the photosynthesis vs. irradiance (P-I) measurements, while the roots and rhizomes were prepared for respiration measurement in the dark.

To determine the leaf growth rates, shoots in two complete clones per aquarium were punched with a syringe needle just above the leaf sheath junction before the end of the experiments. After six days, these clones were collected. The same procedure was followed as for non-punched shoots, but length and width of new leaves produced were measured. New leaves were distinguishable due to the absence of a punched hole. Relative Growth Rates (RGR) were calculated after Hunt (1982):

$$RGR = \ln (LA_f / LA_i) / t * 100 = \% \text{ per day} \quad (1)$$

Where: LA_f = total leaf area of shoots, LA_i = LA_f minus new leaves formed on marked leaves, and t = time duration between leaf marking and harvest. Leaf elongation rate (cm $shoot^{-1}$ d^{-1}) was calculated as the total length of new leaves formed over the marking period. For *H. uninervis*, the leaves were too narrow to punch, instead we did *in situ* measurements of leaf length in 2 clones for every aquarium. The differences in leaf length were calculated based on the leaf position, which was mapped earlier. The leaf plastochron interval (days) was calculated according to Duarte et al. (1994) as:

$$\text{Leaf plastochron interval} = \frac{\text{number of punched shoots x marking period}}{\text{total number of new leaves}} \quad (2)$$

Leaf longevity was calculated as the product of the plastochron interval and the average number of leaves per shoot.

To determine Photosynthesis-Irradiance (P-I) curves of the cultured plants, shoots without the rhizomes and roots were incubated in clear perspex tubes in a flow-through

system. Details of the procedure are found in Chapter 2. Four tubes were used representing four treatments (2 light x 2 sediment conditions) arranged randomly in a glass aquarium filled with seawater. Fitting of the curves followed the Michaelis-Menten rectangular Hyperbola model (Lederman and Tett, 1981; Hootsmans and Vermaat, 1994):

$$P = (P_{max} \times I) / (K_m + I) - R \quad (3)$$

Where: P = net photosynthetic rate (mg O_2 g^{-1} DW h^{-1}); I = Irradiance (µmol PAR m^{-2} s^{-1}); P_{max} = gross maximal photosynthetic rate (mg O_2 g^{-1} DW h^{-1}); K_m = the half saturation constant (µmol PAR m^{-2} s^{-1}); and R = respiration rate (mg O_2 g^{-1} DW h^{-1}). From equation 3, the following derived parameters were obtained: Light compensation point (LCP) = Irradiance level at zero photosynthetic rate and $\alpha = P_{max} / K_m$.

To determine respiration of the above and belowground compartments, clones were separated as shoot, rhizomes (vertical and horizontal rhizomes) and roots for *T. hemprichii*. For *H. uninervis*, clones were separated as shoot, then the remaining rhizomes with the roots still intact, were cut at the 5th short shoot (the initial shoot number) to represent old and new growth. However, there was no significant difference between the old and new parts for *H. uninervis*, thus data were pooled as belowground respiration (t-test, $p>0.05$). Then they were placed separately in D.O. bottles (300 ml cap) and were incubated in the dark for 2-3 hours. The D.O. bottles were placed in a water basin and were agitated by swirling every 5-10 minutes. All incubations were done in the morning from 0800 to 1100hrs. D.O. analyses followed the Winkler's procedure (Carpenter, 1965). Rate of respiration was calculated as the difference in dissolved oxygen level before and after the incubation, normalized to the biomass of the tissues, all over the period of incubation in hours. After the incubation, the shoots were carefully removed from the D.O bottles, before fixing to be used in the P-I measurements. After all the measurements, small fresh samples of the shoot including the non-photosynthetic leaf parts (approx. 1-3 g) were taken for chlorophyll extraction using ethanol (Wintermans and deMots, 1965). The remaining tissues were measured for fresh weight and were oven dried for at least 48 hrs at 50° C for dry weight measurements.

Daily carbon balances of the two species were estimated with a modification of the approach of Vermaat and Verhagen (1996) using data from P-I curves, biomass, and daily irradiance levels. Shoot respiration was assumed to be constant during the day and was reduced to 52% during night time to correct for temperature effect (Marsh et al., 1986). The daily whole plant carbon balances (CB_p) were calculated as follows:

$$CB_p = C_{gain\ or\ loss} - C_{demand} \quad (4)$$

$$C_{gain\ or\ loss} = (\Sigma_{24h}\ P_{net} \times PQ) / (biomass_{shoot} \times 0.35) \quad (5)$$

$$C_{demand} = (Respiration_{\ Root+Rhizome} \times 24h \times PQ) / (biomass_{Root+Rhizome} \times 0.35) \quad (6)$$

Where: $C_{gain\ or\ loss}$ = the daily net carbon gain or loss of the aboveground shoot material from the average light curve estimated during the culture period (Fig. 5);

Σ_{24h} P_{net} = Sum of net photosynthesis calculated using equation 3 in 24 hours; C_{demand} = the daily carbon demand of the below ground tissues (roots and rhizomes); $Respiration_{Root+Rhizome}$ = dark respiration rates of the roots and rhizomes; PQ = photosynthetic quotient set at 1.15 mol O_2 / mol C (after Vermaat and Verhagen, 1996). Thus the conversion factor from mg O_2 to mg C was 0.3261. All biomass was converted to carbon values using a conversion factor of 0.35 (% organic carbon content of dry weight; Chapter 2). Daily irradiance levels were measured for 5 days using LICOR 2pi quantum sensor and a datalogger during April 2000. Average irradiance was then estimated and subsequently corrected by the experimental shading screens. Both shoot respiration readings (D.O. bottles vs P-I) predicted productivity of *T. hemprichii* quite well. But in *H. uninervis*, respiration values obtained from D.O. bottles seriously underestimated production rates in the light (L+R-) and overestimated production rates in the shaded (L-R-). Thus, for purposes of calculation and comparison, only respiration values obtained from Michaelis-Menten model (equation 3) were used.

Data from the end of the culture period, per clone or for every replicate (set of 2-3 clones) depending on the measured parameters, were subjected to 3-way ANOVA testing for differences between species, light and sediment conditions. A 2-way ANOVA was performed when the species effect was significant, testing for the effect of light and sediment conditions within species. Multiple comparison tests using Tukey's HSD were done to test for significant differences among the four treatments: 2 light (shaded and unshaded) x 2 sediment conditions (oxidized and reduced). Values were log- or square root-transformed whenever necessary to homogenize the variances.

Results

There was no substantial change in the redox values obtained during the culture period with the middle (3 cm) and bottom sediments (8 cm), generally being the same (Fig. 1, ANOVA, $p>0.05$ results not shown). The surface (1 cm), however, was slightly positive in the reduced aquaria. Similarly for the oxidized sediments, values remained relatively constant with time with the lower 3cm more positive (>+360) than the surface sediment (+310). The roots of the two species are generally below the 3cm depth although the apical meristems are situated near or at the sediment surface.

The shoot biomass of *T. hemprichii* was roughly two folds higher than that of *H. uninervis* (3-way ANOVA, $p<0.01$, Tables 2-3, Fig. 2) with the factor "species" explaining 15% of the variation. Neither shading nor sediment conditions influenced shoot biomass, but the interaction between species and shading was significant (7%) indicating differences in their response to light. Two-way ANOVA indicated shading as the main factor for shoot differences in *T. hemprichii* ($p<0.01$ explaining 20% of the variation) while no differences were seen for *H. uninervis* ($p>0.05$, results not shown).

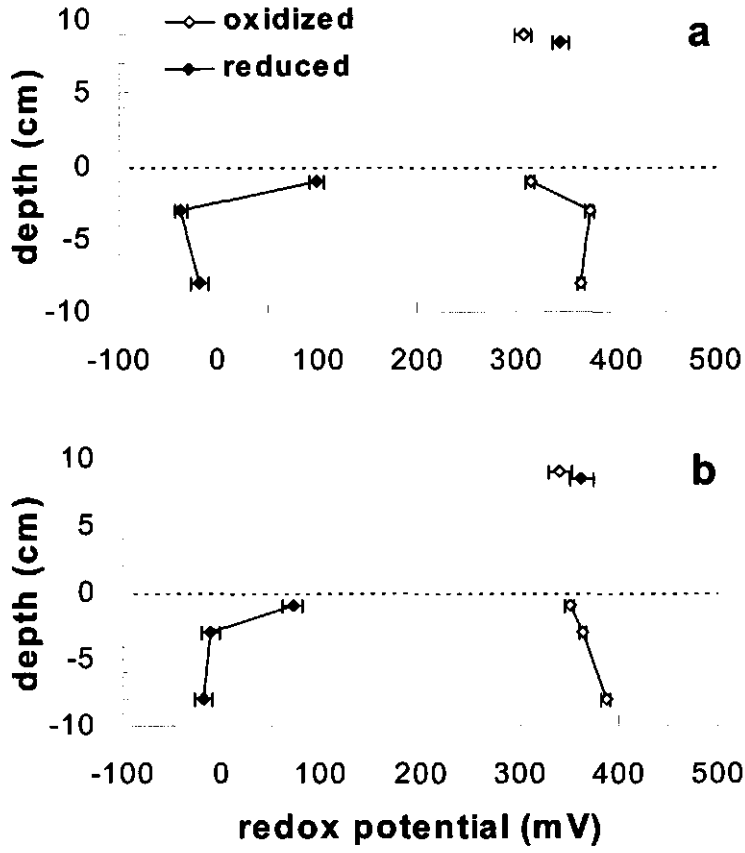

Fig. 1. Average (± SE) redox potential (Eh, mV) of the water column (0 to +10 cm) and sediments (0 to –10 cm) in (a) *T. hemprichii* and (b) *H. uninervis*. Surface of the sediments is 0 depth. There was no significant differences in redox potential between shaded and unshaded aquaria, thus data were pooled for the same sediment type.

Root biomass was significantly different among the two species (3-way ANOVA; $p<0.001$; Table 2; accounting for 41% of the variation) with *T. hemprichii* having 5-8 times higher quantities than *H. uninervis*. The effect of shading was significant describing 24% ($p<0.001$) of the variation followed by the sediment effect (4%, $p<0.05$). For *T. hemprichii*, production of roots was mainly influenced by shading, but roots were significantly longer in the unreduced sediment which was further enhanced without shading (Table 4). Sediment type influenced root length in *H. uninervis*. Rhizome production of *T. hemprichii* showed a highly significant species effect (89%, $p<0.001$) but was only slightly influenced by shading (1%, $p<0.05$) and not affected by the sediment type ($p>0.05$).

Table 2. Results of 3-way ANOVA examining the effects of species, shading and sediment conditions (Redox) and their interactions on the biomass and growth of *T. hemprichii* and *H. uninervis*. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS/ total SS) and the level of significance (*p*). Bold numbers with $p<0.05$ are considered significant. RGR is relative growth rate.

Biomass		**Leaves**		**Rhizomes**		**Roots**	
Factors	***df***	**%**	***p***	**%**	***p***	**%**	***p***
Species	1	15	**0.003**	89	**<0.001**	41	**<0.001**
Shading	1	4	0.105	1	**0.022**	24	**<0.001**
Redox	1	2	0.233	1	0.113	4	**0.015**
Species x Shading	1	7	**0.034**	<0	0.192	1	0.133
Species x Redox	1	2	0.313	<0	0.730	2	0.072
Shading x Redox	1	5	0.070	<0	0.146	<0	0.623
Species x Shading x Redox	1	<0	0.758	<0	0.730	1	0.197
Within + Residual	44	65		9		27	
Total	51	100		100		100	

Growth parameters		**Leaf RGR**		**Leaf elongation**		**Leaf lifespan**	
Factors	***df***	**%**	***p***	**%**	***p***	**%**	***p***
Species	1	<0	0.833	11	**0.009**	3	0.107
Shading	1	19	**0.003**	6	0.050	46	**<0.001**
Redox	1	2	0.342	5	0.087	<0	0.517
Species x Shading	1	4	0.148	8	**0.026**	12	**0.001**
Species x Redox	1	<0	0.785	3	0.165	<0	0.960
Shading x Redox	1	<0	0.669	4	0.122	<0	0.949
Species x Shading x Redox	1	<0	0.654	1	0.435	1	0.407
Within + Residual	40	75		61		38	
Total	47	100		100		100	

The relative growth rates of the two species were not significantly different, but the shade effect on this growth measure was significant explaining 19% of the variation (3-way ANOVA, $p<0.01$, Table 2). The sediment effect and the interaction between shade and sediments were not significant for the computed relative growth rates. For the leaf elongation rates, the species effect (11%, $p<0.01$) was significant and the shade and species interaction was important as well, explaining 8% of the variation. The estimated plastochron interval, on the other hand, was not significantly different among species but was affected by shade levels (46%, $p<0.001$) as well as by species and shading interaction (12%, $p<0.01$). These results strongly suggest that shade alone resulted in a decrease in growth rates and a subsequent increase in plastochron interval for both species.

Table 3. Biomass characteristics (mg DW clone^{-1} ± SE) of *T. hemprichii* and *H. uninervis* under different light (L+ = unshaded; L- = shaded) and sediment conditions (R+ = oxidized, R- = reduced). *p* values are shown and bold numbers indicate significant differences among treatments (one-way ANOVA, $p<0.05$). Values with the same superscripts are not significantly different (Tukeys HSD).

	Thalassia hemprichii				***Halodule uninervis***			
Parameters	**L+R+**	**L-R+**	**L+R-**	**L-R-**	**L+R+**	**L-R+**	**L+R-**	**L-R-**
Leaf (mg/clone)	122 ± 9^{ab}	99 ± 14^{a}	182 ± 21^{b}	100 ± 21^{a}	66 ± 12	95 ± 16	90 ± 8	76 ± 18
Rhizome (mg/clone)	769 ± 92	732 ± 33	1013 ± 116	818 ± 82	111 ± 19	95 ± 17	147 ± 20	91 ± 19
Roots (mg/clone)	194 ± 14^{c}	78 ± 8^{ab}	109 ± 6^{b}	52 ± 7^{a}	46 ± 6.7^{ab}	34 ± 7.5^{ab}	51 ± 5.5^{b}	27 ± 6.9^{a}
Leaf (%)	12 ± 1	11 ± 1	15 ± 2	10 ± 2	31 ± 8	44 ± 1	33 ± 2	42 ± 6
Rhizome (%)	69 ± 2^{a}	81 ± 2^{bc}	77 ± 2^{b}	84 ± 2^{c}	50 ± 6	43 ± 3	48 ± 2	46 ± 8
Roots (%)	19 ± 2^{a}	9 ± 1^{b}	9 ± 1^{b}	6 ± 1^{b}	19 ± 3	14 ± 3	19 ± 2	12 ± 14
Root-Rhizome : Shoot ratio (RSR)	8.0 ± 0.7	9.0 ± 0.8	6.8 ± 1.0	10.6 ± 1.4	3.0 ± 1.0	1.3 ± 0.1	2.0 ± 0.2	1.6 ± 0.5

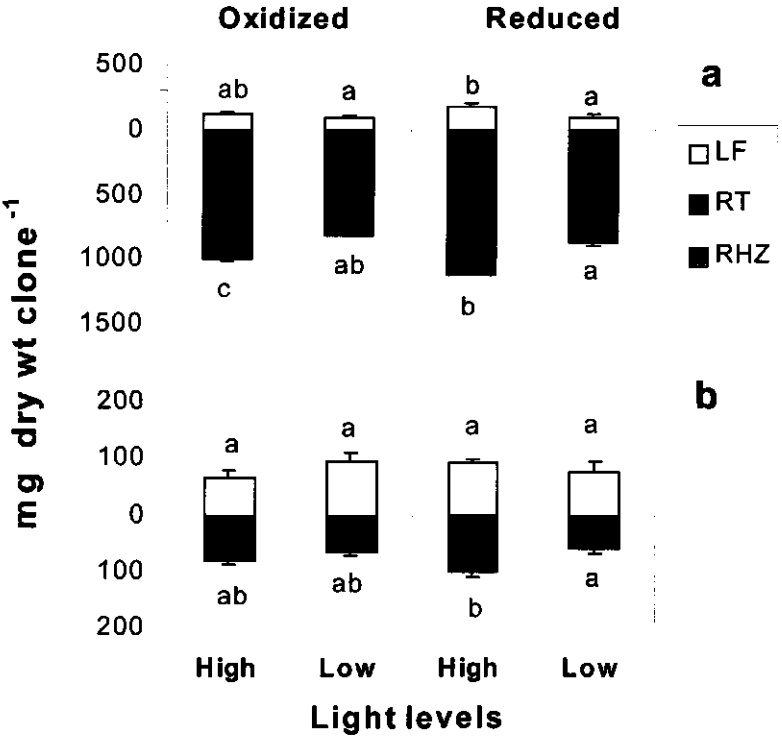

Fig. 2. Average biomass of the above and belowground tissues of (a) *T. hemprichii* and (b) *H. uninervis* at the end of the experiment grown in different light (High = unshaded and low = 78% shading) and sediment conditions (oxidized and reduced). Error bars are standard errors of mean (n=3). Bars with similar letters indicate no significant differences using Tukeys HSD. Letters in the belowground are for root biomass. Rhizome biomass in both species was not significantly different among treatments. The root-rhizome : shoot ratios were also not significantly different among treatments for both species (one-way ANOVA $p>0.05$). LF = leaves, RHZ = rhizomes, RT = roots.

Saturated photosynthetic rates (P_{max}) did not show differences among treatments but did differ between species (Tables 5-6, Fig. 3, 3-way ANOVA, $p<0.05$, explaining at least 23% of the variation). Likewise, the initial slope of the P-I curves was significantly different between species but not among treatments and their interaction effect. Light compensation points were relatively constant and did not show any effect. In contrast, the half saturation constant (K_m) exhibited differences between species ($p<0.001$, explaining 35% of the variation) and shade levels ($p<0.05$, 10%). The interaction between shading and sediment conditions ($p<0.05$, 12%) was also

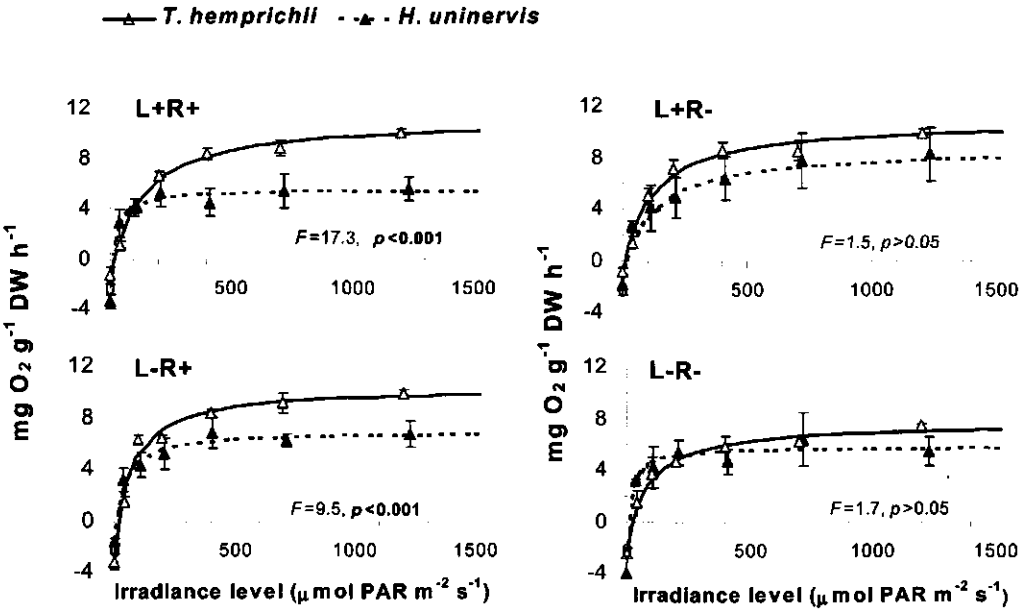

Fig. 3. Photosynthesis vs. irradiance (P-I) curves of *T. hemprichii* and *H. uninervis* under different light (L+ = unshaded; L- = shaded) and sediment conditions (R+ = oxidized ; R- = reduced). *F*-values comparing curves for the two species including the significance levels (*p*) are shown in each graph. Separate multiple comparison test, using *F*-statistics among fitted P-I curves of the different treatments in each species were also made: the P-I curve of L-R- was significantly different from the rest in *T. hemprichii*, but no significant differences were observed among P-I curves in *H. uninervis* ($p>0.05$).

significant, as well as the 3-way interaction ($p<0.01$, 17%). Similarly, shoot respiration differed significantly between species ($p<0.001$, 32%), and shade levels ($p<0.05$, 5%), while the sediment effect was not significant. The dark respiration rate of the non-photosynthetic tissues (roots and rhizomes) was strongly influenced by sediment oxygenation describing 10% of the variation, followed by the shade effect (2.5%, $p<0.05$). Still, the difference between species was strong (34%, $p<0.001$, Tables 5-6). The bulk of the respiration from *T. hemprichii* is from the roots accounting for at least 88% of the belowground respiration (results not shown). For reduced sediments, the influence of shading was not significant between species (*F*-stat, $p>0.05$, Fig. 3) whilst on oxidized sediments, *T. hemprichii* P-I curves were different from those of *H. uninervis*. The lower K_m, steeper slope and lower P_{max} of *H. uninervis* indicate that it is more easily shade acclimated than *T. hemprichii* (sensu Boardman, 1977).

Table 4. Average growth and morphometrics of *T. hemprichii* after 9 weeks in culture and *H. uninervis* after 6 weeks in culture under different light (L+ = unshaded; L- = shaded) and sediment conditions (R+ = oxidized, R- = reduced). *p* values written in bold numbers are significantly different (one-way ANOVA., $p<0.05$). Values are subsequently tested for Tukeys HSD and those with the same superscript letters are not significantly different ($p>0.05$).

	Thalassia hemprichii				***Halodule uninervis***			
Parameters	L+R+	L-R+	L+R-	L-R-	L+R+	L-R+	L+R-	L-R-
Plastochrone interval (days)*	9.2 ± 1.5^{a}	37.4 ± 9.1^{b}	9.2 ± 0.9^{a}	28.8 ± 8.0^{b}	15.1 ± 2.7	17.7 ± 3.7	9.6 ± 0.7	18.3 ± 2.0
Leaf elongation rate (mm d^{-1})	3.1 ± 0.6^{ab}	3.0 ± 0.8^{a}	6.4 ± 0.6^{b}	3.4 ± 0.8^{a}	2.6 ± 0.3	3.1 ± 0.6	3.2 ± 0.6	2.9 ± 0.5
Leaf relative growth rate (% d^{-1})	3.1 ± 0.2^{ab}	2.1 ± 0.4^{a}	3.4 ± 0.2^{b}	2.2 ± 0.4^{a}	2.8 ± 0.2	2.4 ± 0.4	3.0 ± 0.4	2.5 ± 0.6
Leaf longevity (days)	34 ± 6^{a}	125 ± 25^{b}	39 ± 7^{a}	100 ± 21^{ab}	22 ± 4	39 ± 5	21 ± 4	36 ± 11
Rhizome elongation rate (mm d^{-1})	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	1.1 ± 0.3^{a}	1.9 ± 1.0^{ab}	3.6 ± 0.8^{b}	1.1 ± 0.2^{a}
Rhizome Internode distance (mm)	2.5 ± 0.2	2.9 ± 0.2	2.4 ± 0.3	2.6 ± 0.26	n.d.	n.d.	n.d.	n.d.
Rate of internode prod'n per day	0.5 ± 0.1^{c}	0.3 ± 0.0^{ab}	0.5 ± 0.1^{bc}	0.3 ± 0.0^{a}	n.d.	n.d.	n.d.	n.d.
Ramet distance (mm)	36 ± 1	38 ± 3	34 ± 2	37 ± 2	18 ± 3	22 ± 3	18 ± 2	18 ± 3
Mean number of new roots/clone	23 ± 2^{a}	12 ± 1^{b}	20 ± 2^{a}	12 ± 1^{b}	n.d.	n.d.	n.d.	n.d.
Mean length of roots (mm)	84 ± 4^{c}	65 ± 6^{b}	50 ± 5^{ab}	38 ± 3^{a}	89 ± 13^{a}	90 ± 6^{a}	45 ± 3^{b}	43 ± 2^{b}

for Plastochron interval in *H. uninervis*, shading effect was significant (2-way ANOVA explaining 25% of the variation), whilst shading effect was significant for the number of apical nodes in *T. hemprichii* (2-way ANOVA, $p=0.03$ explaining 13% of the variation)

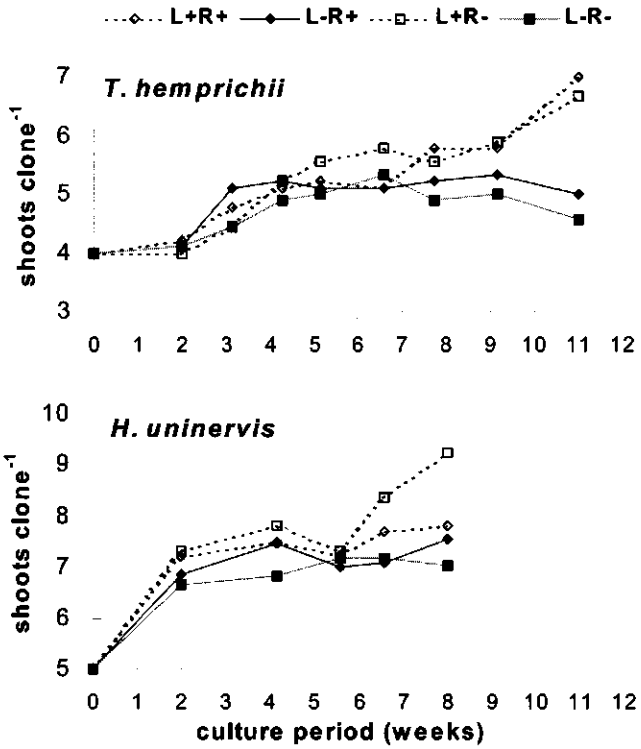

Fig. 4. The average number of shoots per clone in *T. hemprichii* and *H. uninervis* during the experimental period (n=3). Treatment codes as in Fig. 3.

Total chlorophyll content was strongly influenced by light levels and was significantly higher in the shaded plants (3-way ANOVA, $p<0.001$, Tables 5-6) describing 35% of the variation. This was followed by the sediment type (7%, $p<0.001$) and the interaction between species and sediment type (4%, $p<0.01$). The chlorophyll a:b ratio, was shown to be significantly different among species (36%) but was also influenced by light (10%, $p<0.001$). The chlorophyll a:b ratio was highest in the unshaded aquaria with oxidized sediments, and lowest in shaded *T. hemprichii.*

The number of new shoots produced was generally lower in the shaded plants for both species, whilst shoot mortality was highest in the shaded plants grown on reduced sediments. Thus the balance of shoot recruitment and mortality during the culture period was only negative in the treatments combining shading and reduced sediment (Fig. 4, Table 7). The pronounced mortality suggests a declining population if the experiment were extended. Moreover, branching of *T. hemprichii* was enhanced in the light on oxidized sediments, while very limited branching was observed for *H. uninervis.*

Table 5. Results of 3-way ANOVA examining the effects of species, shading levels, and redox potential of sediments and their interactions on the fitted photosynthesis curve parameters, respiration and chlorophyll contents of *T. hemprichii* and *H. uninervis*. Degrees of freedom are similar for values in the same row. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS/ total SS) and the level of significance (*p*). Bold numbers with $p<0.05$ are considered significant. Degrees of freedom are similar in the row unless indicated.

		P_{max}		K_m		Slope	
Factors	*df*	%	*p*	%	*p*	%	*p*
Species	1	23	**0.028**	35	**<0.001**	29	**0.001**
Shading	1	1	0.712	10	**0.018**	3	0.217
Redox	1	1	0.677	1	0.416	1	0.587
Species x Shading	1	<0	0.977	<0	0.989	<0	0.888
Species x Redox	1	9	0.142	2	0.249	1	0.725
Shading x Redox	1	1	0.522	12	**0.011**	15	**0.013**
Species x Shading x Redox	1	2	0.552	17	**0.004**	20	**0.006**
Within + Residual	16	63		23		31	
Total	23	100		100		100	

		Respiration		P_{max}/R		LCP	
Factors	*df*	%	*p*	%	*p*	%	*p*
Species	1	4	0.271	13	**0.019**	14	0.087
Shading	1	11	0.062	21	**0.005**	1	0.658
Redox	1	1	0.726	1	0.669	1	0.738
Species x Shading	1	5	0.206	7	0.068	12	0.120
Species x Redox	1	3	0.342	1	0.502	1	0.616
Shading x Redox	1	12	0.050	13	**0.019**	0	0.964
Species x Shading x Redox	1	19	**0.018**	14	**0.015**	1	0.742
Within + Residual	16	45		30		70	
Total	23	100		100		100	

		Shoot respiration		Root+rhizome respiration		Chlorophyll		
Factors	*df*	%	*p*	%	*p*	df	%	*p*
Species	1	32	**<0.001**	34	**<0.001**	1	<0	0.441
Shading	1	5	**0.012**	3	**0.043**	1	35	**<0.001**
Redox	1	<0	0.504	10	**<0.001**	1	7	**<0.001**
Species x Shading	1	<0	0.785	<0	0.431	1	<0	0.986
Species x Redox	1	<0	0.592	1	0.751	1	4	**0.002**
Shading x Redox	1	<0	0.527	1	0.289	1	<0	0.654
Species x Shading x Redox	1	1	0.319	<0	0.746	1	<0	0.814
Within + Residual	44	62		53		112	54	
Total	51	100		100		119	100	

* shoot dark respiration estimated using the D.O. bottles

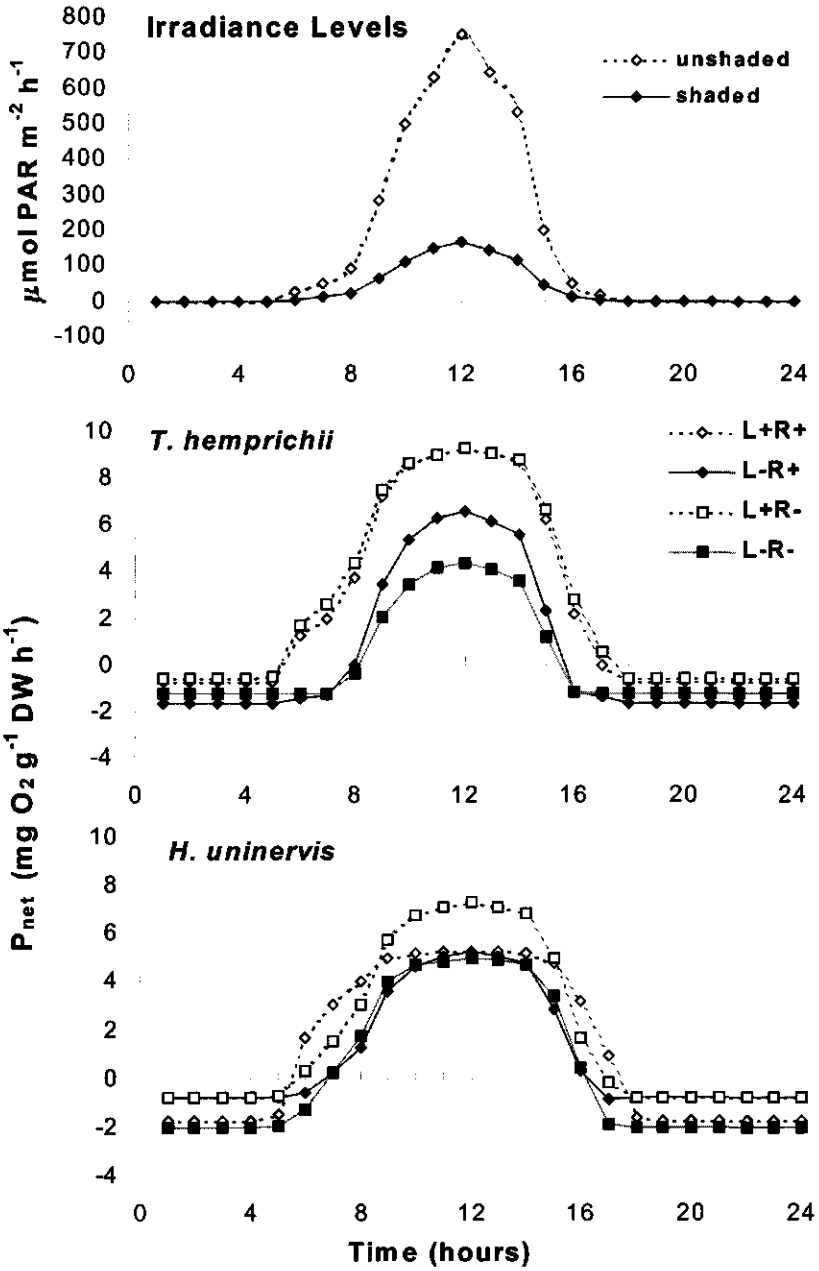

Fig. 5. Estimated daily irradiance (µmol PAR m^{-2} h^{-1}) and net oxygen balances (mg O_2 g^{-1} DW h^{-1}) for the clones of *T. hemprichii* and *H. uninervis* grown in different light (unshaded = L+, shaded = L-) and sediment conditions (oxidized = R+, reduced = R-).

Table 6. Average values (± SE) of photosynthetic capacities, above and below ground dark respiration, and pigment contents of *T. hemprichii* and *H. uninervis* in culture under different light (L+ = unshaded ; L- = shaded) and sediment conditions (R+ = oxidized, R- = reduced). *p* values (one way ANOVA) are shown and bold values ($p<0.05$) indicate significant differences among treatments. Values with the same superscripts are not significantly different (Tukeys HSD). *P* values with single asterisk means the data were log transformed to conform with homogeneity of variance

	Thalassia hemprichii				***Halodule uninervis***			
Parameters	**L+R+**	**L-R+**	**L+R-**	**L-R-**	**L+R+**	**L-R+**	**L+R-**	**L-R-**
P_{max} ($mgO_2gDW^{-1}h^{-1}$)	12.7 ± 1.3	13.6 ± 0.8	11.8 ± 0.2	10.0 ± 0.5	8.9 ± 1.3	8.5 ± 1.1	10.1 ± 2.7	9.8 ± 2.7
K_m ($\mu Em^{-2}\ s^{-1}$)	126 ± 2	63 ± 7	105 ± 9	76.8 ± 33	18 ± 8^{a}	40 ± 14^{ab}	111 ± 42^{b}	14 ± 4^{a}
Respiration ($mgO_2gDW^{-1}h^{-1}$)	1.5 ± 0.7	3.3 ± 0.6	1.2 ± 0.3	2.4 ± 0.3	3.4 ± 0.5	1.6 ± 0.2	1.5 ± 0.8	4.0 ± 1.2
P_{max}/R	16.6 ± 9.8	4.3 ± 0.5	11.5 ± 2.7	4.2 ± 0.4	2.7 ± 0.4^{a}	5.6 ± 0.4^{ab}	10.0 ± 3.5^{b}	2.5 ± 0.2^{a}
P_{max}/K_m	0.10 ± 0.01	0.22 ± 0.04	0.11 ± 0.01	0.13 ± 0.06	0.69 ± 0.23	0.32 ± 0.16	0.13 ± 0.05	0.73 ± 0.15
LCP ($\mu Em^{-2}\ s^{-1}$)	16.7 ± 7.3	19.3 ± 1.6	11.0 ± 2.1	26.8 ± 13.7	9.8 ± 2.3	8.3 ± 2.5	13.5 ± 3.6	9.4 ± 2.9
Shoot respiration*	2.4 ± 0.2	2.0 ± 0.1	2.4 ± 0.2	2.1 ± 0.4	2.1 ± 0.7	1.7 ± 0.6	3.1 ± 0.7	2.0 ± 0.6
Root+rhizome respiration ($mgO_2gDW^{-1}h^{-1}$)	2.2 ± 0.1^{ab}	1.8 ± 0.2^{a}	2.6 ± 0.2^{b}	2.6 ± 0.2^{b}	1.9 ± 0.7	1.1 ± 0.2	3.1 ± 0.6	2.4 ± 0.7
Total Chlorophyll (mg g DW^{-1})	2.8 ± 0.1	3.7 ± 0.1	2.9 ± 0.1	3.7 ± 0.1	2.6 ± 0.1	3.4 ± 0.2	3.3 ± 0.1	4.1 ± 0.1
Chl-a : Chl-b	1.1 ± 0.1^{b}	0.7 ± 0.1^{a}	0.7 ± 0.04^{a}	0.9 ± 0.08^{ab}	1.8 ± 0.05	1.2 ± 0.08	1.5 ± 0.03	1.1 ± 0.02
Total Phaeopigments (mg g DW^{-1})	2.8 ± 0.1	3.5 ± 0.3	3.2 ± 0.1	3.9 ± 0.1	2.2 ± 0.1	3.0 ± 0.5	2.8 ± 0.2	3.8 ± 0.2

* shoot respiration measured using the D.O. bottles incubated in the dark

Table 7. Daily carbon balances for *T. hemprichii* and *H. uninervis* under different light (L+ = unshaded ; L- = shaded) and sediment conditions (R+ = oxidized, R- = reduced) estimated from photosynthesis vs. irradiance curve parameters and daily irradiance.

	Thalassia hemprichii				*Halodule uninervis*			
Parameters	**L+R+**	**L-R+**	**L+R-**	**L-R-**	**L+R+**	**L-R+**	**L+R-**	**L-R-**
Net shoot photosynthesis (mg O_2 g^{-1} DW d^{-1})	58.3	10.3	64.1	3.1	30.4	23.3	44.1	8.9
Predicted shoot RGR (g g^{-1} DW d^{-1})	0.053	0.010	0.059	0.003	0.027	0.021	0.041	0.006
Observed shoot RGR (g g^{-1} DW d^{-1})	0.031	0.021	0.034	0.022	0.028	0.024	0.030	0.025
Aboveground C gain (g C $clone^{-1}$ d^{-1})	0.45	0.10	0.33	0.03	0.43	0.23	0.46	0.11
Belowground C demand (g C $clone^{-1}$ d^{-1})	0.05	0.05	0.05	0.07	0.27	0.18	0.35	0.46
Carbon balance (above – belowground)	0.40	0.05	0.28	-0.04	0.16	0.05	0.10	-0.35
Observed shoot production during the last 3 weeks (mg C $clone^{-1}$ d^{-1})	0.32	-0.07	0.46	-0.11	0.34	0.14	0.12	-0.03

Estimated carbon balances of the two species under different treatments are shown in Figure 5 and Table 7. The daily net oxygen produced was lower in the shaded plants because the amount of irradiance reaching the plants was also low. The resulting net carbon balances therefore in the shaded and oxidized sediments were low but still positive in both species, while it was negative in the shaded and reduced sediments. Based on the calculated carbon balances, the predicted leaf growth rates were relatively close to the observed growth rates in both species, except for the unshaded and reduced sediments where predicted leaf growth rates were much lower than observed. Similarly, the observed shoot production during the last three weeks agreed quite well with the carbon balance model for the unshaded reduced sediment having negative values indicating shoot mortality in both species.

Discussion

The experimental shading in the present study invoked a number of responses in both species, both at the level of photosynthetic capacity and that of growth and morphology. *T. hemprichii* was more pronounced in its response to shading, with clearly significant effects leading to an increase in leaf length and leaf lifespan but a decrease in shoot production, leaf growth, rhizome internode production, as well as

branching rate, all suggesting substantial phenotypic plasticity. These responses are generally similar to those observed elsewhere (Duarte et al., 1997; Lee and Dunton, 1997; Vermaat et al., 1997, Bach et al., 1998; Terrados et al., 1998; Uy et al., in prep., Chapter 2). In contrast, very limited responses to shading were observed in *Halodule uninervis*. The increase in leaf longevity probably was a result of the decrease in net carbon availability in the shaded plants. As a consequence of the reduction in the total available carbon, shoot as well as leaf production were greatly reduced leading to longer leaf lifespan and longer leaves. Chlorophyll content, on the other hand, increased with shading in both species as an adaptive strategy to capture more light (Boardman, 1977; Björkman, 1981; Chapter 2).

Shade acclimation was detected in *T. hemprichii* with a decrease in P_{max} and K_m on reduced sediments with shading. *H. uninervis*, on the other hand appeared not responsive to shading which conform in our earlier findings in the field (Chapter 2). Overall, *T. hemprichii* had higher P_{max}, K_m and light compensation point (LCP) as compared to *H. uninervis*. This latter supports the observation by Enriquez et al. (1995) that LCP values of seagrass generally increase with increasing tissue thickness. The ratio of oxygen consumption per unit dry weight by the leaves over that of below ground tissues in this study ranged from 0.5 to 1.9. These values are low as compared to those reported in *Posidonia australis* and *Posidonia sinuosa* (5.0, Masini et al., 1995); *Zostera marina* (3.0, Kraemer and Alberte, 1993), *Halodule wrightii,* (2.6, Dunton and Tomasko, 1994) and *Thalassia testudinum* (2.4, Fourqurean and Zieman, 1991).

Reduced sediments resulted in shorter roots for both species. Probably, maintaining roots under these conditions is more costly than in oxygenated sediments. A confounding influence of sediment nutrient availability seems improbable since nutrient levels were similar across all treatments. Indeed belowground respiration rates were higher in the plants growing on reduced sediments: compared to oxidized sediments, these were 1.2-1.5 times higher in *T. hemprichii* and 1.7-2.3 times higher in *H. uninervis*. Smith et al. (1988) similarly showed in *Zostera marina* that respiratory rates increased following anaerobic conditions as much as 1.6-1.8 times. However, experiments with emergent saltmarsh macrophytes, *Spartina alterniflora* under controlled redox-pH conditions (+500 to –200mV range) have shown no effects of redox potential on plant respiration or nitrogen uptake (DeLaune et al., 1984).

The combination of shading and reduced sediments also led to net shoot mortality. On unshaded reduced sediments, however, shoot recruitment was higher than in unshaded oxidized sediments, particularly in *H. uninervis*. Other response variables of clonal expansion (number of apical nodes and rhizome elongation rates) were also maximal in the unshaded reduced sediments. In *T. hemprichii*, also leaf elongation rates were maximal in this treatment leading to high leaf biomass. This surprising stimulus of aboveground production may represent a prudent reallocation to leaf material when roots and rhizomes put extra respiratory demands. Most probably, the conditions of this treatment reflect the natural habitat as sediments in seagrass beds are

reported to be generally moderately reduced with Eh values ranging from −100 to 200 mV in the top 10 cm (Terrados et al., 1999).

The root-rhizome: shoot ratio (RSR) of the plants grown on unshaded reduced sediments was similar to that in the field for *T. hemprichii* (Chapter 2). In contrast, for *H. uninervis* it was almost 2 folds lower than in the field. Different explanations are possible: a) the experimental sediment used was more coarse grained and probably more reduced that that of the field site, which may have caused complex interactions of sediments pH, free sulphides (Moriarty and Boon, 1989) and plant response; b) the apical fragments may not have fully matured yet. *In situ* roots can reach up to 40 cm particularly in taller, older shoots of *H. uninervis*.

As a result of the large net carbon gain in plants grown in full light, regardless of sediment conditions, leaf growth, shoots production and rhizome elongation were higher than other treatment. Plants grown in shaded and reduced sediments yielded a negative net carbon balance. This resulted in high shoot mortality, increased leaf longevity due to lack of necessary carbon reserves to produce new leaves, as well as the reduction in the production of new roots in the case of *T. hemprichii*. This supports the findings of Dennison and Alberte (1985) who have shown through experimental manipulation of *in situ* light that larger net carbon gains also led to relatively faster leaf formation rates in *Z. marina*.

The plants grown on the shaded and oxidized sediments were still able to maintain a net positive carbon balance thus providing opportunity for growth even at relatively low light. Observed shoot production however was already reduced which would indicate that shoot production is more costly than maintaining leaf growth in these plants as has been shown in *Zostera noltii* (Vermaat and Verhagen, 1996). This is probably because of the presence of enough internal carbon reserves that can still support the physiological requirements of the remaining shoots. Alcoverro et al. (1999) demonstrated the mobilization of stored carbon reserves in the belowground tissues for *Z. marina* during a period of extreme light limitation. They reported that the *Z. marina* responded to light reduction through reduced root production, depletion of sucrose reserves and a decrease in growth rate. Photosynthesis and respiration of *Z. marina* shoots however were not affected by prolonged carbon limitation, but severe light limitation could lead to the inability to mobilize carbon reserves thus resulting to plant death. Moreover, Zimmerman et al. (1994) estimated that the carbon reserve of *Z. marina* is sufficient to meet requirements of anaerobic metabolism for about 3 days.

This study has shown *T. hemprichii* to be more responsive to light and sediment conditions than *H. uninervis*. Similarly, experiments on redox manipulation by sucrose addition in several southeast Asian seagrass species have singled out *T. hemprichii* as the species mainly affected by oxygen-depleted sediment through a reduction in shoot density and growth rate after two weeks (Terrados et al., 1999), whilst leaf growth rates of *H. uninervis, Z. marina, Cymodocea nodosa* were not affected. *T. hemprichii* was also the most sensitive to siltation (Bach et al., 1998; Terrados et al., 1998) and to burial (Duarte et al., 1997).

We have demonstrated experimentally that the combination of low light and highly reducing sediments could be critical for the growth and development of tropical seagrasses as shown by the high mortality rate reported in this study and as predicted by the daily estimated carbon balance. Clearly, in the light of the ongoing siltation and eutrophication of Southeast Asian habitats (Vermaat et al 1997; Bach et al 1998; Terrados et al 1998), we must assume that seagrasses are declining throughout the region, although clear evidence in terms of hectares lost overtime or reduced depth penetration is lacking.

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Chapter 5

The effects of shading on clonal integration in the seagrasses *Thalassia hemprichii* and *Halodule uninervis*

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Abstract

Physiological integration of tropical seagrasses was assessed in the field using the stable isotope ^{15}N as tracer. Shading screens that reduced incident light by 81-89% were installed *in situ* in 2 separate seagrass meadows dominated by the late successional *Thalassia hemprichii* and the early successional *Halodule uninervis*, respectively. Plots were arranged in a randomized block design and the experimental shading period was 3 months, from January to April 1999. Shoot density was greatly reduced after 3 months of shading: the reduction was 82% for *T. hemprichii* and 70% for *H. uninervis*. Relative growth rates for leaves of both species remained relatively similar but plastochron intervals were slightly higher in the shaded plants resulting in longer leaf lifespan. Nitrogen content was significantly higher in the shaded plants for both species while % organic carbon content was reduced leading to a decrease in C:N ratio with shading. Under shade, the $^{15}NH_4$ uptake of incubated shoots was lower in *H. uninervis* but it was not influenced in *T. hemprichii.* Translocated ^{15}N along the horizontal rhizomes was significantly reduced from 38% to 6% in *H. uninervis* under shading, whilst a slight decrease was observed in *T. hemprichii.* ^{15}N was shared among unpublished). Moreover Stapel et al. (1997) reported an average enrichment of 2% after p*ulse laervis* in full light. The amount of ^{15}N generally decreased rapidly away from the incubated shoots but values increased at the apical shoot suggesting accumulation for growth expansion. This increase in the ^{15}N concentration in the apical shoot, however, was not observed in the shaded plants of both species. The overall effect of shading caused accumulation of nitrogen within the existing tissues instead of the growing rhizome apices, all as a part of a general reduction in growth. Although at a relatively low intensity, physiological integration of the clones was maintained despite severe shading stress that caused shoot mortality and longer leaf turnover as well as decreased nitrogen assimilation.

Keywords: growth, nitrogen, stable isotope, tropical seagrass

Introduction

Seagrasses are fully submerged marine clonal plants that form extensive meadows by vegetative production of new shoots along creeping rhizomes that extend to several meters in total length. Patterns in rhizome branching in seagrasses are discussed in Tomlinson (1974) and Marbà and Duarte (1998) in relation to meadow maintenance and clonal propagation. Several authors (Harper, 1977; Hartnett and Bazzaz, 1983; De Kroon and Van Groenendael, 1990) have used the term clonal growth emphasizing the multiplication of modular units within a single, morphologically and physiologically integrated genetic individual and herewith implying the occurrence of resource translocation between interconnected ramets. Studies on clonal integration have been done quite extensively in terrestrial plants (Tietema and Vroman, 1978; Tietema, 1980; Hartnett and Bazzaz, 1983; Noble and Marshall, 1983; D'Hertefeldt and Jónsdóttir, 1999; see also reviews of De Kroon and Van Groenendael, 1990 and Callaghan et al., 1992) using either ^{14}C, ^{33}P, acid fuchsin dye or differences in morphological architecture. Such studies on seagrass are relatively few with the earlier works done on *Zostera americana* (Harrison, 1978) and *Posidonia oceanica* (Libes and Boudouresque, 1987) using ^{14}C, and recently on *Thalassia testudinum* (Tomasko and Dawes, 1989; 1990) and *Cymodocea nodosa* (Terrados et al., 1997a; 1997b) through experimental manipulation of growth parameters.

The use of the stable isotope ^{15}N in tracing translocation of materials to quantify degree of physiological integration between interconnected ramets is relatively new. Recently ^{15}N was used as tracer in measuring nutrient uptake and nitrogen cycling in *T. hemprichii* (Stapel et al., 1996, Hemminga et al., 1999), in tracing transport along interconnected ramets in several seagrass species (Marba N., unpublished data), and also in determining nutrient sharing in a clonal herb *Fragaria chiloensis* (Alpert, 1996). Growth effects of shading have been studied quite extensively (Tomasko and Dawes, 1990, Czerny and Dunton, 1995; Grice et al., 1996, Vermaat et al., 1997, Hemminga, 1998) and its long-term effect on the same species is subject of another paper by the same authors (Chapters 2 & 3).

This study was designed to determine the effects of shading on the degree of clonal integration in two seagrass species of contrasting lifespan and successional status. We used the early successional and short-lived *Halodule uninervis* (Forsskal) Ascherson and the longer-lived and late successional species *Thalassia hemprichii* (Ehrenberg) Ascherson (Duarte 1991; Vermaat et al., 1995). Here we aim to: 1) quantify translocation of materials along interconnected ramets with stable isotope ^{15}N as tracer; and 2) compare the growth, biomass and nutrient allocation of interconnected ramets under shaded and unshaded conditions.

Materials and Methods

The experiments were conducted in two seagrass beds dominated by either of the two species, *Halodule uninervis* at Naawan and *Thalassia hemprichii* at Sulawan both in Misamis Oriental, Southern Philippines (cf. Chapter 2). Two shading treatments were employed as follows: shaded (81-89% reduction of incident light) and control i.e. without shading. Benthic screens measuring 1.6 x 1.6m were installed for shading, while the controls had the frames minus the screens. A total of 8 plots constituting 2 treatments with 4 replicates each were arranged for each species in a randomized block design within the seagrass bed. Height of the benthic screens was approximately 10cm above the tallest shoot inside the canopy.

The shading experiment ran for 3 months, within the period March to May 1999, in the same seagrass beds as those used in the long-term shading experiments (Chapters 2 & 3). Towards the end of the shading period, shoot density was measured as described in Chapter 2, after which labelling with ^{15}N-NH_4Cl (99% ^{15}N, Compro Scientific) was done. In each plot, three shoots of more or less the same age were selected for labelling with ^{15}N. The selected shoots were at least 50cm from the outside perimeter of the screens and distance between them was maximized, to minimize the chance of interconnection in the belowground network of rhizomes. Incubation was done by enclosing the whole shoot in a transparent polyethylene bag (7.5 cm wide and 35 cm height, ± 1.8 L) fitted at the top end with a one-way stop valve. The polyethylene bag was secured with coloured plastic tie wire at the stem of the shoot exposing only the leaves for ^{15}N labelling. A soft foam material (earplug) coated with a small amount of silicone grease was wrapped around the stem, before tying, to prevent damage to the plant. A stick was then provided to support the whole plant and polyethylene bag. The polyethylene bags moved with the waves allowing mixing inside the chambers.

The incubation period lasted 3 hours after introducing ^{15}N through the one-way injection port. The final concentration of 40 µM $^{15}NH_4Cl$ in the chamber follows that of Stapel (1997) and N. Márba et al. (unpublished), which is almost twice the half saturation constant for ammonium uptake by *T. hemprichii* (Stapel et al., 1996). Incubation was done from 1000 to 1400 hrs in all the plants. In the event of cloudy skies or an unusually low irradiance for a particular day, incubation was postponed to the next successive days until an appropriate irradiance (bottom irradiance >600 µmol PAR m^{-2} s^{-1}) was achieved in the unshaded plots. To avoid contamination of the neighbouring plants after incubation, the labelled water inside the plastic chamber was sucked out carefully to a receiving plastic bag with a hand pump. Before removing the plastic chamber, the incubated shoots were tagged at the vertical rhizomes with a coloured tie wire. A stick with coloured tip was also inserted beside the labelled shoot. After the incubation, 30 randomly chosen shoots in all the experimental plots were punched at the leaf-sheath junction using a syringe needle. They were all collected after 5 days and brought to the laboratory for leaf productivity measurements (after Duarte et al., 1994).

The ^{15}N labelled clones were harvested after 5 days. Harvesting was done carefully and slowly to get the whole clonal plant as complete and intact as possible. We proceeded by fanning the sediments to expose the rhizomes and roots that were carefully traced and removed from the sediments and entangled from other clones by hand. A map of each clone relative to the quadrat was drawn as a guide. In the shaded plots, the clones were cut where rhizomes went beyond the perimeter quadrat (1.6 x 1.6 m). A number of isolated shoots not part of the ^{15}N labelled clones were also collected within the perimeter to determine the natural background levels of ^{15}N and served as the blank sample. Harvested complete clones were brought to a makeshift lab at the site for mapping and measurements of the following: total length of the clone, distances between shoots, and number of internode scales. Immediately after the measurement, whole ramets (shoots + rhizome + roots) were separated to avoid possible further translocation of ^{15}N. Horizontal rhizomes were cut in between the shoots, coded properly and placed individually in aluminum foils or plastic bags. At the MSU-Naawan laboratory, rinsing and cleaning of individual ramets was finished and the following measurements were made: number of leaves, length and width of each leaf per shoot, sheath length, vertical rhizome length and thickness, and number of leaf scars. After the measurements, epiphytes were gently scraped from the leaves with the edge of a glass slide. Then tissues were washed with freshwater only since acid-washing to remove calcareous epiphytes will significantly reduced $^{15}N/^{14}N$ ratios (Bunn et al., 1995). Cleaned samples were dried at 60°C until constant weight. Each ramet therefore had information on age, biomass, position and relative distance from the incubated shoot. Dead rhizomes and shoots were removed and weighed and measured for length and width as well.

The marked shoots were sorted and measured separately. Leaf growth rates were determined by separating the new leaves from the old leaves based on the leaf hole markings. First, the leaves were cut at the leaf-sheath junction and then the leaves were cut again at the leaf hole marks and were separated into new (below the marks) and old materials (above the mark). Length and width was measured and all leaves from the same plot were pooled for dry weight measurements. Relative growth rates were calculated as the natural log of the ratio between total leaves and old leaves (either biomass or leaf area) divided by the incubation period (normally 5 days in this study) multiplied by 100 to be expressed as % increase per day (Hunt, 1982). Leaf growth rates were calculated as the amount of new biomass or leaf area over the number of observation period. The Plastochrone Interval (PI = days) was estimated for each plot by multiplying the total number of shoots measured by the observation period in days and dividing this product by the number of new leaves without hole markings formed (Duarte et al., 1994)

To determine translocation of materials along the interconnected ramets in every complete clone, healthy ramets were selected systematically at every 10-20 cm from the incubated shoot in both directions. Selected oven-dried ramets including the shoot with its rhizomes and roots were ground into powder using mortar and pestle and then

placed in small glass vials for later laboratory analyses at NIOO-CEMO. Total nitrogen and organic carbon content were analyzed using a Carlo Erba NA 1500 CN analyser. The $^{15}N/^{14}N$ ratio was measured with a Finnigan Mat delta S isotopic ratio mass spectrophotometer coupled to a Fisons NA 1500 CN analyser via a co-flow interface. The stable isotope ratios of $^{15}N/^{14}N$ were expressed as the relative per mil (‰) difference between the sample and the standard (nitrogen in air).

$$\delta^{15}N = [R_{sample} / R_{standard} - 1] \times 1000 = ‰$$

Where $R = {}^{15}N/{}^{14}N$. Enrichment with ^{15}N was calculated by subtracting background levels of ^{15}N measured in blank or unlabelled ramets. Then values were normalized to whole ramet dry weight.

Results were analyzed using *t*-tests for comparing control and shaded plots for each species. 2-way ANOVA was used to compare effects of species, shade treatments and their interactions. Tests were considered significant at α=0.05. Linear regression was done to test for the relationship between age, biomass and ramet position from the incubated shoot against tissue nutrient contents. Fitted linear models were subsequently compared for differences between shading treatments using the following *F*-statistic (Vermaat and Hootsmans, 1994):

$$F = \frac{(RSS_{1+2} - (RSS_1 + RSS_2)) / (df_{1+2} - (df_1 + df_2))}{(RSS_1 + RSS_2) / (df_1 + df_2)}$$

Where: RSS_1 = residual sum of squares for dataset 1, RSS_2 = residual sum of squares for dataset 2, RSS_{1+2} = residual sum of squares for the combined datasets, df_1, df_2, df_{1+2} = the corresponding degrees of freedom.

Results

At the end of three months shading period, shoot density was reduced to 82% in *T. hemprichii* and 70% in *H. uninervis* and. The shading effect explained 39% of the variation (2-way ANOVA, p<0.001, Tables 1 and 2). The species effect was also significant (explaining 49%, p<0.001) with *H. uninervis* having 5 times more shoots per unit area than *T. hemprichii* on the average. The number of dead shoots in the harvested intact clones for the unshaded plants were 36% and 46% for *H. uninervis* and *T. hemprichii*, respectively. However, shoot mortality was even higher in the shaded plants for both species (2-way ANOVA, p<0.01, Tables 1 and 2). In the shaded plots, some of the rhizomes were also decaying, resulting in breakage and thus incomplete plants. The longest intact clones harvested for *T. hemprichii* were 0.9 m and 0.6 m for the control and shaded plots, respectively. Whilst longer intact clones were harvested for *H. uninervis* measuring 1.4-1.7 m for the shaded and control plots, respectively.

Table 1. Results of 2-way ANOVAs examining the effects of shading and species (*T. hemprichii* and *H. uninervis*) on shoot density, shoot mortality, leaves, rhizome and root biomass, relative growth rates (RGR), leaf elongation rates and plastochron interval . Degrees of freedom are similar for values in the same row unless indicated otherwise. Presented are the degrees of freedom (df), the % of total variation explained (factor SS/ total SS) and the level of significance (p). Bold numbers with $p<0.05$ are considered significant. Means and standard errors are presented in Table 2.

	Shoot density			**Shoot mortality**		
Factors	df	%	p	df	%	p
Species	1	49	**<0.001**	1	5	0.138
Shading	1	39	**<0.001**	1	22	**0.004**
Species x Shading	1	<1	0.513	1	<1	0.885
Within + Residual	15	12		32	73	
Total	18	100		35	100	

	Leaf biomass			**Rhizome biomass**		**Root biomass**	
Factors	df	%	p	%	p	%	p
Species	1	41	**<0.001**	59	**<0.001**	32	**<0.001**
Shading	1	2	**0.011**	0	0.547	2	0.050
Species x Shading	1	1	0.054	1	0.170	2	**0.016**
Within + Residual	152	56		40		64	
Total	155	100		100		100	

	Leaf relative growth rate			**Leaf elongation**		**Leaf plastochron interval**		
Factors	df	%	p	%	p	df	%	p
Species	1	53	**<0.001**	91	**<0.001**	1	5	0.378
Shading	1	<1	0.700	1	0.345	1	26	0.056
Species x Shading	1	12	0.083	1	0.031	1	<1	0.902
Within + Residual	9	32		7		13	69	
Total	12	100		100		16	100	

Leaf relative growth rates expressed both as leaf area and biomass were significantly different between species (species effect explaining 52-55% of the variation, 2-way ANOVA, $p<0.005$, Tables 1and 2). However, the shading effect was not significant, and neither was the interaction between species and shading, indicating no substantial influence of shading on leaf growth rates in both species. The plastochron interval was longer (1.6-1.7x) in the shaded plants (Tables 1 and 2, almost significant at $p=0.056$).

Table 2. Average values ± standard errors of the different parameters measured in the control and shaded plots of *T. hemprichii* and *H. uninervis* after 3 months of shading. Results of *t*-test for each species are shown as *p* values; bold numbers indicate significant differences at $p<0.05$.

	Thalassia hemprichii			***Halodule uninervis***		
	Control	Shaded	*p*	Control	Shaded	*p*
Shoot density (shoot m^{-2})	571 ± 86	102 ± 19	**0.003**	2652 ± 127	799 ± 267	**0.002**
% shoot mortality*	45.7 ± 5.6	63.4 ± 8.1	0.091	35.7 ± 5.5	55.1 ± 4.2	**0.011**
Biomass parameters						
Leaves (mg $ramet^{-1}$)	151 ± 12	106 ± 18	**0.037**	25 ± 2	18 ± 2	**0.007**
Stem (mg $ramet^{-1}$)	73 ± 12	100 ± 11	0.158	n.d**.	n.d.	
Rhizomes (mg $ramet^{-1}$)	158 ± 10	156 ± 12	0.870	64 ± 4	51 ± 5	**0.036**
Roots (mg $ramet^{-1}$)	49 ± 6	75 ± 10	**0.028**	15 ± 2	11 ± 2	0.121
Total (mg $ramet^{-1}$)	433 ± 28	437± 34	0.970	104 ± 6	80 ± 6	**0.011**
% Leaves or shoot	34 ± 2	22 ± 3	**<0.001**	23 ± 1	22 ± 2	0.663
% Stem	15 ± 2	23 ± 2	**0.002**	n.d.	n.d.	
% Rhizomes	40 ± 2	38 ± 2	0.725	60 ± 2	62 ± 1	0.339
% Roots	11 ± 1	17 ± 2	**0.002**	17 ± 2	16 ± 2	0.611
Root-Rhizome : Shoot ratio***	2.3 ± 0.2	5.6 ± 0.9	**<0.001**	3.4 ± 0.2	4.4 ± 0.4	**0.050**
Leaf relative growth rates (% d^{-1})	3.6 ± 0.3	3.3 ± 0.3	0.539	4.6 ± 0.4	5.7 ± 0.5	0.180
Leaf growth rates (mg DW $shoot^{-1}$ d^{-1})	6.8 ± 0.4	5.9 ± 1.1	0.637	0.4 ± 0.1	0.4 ± 0.2	0.872
Plastochron Interval (days)	9.1 ± 1.6	15.5 ± 2.3	0.066	11.6 ± 1.6	18.9 ± 5.6	0.207

* mortality was calculated as percent of dead shoots over live shoots
** n.d. = no data
*** calculated as (stem + roots + rhizomes) / leaves

For the biomass categories leaves, rhizomes (vertical + horizontal rhizomes) and roots, a significant difference between species existed (2-way ANOVA, $p<0.001$, Tables 1 and 2) with the difference for rhizomes being largest (59% of the variance) followed by leaves (41%) and roots (32%). A shading effect was only significant for the leaves (2.4%, $p<0.05$) and barely so for the roots (1.7%, $p=0.05$). As a consequence of the reduction in leaf biomass, the root+rhizome: shoot ratio (RSR) increased in both species (Table 2).

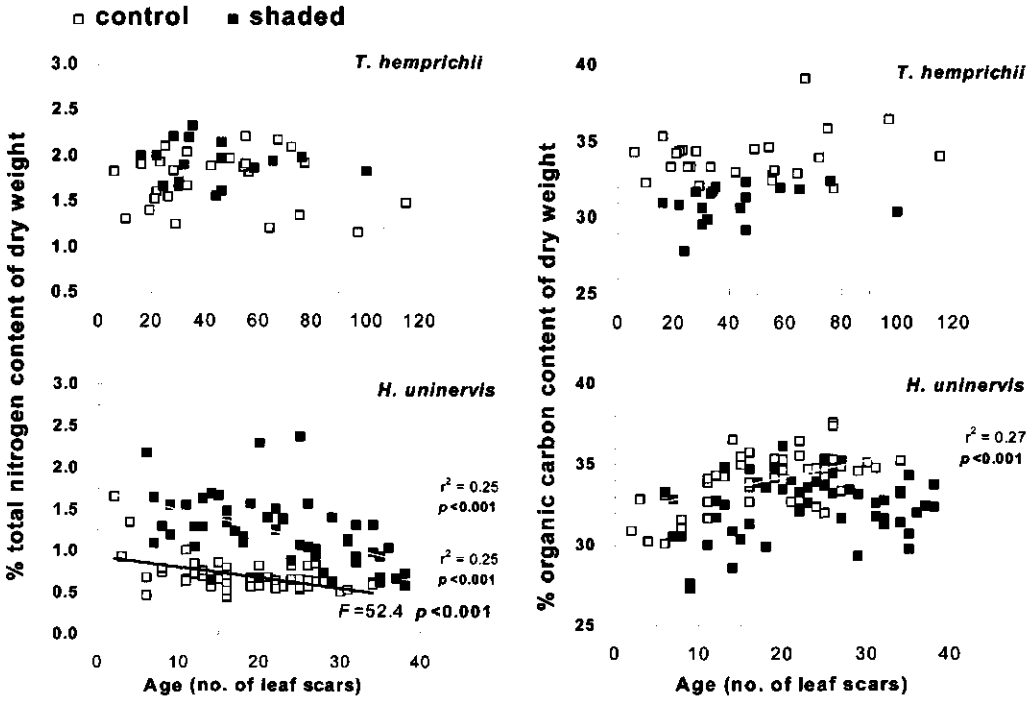

Fig. 1. Relationship of the % total nitrogen and % organic carbon contents of dry weight in the control and shaded plots of *T. hemprichii* and *H. uninervis* against age of the ramets in number of leaf scars which is also equivalent to plastochron interval. Fitted lines of the regression are shown when significant ($p<0.05$): gray line is for the control and the dark line is for the shaded plots. Only the fitted equations of the control and shaded plots of total N content for H. uninervis were significantly different ($F=52.4$, $p<0.001$).

The total nitrogen content of the whole ramet differed significantly between species accounting for 52% of the variation ($p<0.001$) with *T. hemprichii* having at least a 2 fold higher nitrogen content than *H. uninervis* (Tables 3 and 4, Fig. 1). The shading effect was significant as well, explaining 10% of the variation, as was the interaction between species and shading (explaining 1%, $p<0.001$, Tables 3 and 4). The organic carbon content however was similar in both species but the shading effect was significant (explaining 8% of the variation, $p<0.001$). The nitrogen and carbon content were regressed against shoot position or distance from the incubated ramet and results were not significant for both species indicating that the addition of $^{15}NH_4Cl$ did not influence total N content. In *H. uninervis*, total N content declined with increasing age (Fig. 1) whereas carbon content increased. In *T. hemprichii*, no significant correlation with age was observed for nitrogen either for organic carbon content.

Table 3. Results of 2-way ANOVA examining the effects of shading and species (*T. hemprichii* and *H. uninervis*) on $\delta^{15}N$ contents of the incubated and unlabelled ramets, direction of transport e.g. acropetal (towards the growing meristem) and basipetal (opposite direction, towards older ramets), % nitrogen and organic carbon content of dry weights. Degrees of freedom are similar for values in the same row unless indicated. Presented are the degrees of freedom (*df*), the % of total variation explained (factor SS/ total SS) and the level of significance (*p*). Bold numbers with $p < 0.05$ are considered significant. Means and standard errors are presented in Table 4.

	$\delta^{15}N$ of incubated ramets			Uptake rate		$\delta^{15}N$ of unlabelled ramets (background)		
Factors	*df*	%	*p*	%	*p*	*df*	%	*p*
Species	1	86	**<0.001**	82	**<0.001**	1	13	**0.039**
Shading	1	6	**0.003**	5	**0.042**	1	2	0.455
Species x Shading	1	1	0.104	1	0.650	1	7	0.119
Within + Residual	14	7		12		26	78	
Total	18	100		100		31	100	

	% ^{15}N remaining in incubated ramets			% acropetal transport		% basipetal transport	
Factors	*df*	%	*p*	%	*p*	%	*p*
Species	1	1	0.663	7	0.214	19	0.057
Shading	1	11	0.111	27	**0.020**	2	0.477
Species x Shading	1	34	**0.011**	20	**0.039**	17	0.074
Within + Residual	14	54		46		62	
Total	18	100		100		100	

	% organic carbon			% total nitrogen		C:N ratio	
Factors	*df*	%	*p*	%	*p*	%	*p*
Species	1	<1	1.000	52	**<0.001**	40	**<0.001**
Shading	1	8	**<0.001**	10	**<0.001**	16	**<0.001**
Species x Shading	1	2	0.056	1	**0.027**	7	**<0.001**
Within + Residual	175	90		37		37	
Total	178	100		100		100	

The $\delta^{15}N$ in the incubated ramets for the two species was significantly different (species effect explaining 86% of the variation; 2-way ANOVA, $p<0.001$, Tables 3 and 4, Fig.2), whilst the shading effect was also significant ($p<0.01$, explaining 6% of the variation). For the naturally occurring $\delta^{15}N$ or background levels, the species effect was significant as well ($p<0.05$, explaining 13% of the variation) but the shading effect was not ($p>0.05$). *T. hemprichii* had slightly higher background levels of $\delta^{15}N$ than *H. uninervis*. However, the reverse was true for the incubated ramets with *H. uninervis* having 4.8 and 6.2 times higher $\delta^{15}N$ values than *T. hemprichii* for the control and

Table 4. Average values ± standard errors of ^{15}N allocation measured in the control and shaded plots of *T. hemprichii* and *H. uninervis*. Results of *t*-tests comparing treatments within each species are shown as *p* values; bold numbers indicate significant differences at $p<0.05$.

	Thalassia hemprichii			*Halodule uninervis*		
	Control	Shaded	*p*	Control	Shaded	*p*
$\delta^{15}N$ of incubated ramets (‰)	2176 ± 211	1219 ± 178	**0.047**	10490 ± 590	7644 ± 279	**0.003**
$\delta^{15}N$ of unlabelled ramets (‰)	6.3 ± 0.4	7.0 ± 0.6	0.322	5.9 ± 1.4	4.0 ± 0.9	0.265
% ^{15}N of Total N in incubated ramets	1.2 ± 0.1	0.8 ± 0.1	**0.047**	4.1 ± 0.2	3.1 ± 0.1	**0.003**
% ^{15}N of total N in unlabelled ramets	0.369 ± 0.0	0.369 ± 0.0	0.305	0.369 ± 0.0	0.368 ± 0.0	0.172
Total ^{15}N retrieved (μg)	104 ± 33	46 ± 21	0.266	46 ± 5	31 ± 3	0.062
% ^{15}N retrieved	9.7 ± 3.1	4.3 ± 1.9	0.267	4.3 ± 0.5	2.9 ± 0.3	0.062
Leaves uptake rate (μmol g^{-1} DW h^{-1})	12.7 ± 2	7.7 ± 1	0.251	40.1 ± 3	32.5 ± 2	0.071
% ^{15}N remaining in the incubated ramet	77.5 ± 3	83.8 ± 12	0.562	62 ± 7	94 ± 1	**0.009**
% ^{15}N exported	22.5 ± 3	16.2 ± 12	0.562	37.4 ± 7	6.0 ± 1	**0.009**
% acropetal transport	11.7 ± 4	13.2 ± 12	0.892	35.9 ± 7.1	2.8 ± 0.2	**0.006**
% basipetal transport	10.7 ± 1	3.9 ± 3	0.065	1.5 ± 0.5	3.3 ± 0.9	0.107
% carbon of DW	35.0 ± 1.1	31.5 ± 0.3	**0.013**	33.9 ± 0.2	32.6 ± 0.4	**<0.001**
% nitrogen of DW	1.8 ± 0.1	2.7 ± 0.1	**0.010**	0.7 ± 0.0	1.3 ± 0.4	**0.006**
C:N ratio	20.3 ± 0.6	15.5 ± 0.4	**<0.001**	50.7 ± 1.5	28.5 ± 1.3	**<0.001**

shaded plots respectively (Tables 3 and 4, Fig. 2). The distribution of ^{15}N along interconnected ramets in few of the samples successfully harvested is shown in Fig. 3. For both species, the enrichment generally decreased with increasing distance from the incubated shoots but the well-lit control clones generally ended with an increased ^{15}N concentration (2-8 folds) at the apical ramets. In the shaded plants in contrast, the ^{15}N enrichment continuously decreased without any apical increase.

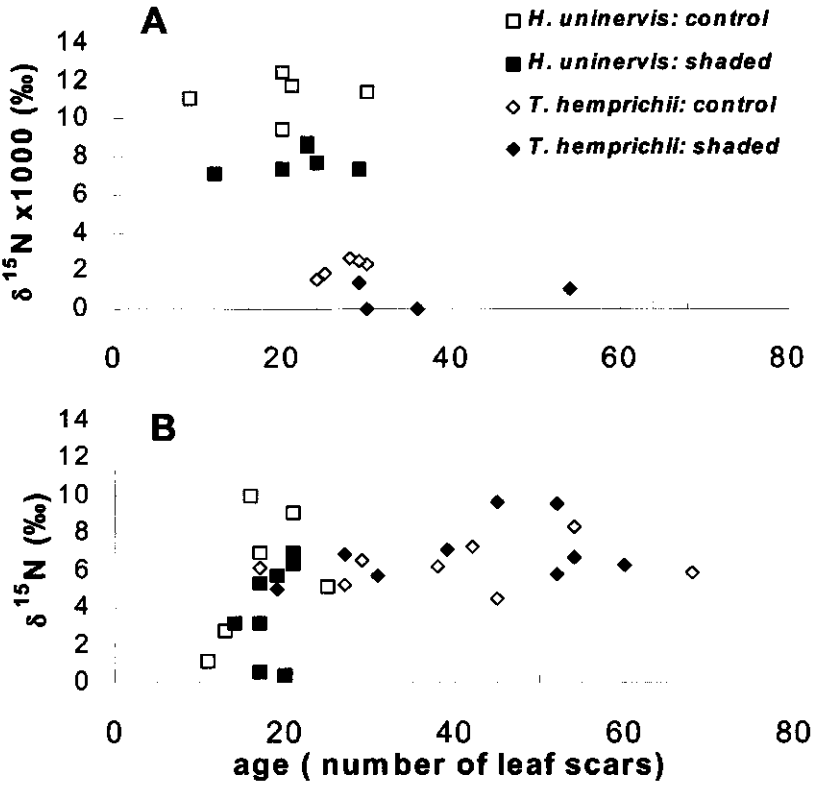

Fig. 2. $\delta^{15}N$ values of (a) incubated and (b) background, naturally occurring ^{15}N in the control and shaded plots of *T. hemprichii* and *H. uninervis* relative to shoot age expressed in number of leaf scars which is equivalent to leaf plastochron. Note: one outlier incubated shoot of *H. uninervis* was removed

Uptake rates of the clones were estimated by assuming that the total ^{15}N retrieved after 5 days in the whole clone represent the total ^{15}N uptake of the incubated single shoot. Parts of the clone, however, were not harvested because we only harvested within the experimental shaded areas and probably have missed parts of the extensive rhizome network. Thus uptake rate of incubated shoot were calculated as: Total available mg ^{15}N retrieved from a clone / dry weight of the incubated shoot / molecular weight of ^{15}N / 3 hours incubation x 1000 = µmol ^{15}N g^{-1} dry wt h^{-1}. The ^{15}N uptake rates were significantly different between species (Tables 3 and 4) with *H. uninervis* assimilating more than threefold higher than *T. hemprichii*. The shading effect was significant as well ($p<0.05$, but explained less) with shaded *H. uninervis* assimilating slightly less than the control. No significant difference was seen on *T. hemprichii* between shading levels (*t*-test, $p>0.05$, Table 4).

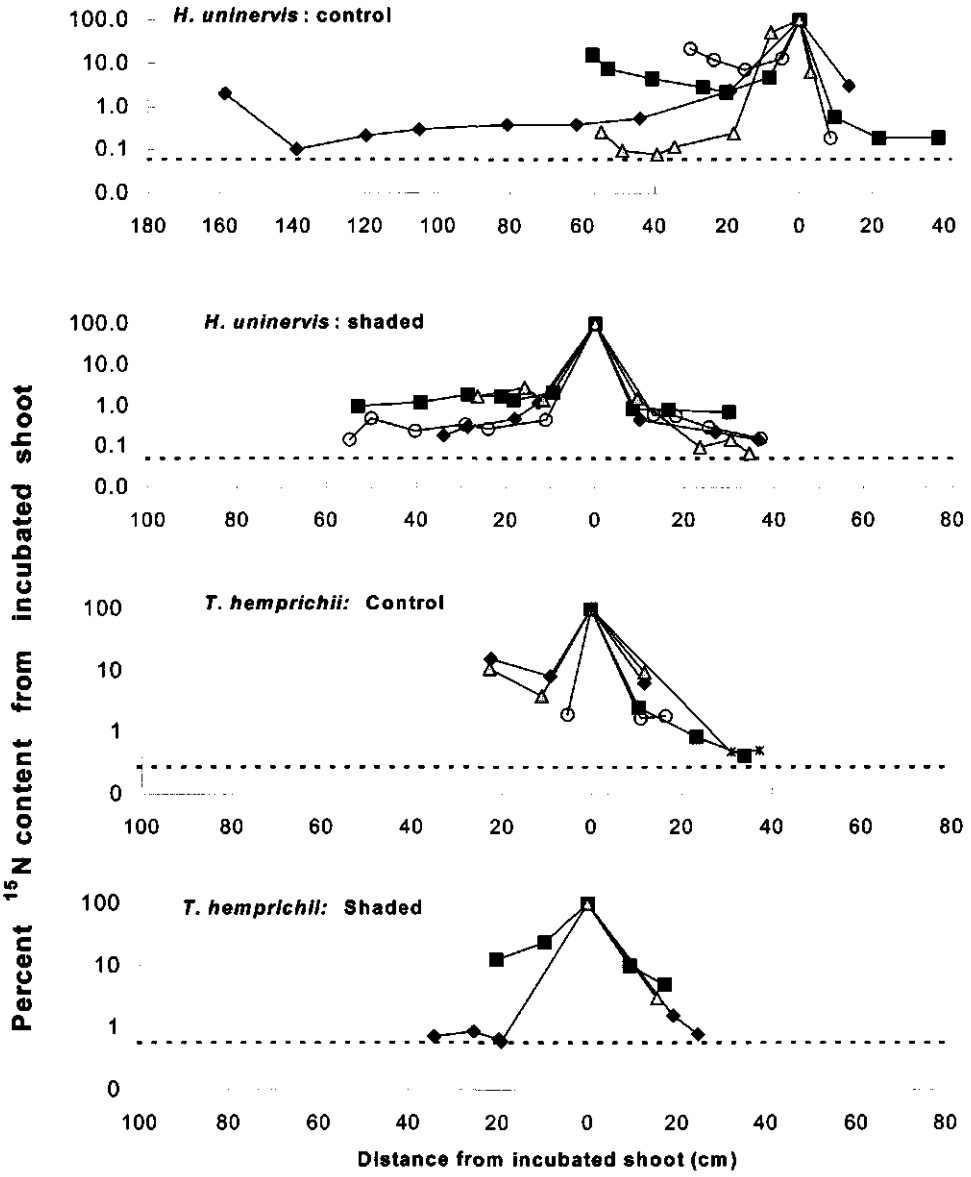

Fig. 3. Distribution of ^{15}N among ramets in control and shaded relative to the incubated shoot at 0 distance for *H. uninervis* and *T. hemprichii*. Values are expressed as percentage of value of the incubated shoot. The straight broken line represents the values of unlabelled ramets or background levels. Distances are in centimeters with younger ramets to the left. For clarity, only 4 out of 7 *H. uninervis* clones are shown.

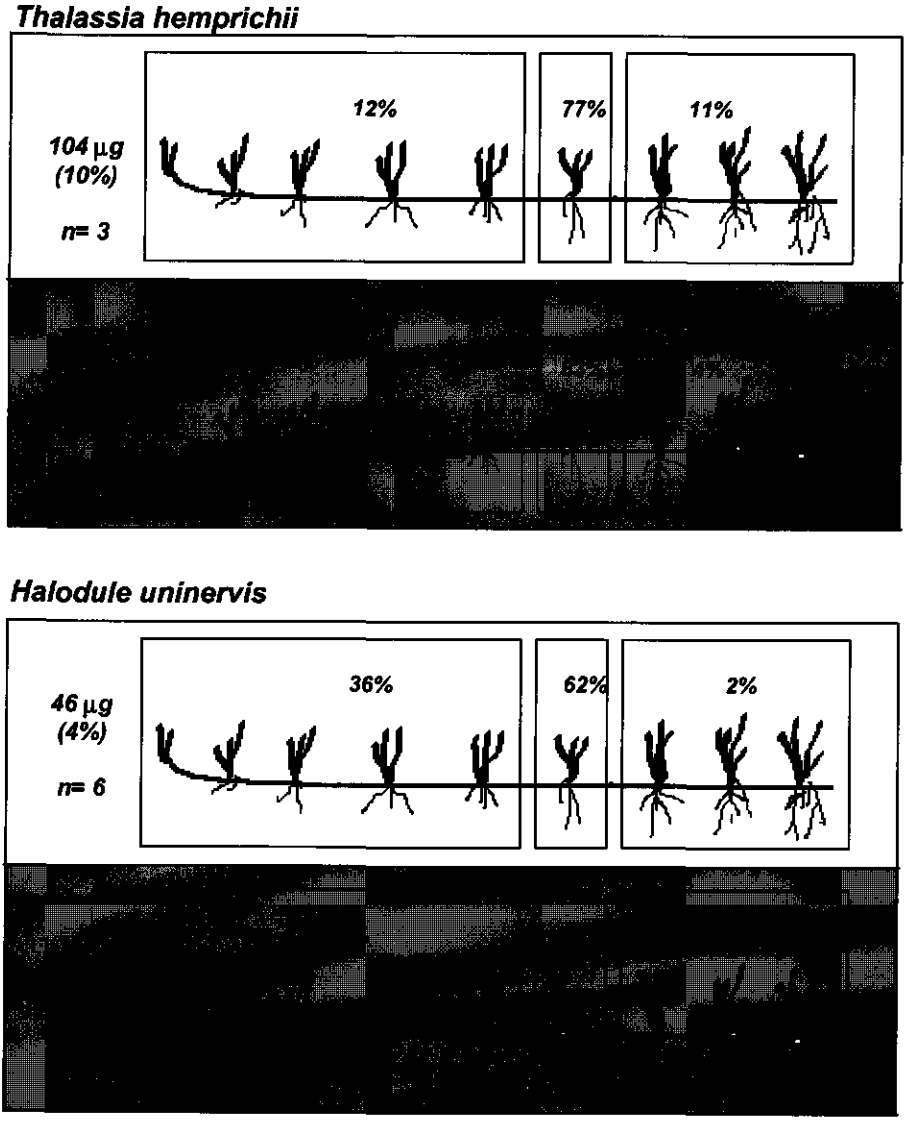

Fig. 4. Diagrammatic presentation of the effects of shading on ^{15}N allocation in clonal fragments of *H. uninervis* and *T. hemprichii* in the control (clear blocks) and shaded (grey blocks) plots. Presented are the total amount of ^{15}N (µg) retrieved and the equivalent percentage of the total amount introduced in parenthesis. The number of clonal fragments (n) for each treatment is also given. The equivalent percent amount of ^{15}N translocated in the three compartments are presented relative to the incubated ramet at the center, with the youngest meristems to the left.

Translocation of ^{15}N from the incubated shoots had occurred in both directions. For *H. uninervis* under full light, acropetal (towards the growing meristems) transport was significantly higher (12 times) than in the opposite direction (Table 4, Fig. 4). The amount of ^{15}N remaining in the incubated ramets was not significantly different between species or shading levels (Tables 3 and 4), but the interaction between species and shading level was significant ($p<0.05$) explaining 34% of the variation. The total amount of ^{15}N retrieved, after 5 days, was less than 10% in the control plots of *T. hemprichii* but was reduced to 4% in the shade. For *H. uninervis*, recovery was generally less than 4% of the amount given during the incubation (Fig. 4). *H. uninervis* exported an average of 37% of the assimilated ^{15}N to the ramets sharing the same horizontal rhizomes, whilst in the shade, this exported ^{15}N was reduced to 8%. For *T. hemprichii*, the amount of ^{15}N exported from the incubated ramets was 15% and 23% for the control and shaded plots, respectively and these proportions were not significantly different (*t*-test, $p>0.05$).

Discussion

The three months of experimental shading was enough to reduce shoot density to less than 30% of the control in both species. Shoot reduction occurred considerably faster than in a previous experiment: 5-6 months (Chapter 2). This is probably because the present experiment started during the first quarter of the year when low tide occurred during the day. Although the plots were situated in relatively deeper water, some of the plots were still partially exposed. Daytime low tide exposure reportedly causes substantial shoot loss (61-90%, Stapel et al., 1997; Erftemeijer and Herman, 1994) and may have enhanced the shade stress.

The increase in root+rhizomes:shoot ratio (RSR) with shading was not expected. Possession of large belowground biomass of plants under shading stress increases the respiratory demand, which could be a considerable burden to the carbon balance of the plant and may limit its potential for biomass production (Hemminga, 1998). Previous results showed a reduction in RSR in *T. hemprichii* after one year shading, while *H. uninervis* showed no response (Chapter 2). The probable reason for the presently increased RSR, was the significant loss in aboveground biomass (shoots) while the belowground tissues were still present (not yet decayed). We speculate that the rhizome respiration may still be sustained because of available reserves, while the shoot production is temporarily reduced during the 3 months of shading. Thus if conditions return to normal, recovery may still be possible from surviving belowground rhizomes.

Growth rates of both species were not significantly affected by shading after 3 months although a substantial amount of biomass was already lost. The apparent absence of a response in leaf growth rate to shading is similar to our previous findings under field conditions (Chapter 2) and also to those reported under field conditions elsewhere (Gordon et al., 1994; Bach et al., 1998; Rollon, 1998). However, in a separate experiment conducted under laboratory conditions, growth rate in *T.*

hemprichii was significantly reduced with shading while *H. uninervis* still maintained a constant RGR (Chapter 4). A possible reason could be the presence of a strong physiological integration of the *in situ* shaded plants with parts of their rhizomes located outside of the shade perimeter measuring 1.6 x 1.6 m. In the laboratory experiments, whole clones were contained in shaded glass aquaria without any possibility of getting support from unshaded ramets. We have some evidence for the presence of support from ramets located outside of the shade perimeter for *H. uninervis*. For two of the shaded clones, we found that part of it was actually beyond the shades. All ramets inside the shade had tissue N content very similar to those of unshaded plants (cf. Table 4). We excluded these clones from our analyses, but the striking difference in N content suggests strong physiological integration.

Apparently, there is an accumulation of nitrogen in tissues of the shaded plants for both species. Plants growing in full sunlight have lower nitrogen content because they probably require more nitrogen for growth, suggesting that these plants may become nutrient limited. Although relative growth rates was not affected by shading but the production of leaves or the plastochron interval tend to be longer as compared to the control plants. Moreover, shaded plants tend to have higher chlorophyll content (Björkman, 1981; Chapter 2) per unit leaf biomass. Nitrogen is an important nutrient for the construction of the photosynthetic apparatus, which as a consequence, might be expected to increase thus explaining higher nitrogen content in shaded plants (Hopkins, 1995). In a parallel experiment in both species exposed to long-term in-situ shading, leaf N content also increased with shading (Chapter 2) and the same is reported for other seagrasses (Grice et al., 1996; Moore and Wetzel, 2000).

Nitrogen (as ^{15}N) was shared among ramets but the largest net transfer occurred acropetally, particularly in *H. uninervis* under full light conditions. Although basipetal transport is also reported in terrestrial plants, particularly in more recent quantitative assessments (Alpert, 1996; D'Hertefeldt and Jonsdottir, 1999), acropetal transport is generally important in land plants (Tietema, 1980; Noble and Marshall, 1983) as well as seagrasses (Tomasko and Dawes, 1989; Terrados et al., 1997a; 1997b). We could only demonstrate this in *H. uninervis*, but not in *T. hemprichii*. We interpret this as a sign for a higher degree of clonal integration in *T. hemprichii*, but cannot rule out the influence of limited discriminative power of our test, particularly because the shaded *T. hemprichii* had a low number of samples (Fig. 4, Table 4). Terrados et al., (1997b) have shown through experimental manipulation in *Cymodocea nodosa* that the apical shoots are a strong sink for resources and that support for growth of young ramets is coming from much older ramets (at least 50 cm away) sharing the same horizontal rhizome. The acropetal bias in most clonal plants has been attributed to the water potential gradient existing in the rhizomes, since older plants have more extensive roots than the younger plants. Nutrients are thought to be transported through the xylem that is driven by the water potential gradient (Alpert, 1996; Pedersen, 1997).

The $^{15}NH_4$ uptake rates calculated for the two species are within the range of maximum NH_4^+ uptake rates (V_{max}) reported for most seagrass species (5-270 µmol g^{-1} DW h^{-1}; Hemminga et al. 1993, Stapel et al., 1996; Lee and Dunton, 1999; Touchette and Burkholder, 2000). Our results for *T. hemprichii* (8-13 µmol g^{-1} DW h^{-1}) were relatively low compared to the reported maximum ammonium uptake rates for the same species (V_{max}= 32-37 µmol g^{-1} DW h^{-1} from lab measurements, Stapel et al., 1996), but uptake in our experiment may well not proceed at maximum rates.

The naturally occurring $\delta^{15}N$ values in *H. uninervis* tissues (4.0-5.9 ‰) reported in this study are higher than those reported for the same species elsewhere (1.7-3.3 ‰, Grice et al., 1996; Udy et al., 1999; Marbà et al., unpublished). These high values could indicate that the area has a high input source of ^{15}N since the Naawan site is located near a coastal town and a river. For *T. hemprichii*, our values (6.3-7‰) are slightly lower than those reported for the same species in Kenya (8.4 ±1‰, Marbà et al., unpublished). Moreover Stapel et al. (1997) reported an average enrichment of 2% after pulse labelling with ^{15}N from the natural background levels of 0.37% of total nitrogen, which is similar to our study.

Our results suggest that resource sharing in seagrass is species specific: the pioneering species, *H. uninervis* exhibited a stronger acropetal translocation of ^{15}N than the late successional species *T. hemprichii*. The young, growing apices in *H. uninervis* are the strongest sinks for nutrients as in *Cymodocea nodosa* (Terrados et al., 1997a, b) enabling a fast expansion by the growing rhizomes and a rapid colonization of bare space. Reallocation towards basipetal branches could be an adaptive strategy for *T. hemprichii* to maintain its dominance in established meadows without clear colonization fronts. This is supported by the presence of more branching in *T. hemprichii* than in *H. uninervis* observed in this study. Despite the differences in level, a long-distance transport of ^{15}N along the interconnected ramets was apparent in both the control and shaded plants. Over a period of 5 days, the ^{15}N was redistributed over similar distances and numbers of ramets in both treatments. Although we could not fully recover completely intact clones, even the longest fragment of over 1 m in length had $\delta^{15}N$ signatures that were at least 2 orders of magnitude above background levels. Thus we conclude that despite the increased shoot mortality and longer leaf turnover associated with shade stress, the clones of both species maintained physiological integrity over distances in the order of 1 meter.

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Chapter 6

Effects of experimentally reduced vitality of seagrasses on periphyton load in two contrasting habitats

Uy, W.H., Vermaat, J.E.

Abstract

In two separate experiments, the rhizomes of *Thalassia hemprichii* (Ehrenberg) Ascherson were severed to simulate reduced clonal vitality and determine effects on leaf periphyton load. The rhizomes were either cut individually, isolating a single shoot (experiment 1), or randomly by digging blades in two habitats within the seagrass meadow of Sulawan at Southern Philippines (experiment 2). The habitats were an exposed reef crest and a sheltered subtidal pool. Periphyton accrual was determined on both the seagrass leaves and artificial substrates in the two sites. Isolating an individual ramet led to a significant reduction in leaf length, width and growth rate. Cutting the rhizomes randomly reduced leaf surface area and shoot density but did not affect leaf relative growth rates of the remaining shoots. In the first experiment, periphyton cover was higher on cut shoots than on the controls in the crest area but not in the subtidal pool. In the second experiment, random cutting led to significantly higher periphyton cover in the tidal pool. Our preliminary results suggest that rhizome severing indeed may lead to reduced shoot vitality and subsequently to an enhanced periphyton cover.

keywords: epiphytes, growth dynamics, rhizome severing, *Thalassia hemprichii*

Introduction

Seagrass beds are among the most productive of ecosystem (Fortes 1989; Hemminga and Duarte, 2000). The leaves of seagrasses provide an important substratum for a variety of periphytic organisms, ranging from bacteria to algae and invertebrates (Borum, 1985; Heijs, 1985; Borowitzka et al. 1990; Sterrenburg et al., 1995). These epiphytes or periphyton may contribute up to 24% of the total aboveground biomass (Heijs, 1985) and up to 60% to the total aboveground production of seagrass beds (Morgan and Kitting, 1984). They are ecologically important because they protect seagrass from desiccation during low tide (Penhale and Smith, 1977) and excess insolation (Trocine et al., 1981). Periphyton may also reduce water movements (Borowitzka et al., 1990) and act as an effective sediment trap (Gacia et al., 1999; Vermaat et al., 2000). Although most seagrass must enter the detrital food web before it is available to higher trophic levels (Phillips and Meñez, 1988), periphyton production can be a direct source of carbon available to higher trophic levels through grazing (Cebrian et al., 1996; 1998; Alcoverro et al., 1997; Deocadez, 2000). However, high levels of periphyton may also cause a severe stress on the seagrass due to shading and impeding of nutrients and organic carbon supply (Sand-Jensen, 1977; Borum, 1985), which may lead to large scale decline (Cambridge et al., 1986).

Seagrass meadows provide a habitat for large numbers of important mollusks, other invertebrates and fish (Fortes, 1989). Collection of burrowing bivalves, using digging blades, is one of the activities remaining uncontrolled in the study area. Bivalves are collected by probing the sediments randomly with a knife tied to a long wooden stick. Once a collector hits something, the bivalve is dug up and a hole is left in the seagrass meadow. This method of bivalve collection cuts through the seagrass rhizomes and possibly reduces the growth of the seagrass, since it fragments clones, thereby reducing exchange through rhizome connections and hence affecting clonal integration (chapter 5). Reduced leaf growth may extend the period of exposure to periphyton colonization of individual leaves, thereby increasing overall periphyton load.

Here we consider the question if rhizome cutting reduces seagrass vitality: what are its effects on seagrass dynamics and the attached periphytons? Previous experiments have shown that shading reduces leaf carbon fixation (Chapters 2 and 4) and allocation (Chapter 3), as well as physiological integration among ramets sharing the same horizontal rhizomes (Chapter 5) in the two species of seagrass, *Thalassia hemprichii* and *Halodule uninervis*. These are clear indications of reduced plant vitality. An artificial severing of clonal links may have similar effects at individual shoot and the whole clone level. We assessed the effect of such reduced plant vitality by experimental cutting at 2 levels: (a) by severing individual shoots and (b) at the community level, by randomly cutting within a quadrat.

Materials and methods

Two separate experiments were conducted at the seagrass meadow of Sulawan, Misamis Oriental, Southern Philippines (8°26' N, 124°17' E, see Chapter 2 for a general description of the area). The study focused on the dominant species, *Thalassia hemprichii* (Ehrenberg) Ascherson. Two sites or habitats were selected: (1) a semi-exposed seagrass meadow near the reef crest and (2) a shallow sheltered subtidal pool, a depression between the reef crest and the mangrove reforestation area with an average depth of 0.3 m during lowest low tide.

Experiment 1. Effects on single shoots

The experiment was conducted during the period from June to August 1999. Shoots of *Thalassia hemprichii* were randomly selected at the two sites in Sulawan. A preliminary investigation had shown that collecting and replanting of the individual shoots after cutting the rhizome and roots significantly reduced growth and development of the leaves. A single ramet was therefore isolated by cutting the horizontal rhizomes, using scissors, in both directions without disturbing the roots. After cutting, the shoots were tagged loosely with a colored tie wire around the vertical rhizome or stem. A stick with number was inserted beside the shoot for identification purposes. Observations were made on both severed plants and control or undisturbed shoots. After five weeks, all shoots were harvested carefully by hand and placed in plastic bags. A total of 12 severed shoots and 12 control (unsevered) plants were collected from each habitat.

At the laboratory, leaf morphology (length and width) was measured and all the periphyton were scraped, from both sides of the leaves, using the edge of a glass slide. First the collected shoots were divided equally into three groups (3-4 shoots per group), representing 3 replicates per treatment for each habitat. Then the leaves, in each group, were sorted according to age or leaf number (i.e. youngest to oldest leaf). The scraped periphyton, for each leaf number, was placed in small plastic containers with formalin (5% final concentration) and properly labeled. Microscopic examination was done, in a petri dish under a dissecting microscope, to determine periphyton density and composition per shoot. We distinguished diatoms, filamentous algae, foraminiferans, cyst or egg clusters, crustaceans, nematodes, polychaetes and a rest category 'others'. After the measurements, we collected the periphyton material by filtering through a GF/C filter using a vacuum pump, which was then oven-dried at 50°C until constant weight.

Experiment 2. Effects on the seagrass meadow

The experiment was conducted from November 1999 to January 2000. The same two sites were selected and fixed quadrats (1 x 1 m) were set-up in a randomized block design. Each quadrat was marked with a wooden peg at the corner and divided into 25 small subquadrats (0.2 x 0.2 cm) using a nylon rope. Severing of the rhizomes was done by probing or stabbing the sediments using digging blades or knife (15 x 3.5 cm) up to 15 cm deep within the seagrass meadow. Three treatments were used in the experiment with three replicate each: high (8-10 stabs per subquadrat), low (3 stabs per

subquadrat) and control, no digging or cutting. After the rhizome cutting, the nylon ropes were removed. Shoot density, growth and morphology of the shoots were monitored after 4 and 10 weeks. Shoot density was measured in 5 pre-selected subquadrats (20 x 20 cm) within the 1x1 m quadrat. Growth rate was measured using the leaf marking technique (see Chapter 2). At the end of the experimental period, core samples were harvested in each plot and were brought to the laboratory for measurements. Samples were cleaned with tap water and sorted according to species. The number of cut rhizomes for every core was counted, except for the freshly cut rhizomes due to coring. The number of apical nodes was counted including the number of dead shoots. Shoot morphology was measured as in Chapter 2. After the measurements, periphyton was scraped from the leaves and placed in small plastic vials with formalin for later analyses.

To determine accumulation of periphytons with time, artificial substrates were installed for a period of 6-8 weeks. Plastic strips measuring 10 mm x 70 mm were tied to a stick inserted in the sediment, exposing only the plastic strip that stood upright moving with the current, thus imitating seagrass leaves. The artificial substrates were collected every week. All samples were collected carefully and placed in plastic bags and kept in the refrigerator for analyses the following day. Periphyton load was measured as percent light attenuance (cf Vermaat and Verhagen, 1996) and dry weight. Light attenuance was measured at the lab using a projector lamp (Sylvania, Tungsten Halogen, 82V-360W) mounted on a specially built box with blower. Irradiance during the measurements was set at 200 $\mu mol\ m^{-2}\ s^{-1}$. The plastic strips were immersed in seawater at 1 cm depth and over the light sensor head. The distance between the light sensor and the source light was fixed before measurement was carried on. Irradiance was read at 5 preset points on the plastic strips in all the samples. After light readings, the periphyton was scraped from the plastic strips and placed in small plastic containers with formalin. Subsequent collection was done by filtering the periphyton samples through a GF/C filter using a vacuum flask connected to a suction pump. Before the filtering of samples, foraminiferans were removed and counted. The Whatman GF/C filters with the periphyton were dried in the oven at 60°C until constant weight. Epiphyton attenuance and biomass were expressed as follows:

$$\text{Attenuance (\%)} = (\text{light}_{\text{periphyton + plastic}} - \text{Light}_{\text{blank plastic}}) / \text{Light}_{\text{blank}} \times 100\%$$

Statistical analyses

Statistical significance of rhizome severing, habitat type and leaf age, and their interaction effects was determined using 3-way ANOVA, for experiment 1 (single shoot). For the second experiment (population level), a 2-way ANOVA was used to test for significant differences between rhizome severing and habitat, and their interaction effects. A non-linear regression was calculated to determine relationships between periphyton biomass and percent light attenuance.

Table 1. Average (± SE) morphometrics of *T. hemprichii* and periphyton load, 5 weeks after rhizome cutting of individual shoots.

	Reef crest		Pool	
	severed	**control**	**severed**	**control**
Leaf length (mm)	25.7 ± 3.2	38.6 ± 5.8	67.4 ± 11.3	69.7 ± 6.2
Leaf width (mm)	5.0 ± 0.4	6.9 ± 0.7	8.3 ± 0.3	9.1 ± 04
Leaf surface area (cm^2 $leaf^{-1}$)	1.4 ± 0.3	2.8 ± 0.7	5.9 ± 1.1	6.5 ± 0.8
Number of leaves	3.8 ± 0.2	3.7 ± 0.2	4.2 ± 0.4	4.3 ± 0.2
Periphyton load (mg DW cm^{-2} leaf)	1.24 ± 0.2	0.61 ± 0.2	0.15 ± 0.06	0.10 ± 0.03

Table 2. Results of 3-way ANOVA examining the effects of site, rhizome severing, leaf age and their interactions on leaf surface area and periphyton biomass on *T. hemprichii* at Sulawan, Misamis Oriental. Presented are degrees of freedom (df) and the percent of the variation explained (factor SS/ total SS) and the level of significance (p). Bold numbers with p <0.05 are considered significant. Mean Values are presented in Table 1.

	Leaf surface area			Periphyton biomass		
	df	**% var**	***p***	**df**	**% var**	***p***
Site	1	30	**0.000**	1	32	**0.000**
Rhizome severing	1	3	**0.046**	1	7	**0.005**
Leaf age	3	6	**0.012**	3	10	**0.013**
Site x severing	1	<1	0.774	1	4	**0.017**
Site x age	3	<1	0.861	3	7	**0.040**
Cutting x age	3	<1	0.899	3	1	0.718
Site x severing x age	3	<1	0.998	3	1	0.810
Error	136	60		56	38	
Total	151	100		71	100	

Results

The average leaf length of *Thalassia hemprichii* in the subtidal pool was almost twice that in the semi-exposed seagrass meadow. Leaf width was also wider by 32% and the number of leaves per shoot was higher at the subtidal pool (Table 1).

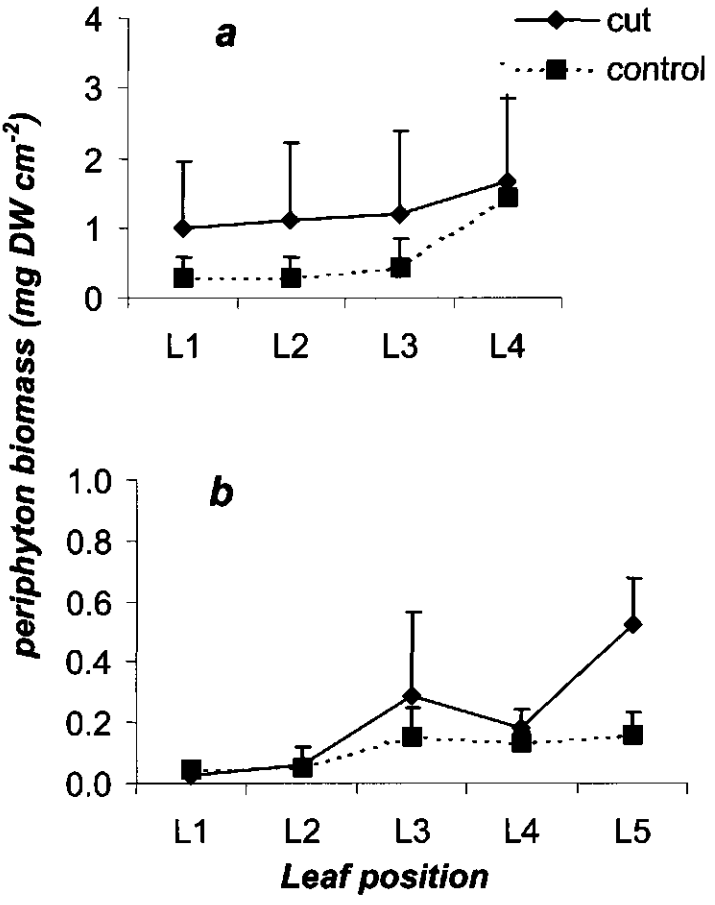

Fig. 1. Average biomass of periphyton in the severed and control seagrass shoots at the (a) semi-exposed reef crest and (b) fully submerged subtidal pool. For the leaf position, L1 is the youngest and L5 the oldest leaf. Error bars are standard errors.

In experiment 1, leaf surface area of *T. hemprichii* was significantly higher in the subtidal pool than in the semi-exposed area (3-way ANOVA, $p<0.001$, Table 2) explaining 30% of the variation. The rhizome severing treatment and the leaf age were significant ($p<0.05$, explaining less than 6% of the variation), but none of the interactions were ($p>0.05$).

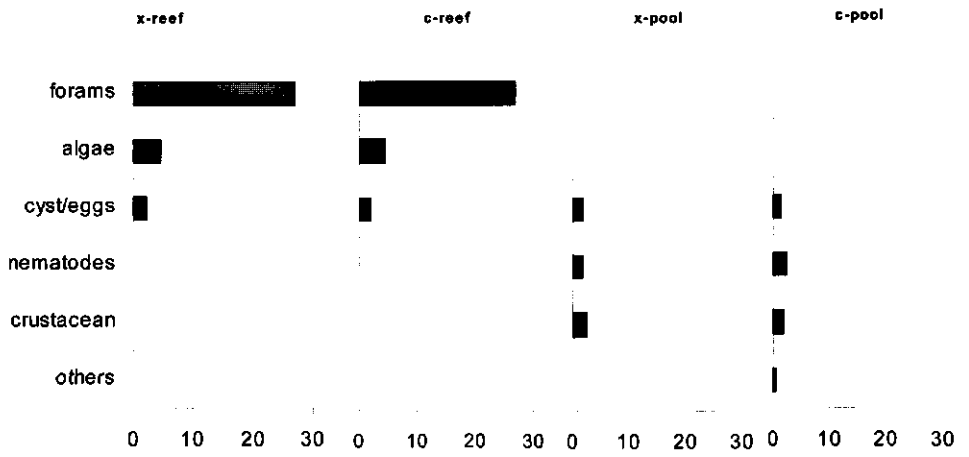

Fig. 2. Periphyton composition (classified according to major groups, y-axis) and density (ind shoot^{-1}, x-axis) in *T. hemprichii* in the (x) severed and (c) control plots of experiment 1 in the two study sites: reef and pool.

Table. 3. Average values ± SE of *T. hemprichii* morphometrics and periphyton load 10 weeks after random rhizome cutting in the seagrass meadow (Experiment 2). Values with different letters are significantly different at $p<0.05$.

	Reef crest			Pool		
Parameters	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Shoot density (m^{-2})	1245 ± 121	1230 ± 46	1093 ± 39	1226 ± 16^{b}	1261 ± 29^{b}	890 ± 160^{a}
Leaf relative growth rate (% d^{-1})	4.5 ± 0.4	4.1 ± 0.1	4.2 ± 0.2	3.5 ± 0.4	3.6 ± 0.3	3.7 ± 0.2
Leaf plastochron Interval (days)	8.7 ± 0.7	8.1 ± 0.3	7.9 ± 0.1	8.4 ± 0.5	8.0 ± 1.0	7.0 ± 0.3
shoot height (mm)	67 ± 2	59 ± 2	57 ± 2	139 ± 6^{a}	119 ± 5^{ab}	89 ± 4^{b}
Number of leaves	3.4 ± 0.1	3.6 ± 0.3	3.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.2 ± 0.1
Leaf width (mm)	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.2	6.8 ± 0.2	6.5 ± 0.2	5.4 ± 0.2
Leaf surface area (cm^{2} shoot^{-1})	2.4 ± 0.2	2.1 ± 0.2	2.1 ± 0.3	6.8 ± 0.7	5.8 ± 1.1	3.7 ± 0.4
New rhizomes (apex core^{-1})	10.7 ± 2	11.0 ± 3	7.0 ± 1	9.0 ± 2	4.0 ± 1	3.3 ± 1
Periphyton load (mg DW cm^{-2} leaf)	4.6 ± 0.8	7.3 ± 3.0	4.2 ± 1.4	1.9 ± 0.7^{a}	2.4 ± 0.6^{ab}	3.5 ± 0.3^{b}

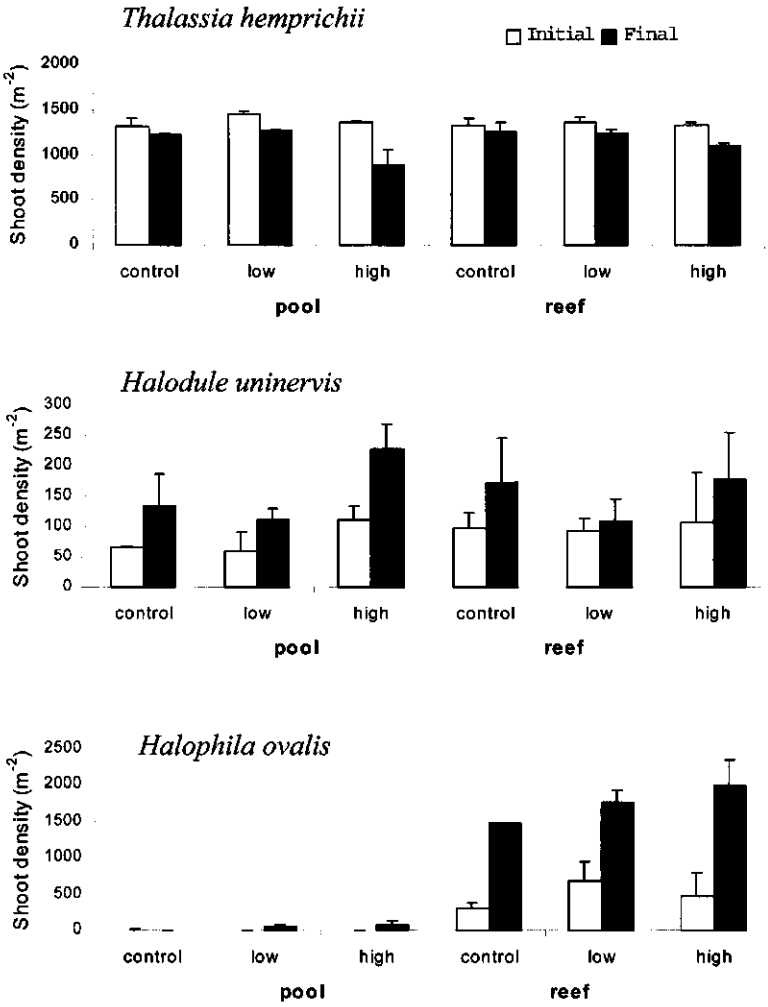

Fig. 3. Average shoot density (± SE) of common seagrass in the rhizome-severed (low and high) and control plots during the initial and final (after 10 weeks).

Periphyton biomass on the leaves was significantly different between the two sites ($p<0.001$) explaining 32% of the variation. The semi-exposed areas had a 6-fold higher periphyton biomass than the subtidal pool. The severing treatment effect on periphyton biomass was significantly different ($p<0.01$), but this was only true at the reef crest (Table 2). The amount of periphyton increased with increasing age of the leaves, although leaf 2 until leaf 4 were not significantly different in terms of periphyton load (Fig. 1).

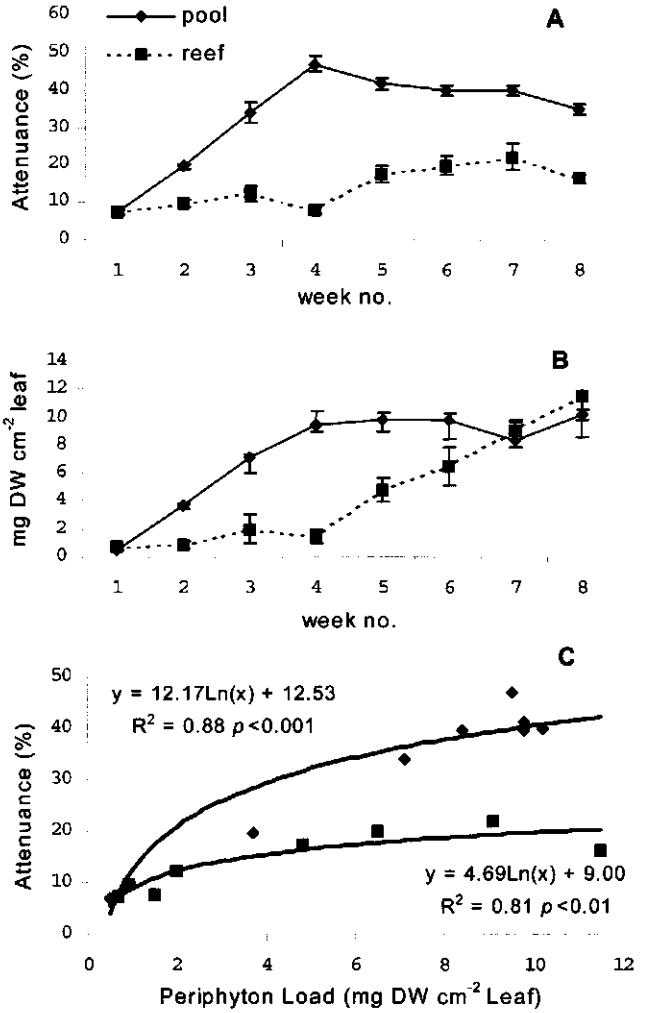

Fig. 4. Periphyton accrual on the artificial leaves (Nov 1999 – Jan 2000) expressed as (A) percent light attenuance, (B) periphyton load and (C) the relationship between light attenuance and periphyton load within *T. hemprichii* beds at Sulawan, Misamis Oriental.

The composition of periphyton communities on the leaves was different between the two sites. Foraminiferans were dominant in the reef crest but rarely occurred in the tidal pool (Fig. 2). Small crustaceans represented by copepods and isopods occurred more frequently in the tidal pool, as did nematodes. Only crustose algae were common in both sites but because of the growth habit (encrusting with no definite shape), counting was not possible.

In experiment 2, random cutting significantly reduced shoot density and increased the shoot height in the tidal pool , but no effect was observed on the reef crest (Table 3). No significant differences in leaf relative growth rate or leaf plastochron interval were noted between the two sites. Periphyton densities were generally higher in the semi-exposed site but did not show any significant differences between treatments. In the pool, however, where periphyton density was relatively low, they were significantly higher in the severed plots (ANOVA, $p<0.05$)

After 10 weeks, shoot densities in the control were reduced by <6% compared to initial shoot density in both sites (Fig. 3). However, rhizome cutting further reduced this to 33% in the pool and 17% in the reef crest. During the experiment, *Halodule uninervis,* density increased in all plots, and also *Halophila ovalis* increased tremendously in density particularly at the reef crest, in all treatments by as much as 157% to 390% of the initial density.

Periphyton accrual on the artificial leaves, in terms of percent light attenuance, was higher in the pool than in the semi-exposed reef (Fig. 4). Similarly, periphyton load expressed per unit leaf area was also higher in the pool. Peak biomass of the periphyton was already achieved during the 4^{th} week in the pool, whilst in the reef, accumulation was relatively slow and biomass still increased during the 8^{th} week. Percent light attenuated by the periphyton was strongly correlated with its biomass. Maximum light attenuance was achieved at almost 50% at the highest periphyton load in the pool, whilst a relatively lower light attenuance (20%) was achieved at the reef.

Discussion

Effects of rhizome severing on the *Thalassia hemprichii* were site-specific. The effect was less in the semi-exposed reef crest. In the subtidal pool, shoot density was significantly reduced and periphyton biomass appeared to be higher per unit leaf area. This is probably linked to the stronger wave action and low tide exposure at the crest, leading to shorter shoots, shorter leaf life span, and hence shorter time spans for colonization by periphytic organisms, as well as selecting for different periphyton taxa. Encrusting algae were dominant on the reef crest and covered large portions of the leaves, making them appear whitish (see Fig.1, Chapter 1), whilst these were rare in the subtidal pool.

Our results indicate that severing of rhizomes did reduce shoot densities but have no effect on the leaf relative growth rates (RGR) of the remaining *T. hemprichii* shoots. However, leaf length and width were significantly affected resulting in a reduction in total leaf surface area. Thus smaller shoots displayed a lower absolute but similar relative growth rate with a decline in size. This latter pattern was observed only at the reef crest.

Periphyton load was significantly increased by rhizome severing in the first experiment, and the effect was most pronounced at the reef crest. In contrast, in the second experiment, the effects on epiphyton were clearer in the pool. Possibly the random digging was more effective in severing rhizomes in the soft sediment of the pool than in the harder rubble of the crest. Though preliminary, our results suggests, indeed, that rhizome severing leads to reduced shoot vitality and subsequently to increased epiphyton cover. However, differences in epiphyton community, substratum-treatment interactions as suggested above, and possibly seasonality in exposure of the crest, prevent us to make overall generalizations.

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Chapter 7

General discussion and conclusions

The functional responses of the two common species of tropical seagrasses to short- and long-term shading were investigated both in the field and laboratory conditions. In the main experimental field study of this thesis, different shade screens were used to simulate deteriorating light conditions. The present study covered different aspects of morphological and physiological responses, and assessed the capacity of local seagrass species to cope with the shading stress that is caused by increased turbidity. This chapter integrates the results of the different studies conducted.

Shoot dynamics

First and foremost, shading significantly reduced shoot density (Chapters 2, 4 and 5). Even during short-term experiments of approximately 3 months (Chapters 4 and 5), shoot numbers declined by as much as 70% and 82% in *Halodule uninervis* and *Thalassia hemprichii*, respectively. Even more so, our long-term shading experiment, lasting for one year, resulted in almost barren plots for *H. uninervis* under the highest shading level (81-89% reduction of incident light). *T. hemprichii* appeared to survive long-term light reduction better, since about a quarter of the original shoots had survived the highest shade level (Chapter 2). The persistence of *T. hemprichii* may well be linked to its more robust rhizomes containing substantial quantities of reserve carbohydrates (up to 43% of dry weight, Vermaat et al., unpublished), which may be mobilized during periods of stress (e.g. Lee and Dunton, 1997). Recovery after removal of the shades indeed was faster in *T. hemprichii*. In both species, recovery in the medium shaded plots (52-67% reduction of incident light) was almost complete after one year. For the highly shaded plots, recovery was slower: for *T. hemprichii* full recovery is projected after 2 years, and this could even last longer for *H. uninervis*. The effect of shading on shoot age structures differed between species. In *T. hemprichii*, densities dropped equally over all age classes. In *H. uninervis*, in contrast, significantly less young or newly recruiting shoots were observed in the shaded plots than in the controls (Chapter 2).

Absolute leaf elongation rates (cm leaf per day) in both species increased with the increase in leaf length with shading. However, relative to the leaf size, the calculated relative growth rates (RGR) expressed as percent leaf biomass or length per day remained relatively constant under field conditions (Chapters 2 and 5). Since RGR was significantly reduced by shading under laboratory conditions (Chapter 4), this apparent absence of a response in leaf relative growth rates under field conditions could possibly

have been due to the fact that we did not cut the rhizome connections at the edges of the experimental plots (as did e.g. Harnet and Bazzaz, 1983; Tomasko and Dawes, 1989; Czerny and Dunton, 1995; Lee and Dunton, 1997).

We had decided to apply a less rigorous measure, and installed a 50 cm wide, shaded perimeter around the actual experimental plot. In chapter 5, we provided evidence that our approach was efficient in preventing re-allocation from outside the shaded plot in most clonal rhizome segments (only one of the seven sampled rhizome pieces of *H. uninervis* suggested re-allocation). We therefore assume that it is not re-allocation from outside the plots, but instead mobilisation from nearby, attached rhizome internodes, that allows some surviving shoots to maintain a continued leaf expansion despite heavy shading. Fitzpatrick and Kirkman (1995) experimentally combined cutting and shading treatments in the comparatively fast growing *Posidonia australis*. They found a reduction in leaf growth with shade, but no effect of cutting. Any translocation, therefore, must have occurred from within the cut perimeter. Bulthuis (1983) offered an alternative explanation: enhanced shoot mortality may have reduced self-shading, which, together with the increased shoot length, may have increased light availability. Since we have not measured light attenuation within the seagrass canopies, unfortunately we cannot evaluate the significance of this latter process.

Architectural properties: morphology and carbon allocation

Both species exhibited substantial plasticity in shoot morphology as a response to low light. In both the long- and short-term shading experiments, leaf surface area increased, and additionally, also stem length of *H. uninervis* increased substantially. Leaf turnover was slower and thus leaf lifespan was longer. The implication could be an increase in periphyton cover on the leaves as a consequence of the longer exposure period (Borowitzka et al., 1990). Excessive periphyton growth has been indicated as a cause for seagrass decline (Borum, 1985, Cambridge et al., 1986), but was generally attributed to increased nutrient load rather than a longer leaf lifespan. In the long-lived Mediterranean seagrass *Posidonia oceanica,* for example, leaf lifespan is almost a year, thus epiphyte accumulation can be considerable, but both *T. hemprichii* and *H. uninervis* have leaf life spans of about a month only (Hemminga et al., 1999; Chapter 4), limiting the scope for periphyton development.

Chapter 3 presents how photosynthetically assimilated carbon was allocated to the different plant compartments using the stable isotope ^{13}C as biotracer. The amount of ^{13}C-$NaHCO_3$ added correspond to only 10% of the total HCO_3 in the seawater medium (normal concentration is 2.2 mM HCO_3 , Sand-Jensen and Gordon, 1984). Despite this relatively small amount, ^{13}C assimilation was already sufficient to quantify translocation in different plant parts. In full light, *H. uninervis* and *T. hemprichii* transported 21% and 34%, respectively, of assimilated ^{13}C from the leaves to the non-photosynthetic stem, rhizome and roots. Under shade, these amounts were reduced by

almost half. However, relative allocation of ^{13}C among non-photosynthetic tissues (rhizomes, stem and roots) was not affected in *T. hemprichii*, whilst in *H. uninervis*, the stem accumulated most of the ^{13}C. It appears that the response to shading of these tropical seagrasses largely conforms to the general pattern of shade acclimation (Boardman, 1977; Björkman, 1981): the photosynthetically assimilated carbon is increasingly used for the maintenance of the leaves at the expense of the below-ground tissues. This may also explain the capacity of the surviving shoots under shade to maintain relative leaf growth rates at a reasonable level. As consequence of the reduced allocation to the rhizomes, new shoot production through branching is reduced thus limiting the capacity to counter high shoot mortality. Finally, below-ground biomass may also have been reduced as a response to low oxygen production of the leaves. Maintaining a large respiratory below-ground biomass would have been impossible at low oxygen production rates due to the shade. A net positive carbon balance must be maintained in order for the plant to survive (e.g. Dennison and Alberte, 1985; Vermaat and Verhagen, 1996; Chapter 4).

Photosynthetic capacities

Measurements of the photosynthetic capacities showed differences in responses of the two species. *T. hemprichii* had consistently higher saturated photosynthetic rate (P_{max}) values than *H. uninervis*, indicating the former as a sun-adapted species (cf Boardman, 1977). However, P_{max} did not show any significant effect during the shading simulation experiments, suggesting photosynthetic acclimation in both species. The light compensation point (LCP) was generally similar for both species within the range of 8-33 $\mu E\ m^{-2}\ s^{-1}$, and this LCP was significantly reduced with shading in the field (Chapter 2), but not under laboratory conditions (Chapter 4). Estimated half saturation constants (K_m) were highly variable and did not show any difference between the two species or shade levels under field conditions. Under laboratory conditions, K_m was significantly higher in *T. hemprichii* and was affected by shading (Chapter 4, Table 4 and 6). Comparing photosynthetic efficiencies ($\alpha = P_{max} / K_m$ = slope), however, *H. uninervis* had higher values than *T. hemprichii,* which may make it more efficient in utilizing low light (Chapters 2 and 4). Individual photosynthesis vs irradiance (P-I) curve parameters, however, must be interpreted with caution, and full P-I curves or diel oxygen balances are to be preferred as means to evaluate the response to light availability.

The apparent lack of response in photosynthetic capacities expressed per unit dry weight may well be related to the observed increase in chlorophyll content with shading. Thus, when photosynthesis vs irradiance parameters were normalized to chlorophyll content, no significant effects were observed. The increase in chlorophyll content during shading is an adaptive strategy to increase the light capturing capacity of leaves under low light (Boardman, 1977). This chlorophyll increase corresponded to an increase in leaf nitrogen content in both species (Chapters 2 and 5), which is not

unexpected, since nitrogen is one of the major components of chlorophyll. The surviving leaves apparently maintained photosynthetic efficiency even at reduced light, indicating photosynthetic acclimation (Chapters 2 and 4). This acclimation is probably accomplished by increasing leaf surface area and chlorophyll content (Chapters 2, 4 and 5). During the long-term shading experiment, temporal differences explained most of the variation in P-I values. Thus it was difficult to attribute changes in P-I parameters with shading.

Interactive effects of shading and sediment anoxia

In the factorial laboratory experiment (Chapter 4), the effect of shading was found to be magnified by adverse sediment conditions. Particularly for *T. hemprichii*, rhizome branching, biomass and root length were all high in oxidized sediment under full light, but photosynthetic capacity was reduced significantly under low light and in reduced sediment. In oxidized sediment, the effect of shading was minimal e.g. a net positive carbon balance was still maintained. In contrast, the combination of shade with reduced sediment resulted in a net negative carbon balance. This may support the postulation by Hemminga (1998), that "successful growth (of seagrass) may coincide with an increasing risk of sulphide toxicity". Seagrass meadows are generally moderately anoxic with Eh values ranging from –100 to 200 mV in the top 10 cm (Terrados et al., 1999). Therefore, in lush, extensive seagrass beds, possibly transient periods of anoxia may already cause stress. Under such conditions, reduced light may well lead to a critically reduced oxygen supply to rhizomes and roots, sufficiently below the respiratory demands to have lethal effects.

Physiological integration

Chapter 5 presents evidence for clonal integration in the two seagrass species through the use of stable isotope ^{15}N. Under normal conditions, *H. uninervis* showed a strong acropetal transport along horizontal rhizomes which is common in most clonal plants (Tietema, 1980; Noble and Marshall, 1983; Tomasko and Dawes, 1989; Terrados et al., 1997). This probably makes *H. uninervis* more efficient in foraging for barren substrates through faster lateral expansion (confirming the higher rhizome growth observed in Vermaat et al., 1995), a feature characteristic of a colonizer (Grime, 1989). In contrast, the late successional *T. hemprichii* showed both acropetal and basipetal transport. In this regard, *T. hemprichii* is probably more effective in keeping clonal fragments together than *H. uninervis*. Moreover, *T. hemprichii* has thicker rhizomes (a factor 2.7, Vermaat et al., 1995), and thus can be considered to have more potential for clonal integration among distant ramets than *H. uninervis*. The greater integration in *T. hemprichii* is achieved, however, at the expense of a reduced potential for rhizome extension. As a consequence of shading, the degree of physiological integration among shoots sharing the same horizontal rhizomes was significantly reduced. However, traceable amounts of ^{15}N were still detected along

interconnected ramets, suggesting still active but less transport of resources. With shading, translocation of ^{15}N along the horizontal rhizomes was reduced from 37% to 6% in *H. uninervis* and from 23% to 17% in *T. hemprichii*. This reduction in ^{15}N transport under shaded conditions may mean that the plants keep resources absorbed by the leaves within the shoot and abstain from export, with shoot requirements acting as the sink of first priority. This can be considered as an adaptive strategy to maintain vital leaf functions under low resource availability. Therefore the few remaining healthy shoots may still have been able to grow and maintain a positive leaf RGR. This is further supported by the observed reduction in photosynthate transport to the rhizome (Chapter 3), which probably reduces the respiratory demand of roots and rhizomes, to meet immediate shoot needs. An apparent plant strategy then must be to maintain functionally photosynthesizing leaves in still surviving shoots.

Rhizome severing and epiphyte load

This chapter 6 assessed the effect of rhizome severing in *T. hemprichii* to simulate reduced shoot vitality and determine its effect on epiphyte load. Horizontal rhizomes were either cut individually isolating a single shoot or randomly with digging blades, locally used to collect burrowing bivalves from the seagrass meadow. Our experiment, although preliminary, indicated a significant reduction in shoot density, but not in leaf relative growth rates as a consequence of rhizome cutting. The latter can be explained by mobilization of carbohydrate reserves in the still connected rhizome fragments that sustained leaf growth. Periphyton cover of the leaves may have been related to the vitality of the leaves or have been due to site-related effects, since it was higher on the severed shoots at the reef crest but it was not affected by cutting at the subtidal pool.

In conclusion

The two seagrass species generally occupy the same shallow coastal waters that may experience high light levels as well as fluctuations. In this respect, both species can be considered as obligate sun species (sensu Boardman, 1977). Björkman (1981) summarized that when grown in full light, plants tend to have higher light saturated photosynthetic rates (P_{max}) and relatively high rates of dark respiration. These characteristics should decline when plants are grown under low light and have the capacity to acclimate. This, however, was not the case in the two seagrass species studied here (Chapter 2). P_{max} and dark respiration did show high variability but were hardly affected by shading. Both species, therefore, appear to have a high potential for photosynthetic shade acclimation. Shade acclimation is probably mainly due to the following (Chapters 2-5): (1) an increase in leaf surface area, (2) an increase chlorophyll content, which, together with (1) will increase light absorption, (3) a reduction in respiratory demand realised by reducing both the quantity of heterotrophic tissue and the respiratory rate itself (Chapter 4), and (4) a reduction in the export of photosynthates out of the shoot (Chapter 3 and 5).

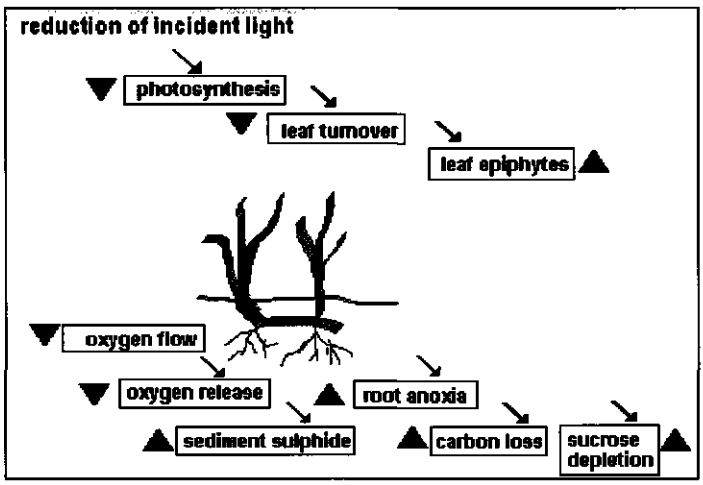

Fig. 1. Cascade effects following light reduction in seagrass plants. Cascades are indicated by sequences of progressively lower placed arrows. The upward or downward pointing filled black triangles indicate an increase or decrease, respectively, of the process (or condition) shown next to the triangles (redrawn from Hemminga 1998).

Results of the different studies conducted support the cascade effect following light reduction proposed by Hemminga (1998), so far largely based on data from the temperate *Zostera marina* (Fig. 1). Both investigated species had a considerable potential for photosynthetic acclimation, particularly in morphometry and chlorophyll content. Differences in survival were apparent from the long-term experiment, with *H. uninervis* experiencing the highest mortality under the highest shading. Short-term,

pulsed events of extremely high turbidity may be easier to cope with than the presently applied longer-term experimental shading. Differences in the capacity to survive these may be evaluated firstly from P-I curves and diel oxygen balances, secondly from carbohydrate reserves, and thirdly from acclimation capacity as derived from our long-term experiment.

Overall, the PI curves of both species predicted survival to be possible at quite low light levels (8-33 $\mu E\ m^{-2}\ s^{-1}$, corresponding to 2-4% of mean daily irradiance, which is lower than the mean level predicted by Duarte (1991: 11% of surface irradiance). However, comparison of individual curve parameters led to conflicting results: in the shade the initial slope of *H. uninervis* was steeper than that of *T. hemprichii*, but the other relevant parameters were hardly different. This similarity was further enhanced when photosynthetic rates were expressed per unit chlorophyll. Morphometry, shoot recruitment and growth patterns, however, were quite different between the two species. This closely agrees with the findings of Vermaat et al. (1997), who found comparatively little variation in photosynthetic performance among 7 seagrass species from different latitudes compared to morphometric and growth characteristics.

This dissertation has shown that photosynthetic capacities and relative growth rates are comparatively poor predictors of the long-term effects of light deterioration in our coastal waters. Rather, more conspicuous responses were observed in shoot morphology and shoot dynamics as a consequence of shading, shoot density decreased, leaf length increased, shoot recruitment dropped and chlorophyll content of the leaves of surviving shoots increased. Increased shoot mortality leading to reduced density was due to a substantial reduction in physiological integration, i.e. the translocation of carbohydrates and nutrients among shoots sharing the same horizontal rhizomes was significantly reduced.

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above all to God for all the blessings...

Daghan salamat sa inyo nga tanan...

Wilfredo Hojilla Uy

Curriculum Vitae

Wilfredo Hojilla Uy was born on November 8, 1959 in Cotabato, Southern Philippines. Wili, as he is known to friends, finished primary and secondary school in a Chinese High School in Cotabato City, and went to Cagayan de Oro City to finish Bachelor of Science in Marine Biology at Xavier University in March 1980. After college, he joined the Bureau of Fisheries and Aquatic Resources as a research assistant to a tuna research project for about a year. Then he went to work as a prawn hatchery technician to a private firm for a couple of months before finally joining the Mindanao State University – Institute of Fisheries Research and Development (now MSU at Naawan). While at MSU-Naawan, Wili was involved in several research projects of the Institute, among these are coral reef, artificial reefs, seaweeds and seagrass resource inventory and assessment. In 1987, he was awarded a scholarship by the Philippine Council for Agricultural Resources, Research and Development (PCARRD) to pursue an MSc in Marine Science at the Marine Science Institute of the University of the Philippines (UP-MSI) where he did his MSc thesis on carpospore production and sporeling growth in a seaweed, *Gracilaria* sp. During this period, he was awarded a research fellowship by the Smithsonian Institution in Washington D.C. for 3 months to work on seaweed taxonomy.

When he returned to work to MSU-Naawan, he continued his research on the production of seedlings from spores for seaweed cultivation aside from being an instructor at the School of Marine Fisheries & Technology of MSU at Naawan. In June 1995, he received another scholarship from the Department of Agriculture, Bureau of Agricultural Research to pursue a Ph.D. degree at UP-MSI. He completed his course work in 2 years but was not able to complete the program since he applied for the Phil-Dutch Ph.D. Fellowship and was awarded last January 1997 by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). It was a 'sandwich' program where he did all experimental and field works in Mindanao, while writing and the analyses of nutrients and stable isotopes were done in the Netherlands. His dissertation focused on understanding how seagrass respond to deteriorating light conditions as a consequence of increased siltation brought about by increased human pressure along the coastal areas.

Wili is blessed with 3 children Lyle, Nico and Kyla and is happily married to a lovely lady, Arleen Bautista-Uy.

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Samenvatting

Het effect van langdurige beschaduwing op Filippijnse zeegrassen

De toenemende belasting met nutriënten (eutrofiëring) en slib (siltatie) is een belangrijk milieuprobleem in de kustwateren van Zuidoost Azië. Zowel nutriënten- als slibbelasting leiden tot verhoogde troebelheid en daarmee tot afnemende lichtbeschikbaarheid voor de oorspronkelijk zeer uitgebreide zeegrasvelden langs de Filippijnse kusten. Ook van elders in de wereld worden dramatische reducties in het zeegrasareaal gerapporteerd. Filippijnse zeegrasvelden zijn van groot belang voor lokale vissersgemeenschappen maar ook voor de economie op grotere schaal. Daarom kan de grootschalige achteruitgang onverwachte maatschappelijke gevolgen hebben.

Een blijvende lichtvermindering kan op langere termijn zowel leiden tot acclimatisering bij overlevende soorten als tot successie naar gemeenschappen die beter aangepast zijn aan schaduw. Lange termijn beschaduwingseffecten in tropische zeegrasgemeenschappen zijn tot nu toe niet onderzocht, ondanks het voor de hand liggende verband met eutrofiëring en slibbelasting. In deze studie is in detail onderzocht wat de mogelijkheden tot acclimatisering zijn in twee algemene Zuidoost-Aziatische zeegrassoorten, *Thalassia hemprichii* en *Halodule uninervis*. De eerste is een algemene, langlevende en relatief grote soort van latere successiestadia, terwijl de tweede korter-levend en kleiner is en een onduidelijker successiestatus heeft. Op twee plaatsen langs de kust van Mindanao is een lange termijn beschaduwingsexperiment opgezet, met drie beschaduwingsniveau's in een randomized block design (0%, 60% en 85% lichtreductie). Een groot aantal aspecten van de groei en ontwikkeling van deze twee soorten zeegras is gedurende een experimenteel beschaduwingsjaar en een daaropvolgend hersteljaar zonder beschaduwing gevolgd: morfometrie, scheut-dynamiek, klonale integratie, fotosynthese-capaciteit, reservesuikergehaltes en nutriëntenverdeling. Bovendien was de interactie met reducerende sediment-omstandigheden ook onderdeel van studie.

Allereerst leidde de zwaarste beschaduwing tot een geprononceerde mortaliteit in de scheuten terwijl nieuwe recrutering grotendeels uitbleef. *Thalassia hemprichii* overleefde de beschaduwing beter dan *Halodule uninervis*: respectievelijk 18% en 5% van de oorspronkelijke scheuten overleefden één jaar. Dit is waarschijnlijk te wijten aan de robuustere wortelstokken van de eerste soort, die een hechtere klonale (of fysiologische) integratie realiseerden en waaruit meer reservesuikers gemobiliseerd konden worden. Opmerkelijk genoeg werd in de kleine aantallen overlevende scheuten de bladgroei (als RGR) gehandhaafd op het niveau van onbeschaduwde controle scheuten. Na verwijdering van de schaduwnetten herstelde *Thalassia hemprichii* zich sneller dan *Halodule uninervis*. Volledig herstel was echter na het eerste jaar nog niet opgetreden en zal vermoedelijk nog tenminste twee jaar extra kosten.

Beide soorten vertoonden opmerkelijke plasticiteit in scheutmorfologie als reactie op beschaduwing. Bladoppervlak en specifiek chlorofylgehalte namen toe terwijl de blad-turnover afnam. In de fotosynthese-curven ontbrak een duidelijke respons. Onder de schaduwnetten werd beduidend minder koolstof gefixeerd door fotosynthese en ook de allocatie naar wortelstokken en nieuwe scheuten was aanmerkelijk verlaagd. Dit is een algemene respons in planten: de beperkte hoeveelheid gefixeerde koolstof wordt besteed aan het onderhoud van het blad ten koste van andere organen of groei. De langstlevende soort, *Thalassia hemprichii*, alloceerde nog relatief meer naar de wortelstokken dan *Halodule uninervis*. Allocatie-patronen van stabiele isotopen van koolstof en stikstof (^{13}C en ^{15}N) over klonale wortelstok-netwerken bevestigde het verschil in klonale integratie tussen de twee soorten. Beschaduwing had een ernstiger effect als de planten op gereduceerd sediment groeiden. Vierentwintiguurs-zuurstofbalanzen bleven in dat geval negatief, hetgeen de grootschalige scheutmortaliteit onder deze omstandigheden bevestigt.

Dit proefschrift suggereert dat fotosynthese en relatieve (blad-)groeisnelheden slechte voorspellers zijn van de lange termijn effecten van beschaduwing. De duidelijkste respons werd gevonden in scheut-morfologie en -aantalsdynamiek: terwijl de bladlengte en het chlorofylgehalte van de overlevende scheuten toenam, nam de scheutdichtheid af als gevolg van een verhoogde mortaliteit en afgenomen recrutering. De toegenomen scheutmortaliteit is waarschijnlijk deels te wijten aan de afgenomen fysiologische integratie. Translocatie van zowel suikers als nutriënten tussen scheuten aan dezelfde wortelstok nam namelijk aanmerkelijk af.

Sa Laktud

Ang pagdaghan sa nutrina sa katubigan ug ang panbanlas sa kayutaan diha sa tubig-baybayon maoy importanteng hilisgutan sa palibut sa habagatang-subangan sa Asia. Mao kini hinungdan sa sobrang pagkalubog sa tubig mao nga moubos ang igong siga sa adlaw para sa halapad nga tubu-anan sa sagbot-dagat (seagrass) nga kadaghanan nagtubu sa tubig kabaybayonan sa Pilipinas. Ang mga dinagko nga salangputan sa pagkagamay sa sagbot-dagat nabalita na gikan sa tibook kalibutan. Tungod kay kini nga sagbot-dagat adunay importanteng kahimoan para sa katawhan ug sa kinatibuk-an ekonomiya. Ang ilang pagkagamay adunay wala damhang sangputanan sa katilingban. Ang tubu-anan sa sagbot-dagat mahinungdanon kaayo sama sa malamboon nga sistema sa "carbon fixing", binhi-an o semilyahan, ug kan-anan sa mga bililhong isda, ug mga ulang, ug mosilbing salaan sa mga hugaw sa tubig ug batebate.

Sa lugway nga panahon, ang kanunay nga pagkunhod sa siga sa adlaw o kahayag sa mga tanom hinungdan sa pag-anad ug pagkabuhi o pagtubu sa uban pang klaseng tanom diha sa naglandong nga mga lugar. Ang mga epekto sa dugay na nga paglandong sa sistema sa atong sagbot-dagat nagpabilin nga wala pa matun-I, bisan sa tataw nga relasyon sa nagkauswag nga pagdaghan sa nutrina sa katubigan ug pagbanlas sa kayutaan. Kini nga pagtuon, naglantaw sa posibleng tubag ug kasarang sa pag-anad sa dako ug dugay nga paglandong sa duha ka importante apan nagsukwahing sagbot-dagat sa habagatang-subangan sa Asia: *Thalassia hemprichii* ug *Halodule uninervis*. Ang nauna daghan, dako kun itandi sa uban, dugay mamatay, ug dugay mosanay. Ang sunod, mubo og kinabuhi o dali lang mamatay, gagmay ug halos dili mosanay. Ang dako nga galastohan sa lugway nga panahon sa pagsulay-sulay mahitungod sa pagpalandong gihimo ug gimatngonan sulod sa usa ka tuig ug usa ka tuig usab alang sa pagbalik-arang arang sa duha ka lugar sa Amihang Mindanao, ang Pilipinas (Naawan ug Sulawan, ang duha anaa sa Misamis Oriental). Ang mga nahitabo o resulta sa pagpalandong giihap sa nagkadaiyang katumbas o "levels": pagsukod (morphometry), "shoot dynamics", "clonal integration", "photosynthetic capacity", "carbohydrate" ug "nutrient allocation". Ang mga katumbas sa pagpalandong nga eksperemento gibanabana ngadto sa 0 porsento (0%), 60 porsento (60%) ug 85 porsento (85%) nga pagkunhod sa kahayag nga moabot sa sagbot-dagat. Usab, ang mga hitabo sa kanunay nga pag-ubos sa mga kahimtang kanunay gi-obserbahan diha sa puno sa nutrina nga katubigan gilangkob niini nga pagtu-on.

Una, ang pinakataas nga lebel sa landong midangat sa tataw nga pagkamatay sa udlot, ug gamay paingon sa walay bag-ong udlot nga mogawas sa mga sagbot-dagat.. Ang *T. hemprichii,* mabuhi pa kay sa *H. uninervis* (18% batok sa 5% sa orihinal nga udlot nga nagpabilin human sa usa ka tuig), tingali tungod sa mas baskog o lig-on nga mga gamot sa nauna, pagpadayon sa hugut nga "clonal integration" ug sa undanong gidaghanon sa tagana nga "carbohydrates" nga mahimong mabalhinbalhin. Sa mga nabuhi nga udlot, ang mga pagtubu sa dahon napadayon diha sa tulo ka eksperimentong lebel sa paglandong. Human nakuha ang mga pagsulay nga pagpalandong, and

pagbawi o pag-usab sa *T. hemprichii* paspas itandi sa *H. uninervis.* Apan ang tibuok nga pagbawi sa mga tanom nga anaa sa pinakataas nga landong gibanabana nga molungtad gihapon sulod sa duha ka tuig sa *T. hemprichii* ug mas taas pa nga panahon ang sa *H. uninervis.*

Ang duha ka klaseng tanom nagpakita sa mahinungdanung pagkalubay sa hitsura sa udlot, isip maoy tubag sa paglandong: Ang gidak-on sa dahon ug ang tinong sulod sa "chlorophyll" mitaas, ug usab ang paghulip sa dahon mikunhod. Walay klarong tubag sa pagpalandong nga na-obserbahan diha sa "photosynthesis-light curve parameters". Ang piho nga carbon, gikan sa 'photosynthesis' gamay lang diha sa landong, ug ang gigahin sa mga gamot ug bag-ong udlot makusganong nakunhod. Kini susama lang sa kinatibuk-ang tubag sa tanom sa pagpalandong; ang pihong carbon gigamit alang sa pagpadayon o pag-atiman sa mga nagpabilin nga mga dahon, ug ang nagpaingon sa parte sa sagbot nga anaa sa ilalom sa yuta, gamay na lang o halos wala na. Ang dugay mamatay nga *T. hemprichii* nagahinan sa maitanding gidak-on nga bahin sa iyang hugot o sikit nga "clonal integration". Ang pagsubay sa gigahin nga mga "stable isotopes" ^{15}N ug ^{13}C, nga gigamit sa eksperimento, nagpamatuod sa kalainan sa "clonal integration" tali sa duha ka klaseng tanom. Ang epekto sa landong klaro kaayo kung ang tanom nagtubu sa nagkaubos nga mga lawog o baho nga yuta. Ang balanse sa tibuok tanom "diel oxygen" o carbon motumbas sa nagpabiling negatibo, pagpamatuod sa dinaghang pagkamatay sa udlot sa niini nga kahimtang.

Kini nga disertisyon o pagtuon, nagpakita nga ang "photosynthesis" ug ang kalabotan sa pagtubu sa mga sagbot-dagat, maitandi nga ubos nga tima-ilhan sa hataas nga epekto sa pagpagkadaut sa kahayag o pagkalubog sa atong dagat kabaybayonan. Kini tungod kay ang tubag o reaksyon sa mga sagbot-dagat mas mamatikdan sa hitsura sa udlot. Isip sangputanan sa paglandong, ang gidaghanon sa mga sagbot-dagat mo-ubos apan motaas ang dahon ug ang "chlorophyll", ang pagpangudlot mogamay nga maoy hinungdan sa pagkagamay sa gidaghanon. Kana tungod sa undanong pagkaubos sa "physiological integration", sama sa pagbalhinbalhin sa "carbohydrates" ug nutrina diha sa mga pagbahinbahin sa udlot sa mao rang naghigdang mga gamot mahinungdanong mikunhod.