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2
3 **Experimental evaluation of the effects**
4 **of siltation-derived changes in sediment conditions**
5 **on the Philippine seagrass *Cymodocea rotundata***
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1 **Abstract**

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3 This study investigated if siltation-associated changes in the sediments are
4 detrimental to seagrasses. We chose *Cymodocea rotundata* as the test species because it
5 is considered one of the Southeast Asian seagrass species most sensitive to siltation. The
6 approach included the (1) evaluation of the effects of silted sediments on plant growth,
7 evaluation of the effects of *in situ* sulfide additions to the sediment on (2) the production
8 of shoots, rhizomes and roots, and on the elongation rate of the horizontal rhizomes of
9 plants located at the edge of a meadow, and on (3) leaf growth, mass allocation patterns
10 and shoot density in a well-developed seagrass meadow. The results showed that under
11 high light availability major changes in sediment conditions associated with siltation did
12 not negatively affect the plants but enhanced their growth likely by increasing the
13 availability of nutrients. Pore water sulfide concentrations of 1mM reduced by more
14 than half the production of shoots, rhizome and roots, and the elongation rates of
15 horizontal rhizomes of *C. rotundata* plants at the edge of the meadow, but had no
16 effects on leaf growth and shoot density in a well-developed *C. rotundata* meadow.

17

18 **Keywords:** *Cymodocea rotundata*; seagrass biomass and growth; siltation; sulfide

19

1 **1. Introduction**

2

3 Increased development and changes in land use patterns in the coastal zone have
4 resulted in increased sediment loading and eutrophication, which have led to extensive
5 degradation and loss of seagrasses (Short and Burdick, 1996; Short and Wyllie-
6 Echeverria, 1996). These have been particularly important in SE Asia, where siltation of
7 coastal ecosystems has been identified as a major threat to seagrass ecosystems (Fortes
8 1988, Bach et al., 1998, Terrados et al., 1998). In addition to reduced light penetration
9 (Bach et al., 1998), siltation promotes changes in the sediments by increasing the
10 concentration of silt, organic matter, and nutrients (Kamp-Nielsen et al., 2001), which
11 likely result in higher rates of organic matter decomposition and oxygen demand in the
12 sediment, negatively affecting seagrasses (Terrados et al., 1988).

13

14 While the light requirements of seagrasses have been investigated extensively
15 (Dennison and Alberte, 1982; Dennison, 1987; Duarte, 1991; Abal et al., 1994;
16 Zimmerman and Alberte, 1996), only a few studies have focused on the sediment
17 conditions (i.e. size distribution of sediment particles, organic matter content, redox
18 potential, sulfide concentration in pore water) required by seagrasses (e.g. Terrados et
19 al., 1999, Koch, 2001). Seagrass distribution seems to be constrained by silt plus clay,
20 organic matter and sulfide concentration in the sediments (Koch, 2001), as supported by
21 the observation that both the species richness and leaf biomass of Southeast Asian
22 seagrass communities declined sharply when the silt and clay content of the sediment
23 exceeded 15% (Terrados et al., 1998).

24

1 Associated with siltation is an increase in organic matter and nutrients in the
2 sediment (Kamp-Nielsen et al., 2001) that could promote microbial activity thus
3 increasing the rate of oxygen consumption and the accumulation of sulfide. Sulfide has
4 been shown to negatively affect photosynthesis, metabolism and growth of seagrasses
5 (Goodman et al., 1995; Erskine and Koch, 2000; Holmer and Bondgaard, 2001), and the
6 negative impact of siltation on seagrasses may be attributed primarily to the increase in
7 sediment sulfide concentrations. However, sucrose addition to the sediment of a
8 multispecific seagrass meadow in Bolinao, Philippines had contrasting effects
9 depending on the seagrass species (Terrados et al., 1999), which suggests that the
10 adaptations that allow seagrasses to survive in anoxic sediments are species-specific.

11
12 The goal of this study was to test experimentally the responses of the seagrass
13 species *Cymodocea rotundata* to changes in sediment conditions associated with
14 siltation. We selected this species because it has been suggested to be one of the most
15 sensitive to siltation among the species present in SE Asia (Terrados et al., 1998), where
16 it has an important contribution to the seagrass community. We tested this hypothesis by
17 evaluating the effects of silted sediments on the growth of *Cymodocea rotundata* plants
18 grown in mesocosms (Experiment 1). The negative impact of siltation on seagrasses
19 could, however, be due to the increased production of sulfide as an anaerobic by-
20 product of organic matter degradation. We tested this hypothesis by two *in situ*
21 experiments. We added sodium sulfide to the sediment to investigate the effects on the
22 production of shoots, rhizomes and roots and on the elongation rate of the horizontal
23 rhizomes of plants located at the edge of a *C. rotundata* meadow (Experiment 2).
24 Finally, we added sodium sulfide to examine the effects on leaf growth, shoot density
25 and mass allocation patterns in a well-developed *C. rotundata* meadow (Experiment 3).

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3 **2. Materials and methods**

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5 2.1 Study site

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7 The *in situ* experimental additions of sodium sulfide to the sediment
8 (Experiments 2 and 3) were performed in a protected lower intertidal area on coral sand
9 in the southeastern side of Silaqui Island, Cape Bolinao, NW Philippines (16° 27.03' N,
10 119° 55.35' E). Cape Bolinao is located on the northwestern tip of Pangasinan and is
11 surrounded by the most extensive coral reefs in the Lingayen Gulf (McManus et al.
12 1990). It is on the western edge of the Lingayen Gulf, facing the South China Sea on
13 the north and west, and the Caquiputan Strait on the east. Within the reef barrier, the
14 coral cover is patchy and extensive seagrass beds cover an area of approximately 25
15 km² (Fortes 1995).

16

17 We chose the seagrass species *Cymodocea rotundata* as the test-species because
18 an evaluation of the species richness and leaf biomass of seagrass meadows along
19 gradients of siltation in SE Asia provided correlative evidence that this seagrass species
20 is one of the most sensitive to siltation (Terrados et al., 1998), and it is a structurally
21 important component of the multispecific seagrass meadows of the region (Bach et al.,
22 1998).

23

24 2.2 Effects of silted sediments on the growth of *Cymodocea rotundata* (Experiment 1)

25

1 The experiment was performed in the outdoor culture-tank facility of the
2 University of the Philippines Marine Science Institute (UP-MSI) in Bolinao from March
3 to June 1999. Five sprigs (*sensu* Thorhaug, 1985) of *Cymodocea rotundata*, 13 to 21 cm
4 long, bearing 5 to 10 shoots and an undamaged rhizome apex were planted in plastic
5 buckets each filled with a 15-cm thick layer of sediment. Three different sediment types
6 were used: “sandy” sediment collected at the beach just outside the UP-MSI field
7 station, a “highly-silted” sediment collected at a silted site, Santa Barbara (Fig. 1), and a
8 “medium-silted” sediment which was prepared by mixing equal amounts of the previous
9 two sediments (Table 1). Six buckets (replicates) were filled with each of the three
10 sediments and all the buckets (a total of eighteen) were arranged randomly within a 75-
11 cm deep concrete tank providing similar water column conditions for all sediments. The
12 outdoor culture-tank facility had a continuous flow-through of seawater and aeration,
13 which maintained a water depth of 51 cm and an average water temperature of 30°C
14 throughout the experiment.

15

16 Plant growth was measured using a rhizome tagging method. All rhizomes were
17 marked immediately behind the apical shoot to be able to distinguish the new shoots
18 produced during the experiment from the shoots present at the start. The total number of
19 shoots in each bucket was counted initially, and after 7 days, 14 days, and thereafter
20 every 14 days until the end of the experiment. The experiment was terminated after 72
21 days and all plants were harvested and the number of new shoots (‘new’ shoots,
22 rhizomes and roots are defined as those that were between the apex of the horizontal
23 rhizome and the mark) was counted. The length of the leaves, internodes of the
24 horizontal rhizome and roots of the new shoots on each plant were measured. The plants
25 were then separated into the different ramet components (i.e. leaves, rhizome, roots) and

1 dried for 24 hours in an oven at 60° C and weighed. The biomass of leaves, rhizome and
2 roots per shoot were computed by dividing total leaf, rhizome and root biomass by the
3 number of shoots in each bucket, and their sum provided the ramet biomass. The
4 rhizome plus roots to shoot ratio was obtained by dividing the sum of the biomass of
5 rhizome and roots per shoot by the biomass of leaves per shoot. Sub-samples of the
6 dried leaves, rhizome and roots from each bucket were ground to fine powder and were
7 analyzed for nitrogen and phosphorus content. Nitrogen concentration was analyzed
8 using a Carlo Erba EA 1108 CHN analyzer and phosphorus was determined
9 spectrophotometrically (Parsons et al., 1984) after 1 hour ignition of leaf material at
10 550°C and 15 min of boiling of the ash in 1N HCl (Andersen, 1976).

11

12 To characterize the differences in sediment conditions among treatments, one
13 sediment core from each bucket was collected using plexiglass tubes (4 cm in internal
14 diameter and 12 cm long). The sediment cores were processed to determine the
15 percentage of coarse sand (sediment particle size >250 µm), 'fine sand' (sediment
16 particle size between 63 µm and 250 µm), and 'silt' (sediment particle size < 63 µm)
17 using the wet sieving method (Buchanan, 1984), the content of organic matter (weight
18 loss after the ignition at 550°C of a 5g dried subsample), and the total amount of
19 nitrogen and phosphorus in the sediment using methods similar to those used to
20 determine total nitrogen and phosphorus in seagrass tissues.

21

22 The differences in sediment characteristics and plant responses between
23 treatments were tested for significance using analysis of variance (ANOVA) and means
24 were compared using Tukey's Honest Significance Difference test (5 % significance
25 level). If the variances of the means were not homogeneous (as detected by the univariate

1 test of Cochran, Hartley and Bartlett) we applied a log transformation before performing
2 the ANOVA. The statistical analysis of the data was performed using STATISTICA
3 software 2000 (StatSoft Inc., Tulsa, OK USA).

4

5 2.3 Effects of *in situ* sulfide addition to the sediment on the growth of the horizontal
6 rhizome of *Cymodocea rotundata* at the edge of a meadow (Experiment 2)

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8 The experiment was carried out from May to August 2000. Ten horizontal
9 rhizomes of *Cymodocea rotundata* including an undamaged apex were chosen
10 haphazardly along a transect line laid parallel to the shore (1.5 meters apart from each
11 other) at the edge of a monospecific *C. rotundata* bed in Silaqui Island. Sand was
12 fanned away carefully from the rhizome apex and the plants were marked with a cable
13 tie at the third internode (counting from the apex) to be able to distinguish new shoots
14 (i.e. those produced during the experiment) from old shoots (i.e. those present at the
15 initiation of the experiment). Ambient concentrations of sulfide in pore water were
16 experimentally increased by adding sodium sulfide to the sediment. Sodium sulfide
17 flakes were contained inside a perforated plastic bag (with needle sized holes), which
18 was placed inside a 2-cm diameter, 10-cm long PVC pipe. Four PVC pipes containing
19 11.5 g of sodium sulfide each were placed at the sides of the horizontal rhizomes of five
20 of the previously selected plants. This represented an approximated load of 256 g
21 sodium sulfide m^{-2} , twice the loading rate that has been shown to be detrimental to
22 seagrass photosynthesis (Goodman et al., 1995). Sodium sulfide additions were repeated
23 every three weeks for the whole duration of the experiment, which translates into a
24 loading rate of 156 mmol sulfide $m^{-2} day^{-1}$. Four similar PVC pipes, without sodium
25 sulfide, were placed at the sides were placed at the sides of each of the other five plants

1 chosen to control for possible effects of the mechanical disturbance associated to the
2 sulfide additions. Every time sulfide was added to the plants, the PVC pipes in the
3 control plants were also removed and re-inserted so all plants were subjected to the
4 same level of mechanical disturbance. PVC pipes containing sodium sulfide were
5 similarly placed in the sediment at two different sites of an adjacent unvegetated sand
6 area: these two sites were used to monitor the effects of the sodium sulfide additions on
7 pore water sulfide levels in the absence of *C. rotundata* plants.

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9 After 93 days the sand around the experimental plants was fanned away and
10 those surviving (4 control and 3 sulfide treated plants) were collected. The vegetative
11 development of the experimental plants was described in terms of the length and width
12 of the leaves, leaf surface of the shoots, the specific leaf area (g cm^{-2} of leaf), the length
13 of the roots, the specific root weight (g cm^{-1} of root), shoot biomass, and the rate of
14 production of shoots, rhizome and roots.

15

16 Sediment pore water samples were collected to monitor sulfide levels on days 1,
17 5, 10, 19, 28, 33, 40, 47, 66, 78, and at the end of the experiment (day 93) by inserting
18 into the sediment a perforated plastic tubing and slow suctioning of the pore water
19 through a Millipore filter into a syringe. Pore water was then transferred into a cryovial
20 which contained 100 μl of 4.4 % zinc acetate and 100 μl of 1N NaOH. Pore water samples
21 fixed in this way were kept in the cryovials until analysis, which was done within two
22 months following the procedure of Cline (1969). Due to the inherent pulsing nature of
23 the concentration of sulfide in pore water after the sodium sulfide additions, an average
24 concentration of pore water sulfide during the experiment was obtained by pooling all
25 the concentration values determined in each of the treatments. The averages of the pore

1 water sulfide concentrations in the different treatments were compared using the
2 Kruskal-Wallis analysis of variance and plant responses to sulfide additions were
3 compared using a 2-sample t-test.

4

5 2.4 Effects of *in situ* sulfide addition to the sediment on leaf growth, mass allocation
6 patterns, and shoot density in a well-developed *Cymodocea rotundata* meadow
7 (Experiment 3)

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9 The experiment was conducted from May to August 2000. Nine square
10 experimental plots (0.25 m² each) were established 1 m apart from each other in a
11 monospecific *Cymodocea rotundata* bed on the southwestern side of Silaqui Island (Fig.
12 1), and the experimental treatments assigned to them following a complete randomized
13 design. Sodium sulfide was added to the sediment by inserting evenly spaced in each
14 plot 9 PVC pipes containing sodium sulfide similar to those described in Experiment 2
15 in two loading rates, 256 g m⁻² and 512 g m⁻², which correspond to twice and four times
16 the loading rates detrimental to seagrass photosynthesis (Goodman et al., 1995). Sodium
17 sulfide additions were repeated every 14 days, which translates into concentrations of
18 156 and 312 mmol sulfide m⁻² day⁻¹. PVC pipes without sodium sulfide were inserted
19 into the sediment of the control plots and every time sulfide was added, the PVC pipes
20 in the control plots were also removed and re-inserted so that all plots were subjected to
21 the same level of mechanical disturbance. There were three replicates (plots) of each
22 treatment.

23

24 The number of shoots of *Cymodocea rotundata* present in the plots was counted
25 on days 0, 6, 40, 72, and at the end of the experiment (day 111). At least ten shoots of *C.*

1 *rotundata* per plot were randomly chosen to estimate leaf growth using a modified
2 method of Zieman (1974). All leaves on each shoot were marked just above the leaf
3 sheath by punching two colinear holes with a hypodermic needle, and the shoot was
4 tagged with a colored twistee tie. After 6 - 13 days, the marked shoots were retrieved
5 and the distance the holes traveled in every leaf of the shoot was measured to calculate
6 growth. The new length and total leaf length of each leaf in the shoot were measured.
7 There were four leaf marking periods: days 6-19, days 28-37, days 56-69, and days 102-
8 108 after the initiation of the experiment.

9

10 At the end of the experiment all the plants in each plot were collected using a
11 stainless steel corer (with an internal diameter of 20 cm) pushed to a depth of 30 cm.
12 The length of the leaf blade, leaf sheath, internodes of the vertical rhizome and
13 horizontal rhizome and roots were measured from a subsample of 20 rhizome fragments
14 with 5-10 shoots. The plants were then separated into leaf blades, leaf sheaths, vertical
15 rhizomes, horizontal rhizomes and roots and dried at 60°C for 24 hours and weighed.
16 Then, the rhizome plus roots to shoot ratio was obtained by dividing total rhizome and
17 root biomass m^{-2} by the total leaf biomass m^{-2} and, the leaf sheath to leaf blade ratio by
18 dividing total leaf sheath biomass m^{-2} by total leaf blade biomass m^{-2} .

19

20 Pore water samples were collected on days 1, 7, 14, 19, 37, 42, 56, 65, 70, 84,
21 93, 98, and at the end of the experiment (day 111) and analyzed for sulfide
22 concentration as described in Experiment 2. The log-transformed averages of the pore
23 water sulfide and the plant responses in the three experimental plots were compared

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25

1 using one way ANOVA.

2

3 **3. Results**

4

5 3.1. Experiment 1

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7 The biomass of *C. rotundata* ramets was higher in the sediments containing
8 larger amounts of silt in the sediment (ANOVA $F= 4.22$, $p<0.05$, Fig. 1a, Table 2). The
9 increase in size of the ramet was driven by a 30% increase in the mass of the leaves per
10 shoot when plants were grown in the medium- and high-silt sediments (ANOVA, $F =$
11 6.69 , $P< 0.01$, Fig. 1b, Table 2). The mass of rhizome and roots per shoot did not differ
12 significantly (Table 2). As a result, the rhizome plus roots to shoot ratio decreased by
13 30% in the medium- and 40% in the high-silt sediments when compared with the sandy
14 sediment but this was not significant (Fig. 1c, Table 2). The average rate of production
15 of shoots increased from 0.54 ± 0.25 shoots d^{-1} in the sandy sediments to 0.72 ± 0.34 and
16 0.86 ± 0.18 shoots d^{-1} in the medium and high-silt sediments (Fig. 1d, Table 2). The
17 leaves, internodes of the horizontal rhizome and roots were significantly longer in the
18 medium and high silt than in the sandy sediment (Table 2). Phosphorus content in the
19 leaves and rhizomes were significantly higher in the medium and high silt sediments
20 than in the sandy sediments while there was no significant difference in phosphorus
21 concentration in the roots between treatments (Table 2). Nitrogen content in all plant
22 parts did not differ significantly between treatments (Table 2).

23

24 3.2. Experiment 2

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1 The addition of sodium sulfide significantly increased the sulfide concentration
2 in the pore water (Kruskal-Wallis test: $H(2, n=72) = 18.1$; $p < 0.01$, Fig. 2a). The
3 average concentration of sulfide in the sediment pore water ranged from 0.07 to 0.18
4 mM near the apex of the horizontal rhizome of control plants, from 1.00 to 5.63 mM
5 near the apex of the horizontal rhizome of the sulfide treated plants, and from 3.23 to
6 9.27 mM in the adjacent unvegetated sediments where sulfide was added.

7

8 Only three out of the five sulfide-treated plants were used for the morphometric
9 and biomass measurements because two of them were dead and decomposing at the end
10 of the experiment. The addition of sulfide reduced plant growth (two-sample t-test;
11 $p < 0.05$, Table 3) as indicated by a 60%, 74% and 90% reduction in the rates of
12 production of new shoots, rhizome and roots respectively (Fig. 3a, b and c) and a 60%
13 decrease in the elongation rate of the horizontal rhizome (Fig. 3d, Table 3), but it did
14 not significantly affect plant size (Table 3).

15

16 3.3. Experiment 3

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18 The addition of sulfide to the sediment significantly increased the sulfide
19 concentration in the pore water (ANOVA of log transformed $\mu\text{M S}$, $p < 0.05$, Figure 2b).
20 Average sulfide concentration in pore water ranged from 0.04 to 0.08 mM in the control
21 plots, from 0.09 to 0.22 mM in the medium-sulfide plots, and from 0.22 to .63 mM in
22 the high-sulfide plots.

23

24 The experimentally increased levels of pore water sulfide did not affect leaf
25 growth for there were no significant differences either in absolute or relative growth

1 rates of *Cymodocea rotundata* between treatments throughout the whole experiment
2 (Fig. 4a and b). The length of the leaf blades, leaf sheaths, rhizome internodes and roots
3 did not differ significantly between treatments (data not shown). The biomass (g DW m⁻²)
4 of leaf blades and leaf sheaths decreased significantly in the high sulfide treatment,
5 while the biomass of rhizomes and roots did not differ among treatments (Table 4). The
6 leaf sheath to leaf blade ratio and rhizome and root to shoot ratio did not differ
7 significantly among treatments (Table 4). The net change in shoot density during the
8 experiment was not significantly different among treatments, although it showed a
9 decreasing trend from control to high-sulfide plots (Table 4).

10

11 **4. Discussion**

12

13 Siltation is associated with an increase in the amount of silt, organic matter and
14 inorganic nutrients in the water column and the sediment (Kamp-Nielsen et al., 2001),
15 which adversely affects seagrasses in SE Asia (Terrados et al., 1998), as reflected in
16 negative relationships between seagrass biomass and species richness and the amount of
17 silt and clay in the sediment (Terrados et al., 1998). These studies, however, were not
18 able to distinguish whether seagrass loss was due to the effects of siltation on the
19 conditions of the water column, the sediments, or both. Bach et al. (1998) showed that
20 plants transplanted from non-silted to silted sites retained high leaf growth rates and
21 similar net changes in shoot density, independently of the differences in light
22 availability among the sites. This suggested that the silted sediment conditions played a
23 larger role than the light conditions in the loss of seagrasses along the siltation gradient
24 (Bach et al. 1998).

25

Our results support the hypothesis that sediment conditions determine the

1 abundance and growth of seagrasses, and provide insight on how the changes in
2 sediment conditions associated with siltation of coastal ecosystems affect seagrass
3 performance. First, the changes in sediment conditions associated with increased
4 siltation need not be detrimental to seagrasses. The results of Experiment 1 suggest that
5 silted sediments may promote seagrass growth, likely because increased levels of silt in
6 the sediment may be associated with an increased availability of nutrients to seagrasses,
7 which are frequently nutrient-limited in Southeast Asia (Erfemeijer, 1994; Agawin et
8 al., 1996; Terrados et al., 1999; Udy et al., 1999). Indeed, the growth of *Cymodocea*
9 *rotundata* was enhanced in the medium- and high-silt sediments as manifested by the
10 higher biomass of leaves per shoot and ramet, and an increasing trend in the production
11 of shoots. The comparatively low shoot biomass and phosphorus contents in the sandy
12 sediment suggests that plants probably benefited from the higher nutrient availability in
13 silted sediments. The total organic matter and silt content of these sediments were
14 within, or in the high end of, the range of values measured in *C. rotundata* stands in
15 Cape Bolinao (Halun, 2001), which were characterized by low values of organic matter,
16 silt and pore water sulfide when compared with maximum values reported by Kamp-
17 Nielsen et al. (2001) for silted unvegetated sites in the region (Table 5). Hence, it is
18 likely that further increases in silt and organic content could lead to negative responses.

19 Second, pore water sulfide can potentially negatively affect seagrass growth.
20 The results of Experiment 2 show that average sulfide concentration in pore water near
21 1 mM reduced by more than half the production of shoots, rhizome and roots, and the
22 elongation rate of the horizontal rhizome and resulted in a 40 % mortality of expanding
23 *Cymodocea rotundata* clones, which suggests that the active meristematic tissues at the
24 apex of the horizontal rhizomes are vulnerable to sulfide. These results are consistent
25 with previous demonstrations of detrimental effects of sulfide on seagrasses (Goodman

1 et al., 1995; Holmer and Bongaard, 2000), and provide support to the hypothesis that
2 relate seagrass loss with increased levels of sulfide in seagrass sediments (Carlson et al.,
3 1994). These results also suggest that the vegetative expansion and colonization of new
4 habitat by seagrasses may be constrained by sediment conditions (i.e. high
5 concentrations of sulfide in pore water).

6
7 However, the addition of sulfide to the sediment of a developed *Cymodocea*
8 *rotundata* meadow did not cause a similar reduction in leaf growth and shoot density
9 even when the sulfide loading rate was twice that applied to the expanding seagrass
10 clones. This contrasting responses suggest that (1) young shoots in isolated horizontal
11 rhizomes colonizing new substrata might be more susceptible to sulfide toxicity,
12 probably related to their higher respiratory demand (Kraemer and Alberte, 1993) than
13 shoots within a developed meadow, and (2) the effect of sulfide additions to the
14 sediment might depend on shoot density. Indeed, the concentration of pore water sulfide
15 reached higher levels in unvegetated than in vegetated sediments receiving the same
16 sulfide loading rate, and also when sediments with a few and many shoots were
17 compared. These results suggest that the demonstrated the capacity of seagrasses to
18 modify sediment conditions (Lee and Dunton, 2000; Enríquez et al., 2001; Marbá and
19 Duarte 2001) depends on plant abundance. Strong negative effects of increased levels of
20 sulfide in pore water on the *C. rotundata* rhizomes colonizing new habitat, but no
21 effects on a developed meadow suggest that the colonization of strongly reducing
22 sediment would proceed at a much lower rate than that set by the potential elongation
23 rates of the horizontal rhizomes and, furthermore, it would depend on the rate at which
24 plant abundance increases in the meadows and gradually changes sediment conditions
25 to more favorable ones.

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Various evidence indicate that the capacity of seagrasses to modify sediment conditions and, in particular, the state of reduction of the sediment (redox potential, pore water sulfide) depends on the release of oxygen into the sediment by seagrass roots, which in turn depends on the photosynthetic rates of seagrasses and, ultimately, on light availability (Pedersen et al., 1998; Connel et al., 1999; Terrados et al., 1999; Lee and Dunton, 2000; Enríquez et al., 2001). It is probable, therefore, that the effects of sedimentary conditions on seagrass performance might be dependent on the local availability of light. The experimental manipulations of sediment conditions in this study were done under high light availability for water turbidity was low both at Silaqui Island (cf. Bach et al., 1998; Terrados et al., 1998) and in the mesocosm. It can be surmised that the experimental sulfide additions could have had stronger negative effects on the plants if performed under sufficiently low light conditions as to reduce the photosynthetic rate of the plants and the amount of oxygen possibly released by the roots into the sediment.

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Oxygen release by seagrass roots provides a mechanistic explanation for the apparent relationship between seagrass abundance and magnitude of the effect of the additions of sulfide to the sediment. In the sulfide-addition experiments, about 390 mmol O₂ m⁻² day⁻¹ and 780 mmol O₂ m⁻² day⁻¹ would be required to oxidize sulfide at the experimental loading rates of 156 mmol sulfide m⁻² day⁻¹ and 313 mmol sulfide m⁻² day⁻¹, respectively. Given an estimated oxygen flux from *Cymodocea rotundata* roots to the sediment of 90 mmol O₂ m⁻² root d⁻¹ (Pedersen et al., 1998), the root-mediated oxygen released by the monospecific *C. rotundata* bed in Silaqui island would be about 58 (±1.0) mmol O₂ m⁻² d⁻¹ (assuming a root surface of 0.65 m² root m⁻² sediment, cf.

1 Duarte et al., 1998) which is insufficient to oxidize all of the added sulfide. Indeed, the
2 significantly higher sulfide concentrations in the medium- and high-sulfide plots
3 indicate that the oxygen released by the plants was not able to oxidize all the sulfide
4 added into the plots. However, the lack of negative effects of sulfide on the plants
5 suggests that the oxygen release by the plants in the well-developed meadow was
6 enough to maintain an oxic microzone in the rhizosphere, and prevent the toxic effects
7 of sulfide.

8

9 Siltation-associated changes in the sediment are potentially detrimental to
10 seagrasses. Our results show that different components of the changes in sedimentary
11 conditions associated with siltation may have different effects on seagrasses. An
12 increase in siltation of the sediment, as indicated by an increase in the amount of silt and
13 organic matter, may not be detrimental to seagrasses. Pore-water sulfide concentrations
14 of 1mM negatively affected clonal expansion, but had no effects on leaf growth and
15 density of shoots within a dense meadow. Siltation is a complex process that involves
16 simultaneous changes in several environmental conditions, both in the sediment and in
17 the water column, and seagrasses respond to these changes in an integrated manner.
18 Our results provide evidence that siltation may particularly impair the colonization of
19 sediments by sensitive seagrass species, such as that investigated here.

20

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22

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4

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22

1 Table 1. Mean (standard deviation, n = 6) of the concentration of total organic matter, phosphorus,
 2 nitrogen, and grain size distribution of the "sandy", "medium-silt", and "high-silt" sediments used in
 3 Experiment 1. Mean values followed by different letters are significantly different ($p < 0.05$, Tukey HSD
 4 test). * $P < 0.05$

	Sandy Sediment	Medium silt sediment	High silt sediment	ANOVA F value
Organic matter (% of DW)	1.19 (0.00)^a	2.64 (1.08)^{ab}	4.84 (1.74)^b	7.27*
Total phosphorus (% of DW)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	3.34
Total nitrogen (% of DW)	0.01 (0.00)^a	0.02 (0.01)^{ab}	0.04 (0.01)^b	7.32*
Coarse sand (% of DW)	40.22 (2.12)	32.70 (8.70)	23.41 (2.89)	4.28
Fine sand (% of DW)	59.65 (2.11)	61.59 (4.22)	64.87 (0.80)	1.83
Silt (% of DW)	0.14 (0.01)^a	5.72 (4.56)^{ab}	11.72 (3.60)^b	5.61*

5

6

7

1 Table 2. Mean (standard deviation, n = 6) biomass, size, rhizome + root to shoot ratio, shoot production,
 2 and nitrogen (N) and phosphorus (P) content of *Cymodocea rotundata* in the "sandy", "medium-silt", and
 3 "high-silt" sediments used in Experiment 1. Mean values followed by different letters are significantly
 4 different (p<0.05, Tukey HSD test). * p<0.05 ** p<0.01

	Sandy sediment	Medium silt Sediment	High silt sediment	ANOVA F value
Leaflength (cm)	10.24 (4.82)^a	12.59 (6.63)^b	12.43 6.73)^{ab}	4.91**
mg leaves shoot⁻¹	20 (10)^a	30 (0.00)^b	30 (0.00)^b	6.69**
mg ramet⁻¹	50 (7)^a	60 (10)^b	60 (10)^b	4.22*
n shoots day ⁻¹	0.54 (0.25)	0.72 (0.33)	0.86 (0.18)	2.15
Rhizome and root to shoot ratio	1.68 (0.58)	1.24 (0.48)	1.02 (0.24)	3.23
mg rhizome shoot ⁻¹	20 (20)	20 (0.00)	20 (10)	0.47
Internode length (cm)	3.50 (1.37)^a	3.83 (1.56)^b	3.78 (2.02)^b	6.73**
Root length (cm)	12.12 (6.08)^a	13.25 (7.88)^a	14.91 (10.01)^b	6.96*
mg root shoot ⁻¹	10 (0.00)	10 (10)	10 (0.00)	0.75
Leaf P (% of DW)	0.23 (0.01)^a	0.26 (0.03)^{ab}	0.27 (0.02)^b	3.90*
Horizontal rhizome P (% of DW)	0.12 (0.02)^a	0.16 (0.03)^b	0.16 (0.03)^b	7.39*
Root P (% of DW)	0.18 (0.03)	0.20 (0.03)	0.22 (0.03)	2.34
Leaf N (% of DW)	2.33 (0.03)	2.72 (0.40)	2.06 (0.82)	2.66
Horizontal rhizome N (% of DW)	0.72 (0.12)	1.05 (0.14)	1.12 (0.45)	2.86
Root N (% of DW)	1.11 (0.08)	1.15 (0.16)	1.28 (0.13)	2.89

5

6

1 Table 3. Mean (standard deviation) of the different variables used to characterize the response of
 2 *Cymodocea rotundata* plants located at the edge of the meadow to the addition of sulfide to sediment
 3 surrounding the apex of the horizontal rhizome (Experiment 2). The statistical significance of the
 4 differences between control and sulfide-treated plants was analyzed using a two-sample t-test (control
 5 plants, n = 4; sulfide-treated plants, n = 3). *p<0.05

	Control plants	Sulfide-treated plants	Two-sample t-test t (p value)
Leaf length (cm)	6.49 (1.03)	7.57 (1.82)	1.00
Leaf width (cm)	0.37 (0.06)	0.38 (0.08)	0.16
mg DW shoot ⁻¹	20 (10)	20 (0.00)	0.21
mg ramet ⁻¹	58.11 (10.56)	51.42 (2.03)	0.34
n shoots day⁻¹	0.14 (0.04)	0.06 (0.019)	2.87*
Internode length	2.92 (0.95)	2.63 (1.04)	
mg DW rhizome day⁻¹	1 (0.00)	0.4 (0.00)	3.16*
n internodes day ⁻¹	0.17 (0.6)	0.082 (0.02)	2.25
cm rhizome day⁻¹	0.44 (0.15)	0.18 (0.08)	2.70*
Root length(cm)	6.55 (0.50)	6.36 (0.00)	0.43
mg DW root day ⁻¹	1 (0.00)	10 (0.00)	1.89
n roots day⁻¹	0.17 (0.04)	0.068 (0.02)	3.80*

6

7

- 1 Table 4. Mean (standard deviation, n = 3) of areal biomass of the different ramet components, leafsheath
 2 to leaf blade (LS-LB) and rhizome plus root to shoot (RS) ratio and net change in shoot density of
 3 *Cymodocea rotundata* in control, medium-sulfide, and high-sulfide plots at the end of the Experiment 3.
 4 Mean values followed by different letters are significantly different ($p < 0.05$, Tukey HSD test). * $p < 0.05$

	Control plots	Medium-sulfide Plots	High-sulfide plots	ANOVA F value (p value)
Leaf blades (g DW m⁻²)	22.62 (2.20)^b	20.71 (0.22)^b	13.6 (3.96)^a	9.91*
Leaf sheaths (g DW m⁻²)	23.32 (2.91)^b	17.58 (3.20)^{ab}	13.16 (4.30)^a	6.27*
Horizontal rhizome (g DW m ⁻²)	104.74 (5.66)	87.18 (2.84)	81.82 (9.14)	1.69
Vertical rhizome (g DW m ⁻²)	27.45 (15.58)	26.01 (4.10)	18.46 (10.12)	0.66
Roots on vert. rhiz. (g DW m ⁻²)	106.03 (30.14)	107.62 (25.64)	59.47 (20.17)	0.75
Roots on horiz. rhiz. (g DW m ⁻²)	19.88 (43.07)	14.05 (8.67)	25.00 (13.10)	3.20
Dead mass (g DW m ⁻²)	68.89 (3.09)	48.28 (6.59)	37.35 (24.72)	3.48
LS to LB ratio	0.97 (0.03)	1.20 (0.19)	0.05 (0.11)	2.46
RS ratio	5.62 (1.25)	6.15 (0.80)	7.17 (1.37)	1.36
Net change in shoot density (n shoot shoot ⁻¹ day ⁻¹)	0.28 (0.06)	0.21 (0.16)	0.18 (0.08)	0.59

Table 5. Average (\pm standard deviation) sediment characteristics of shallow bottoms devoid of seagrass vegetation in Southeast Asia (including values from Cape Bolinao) and those colonized by *Cymodocea rotundata* in Cape Bolinao

	Unvegetated sediment (Kamp-Nielsen et al., 2001)	Sediment colonized by <i>Cymodocea rotundata</i> (Halun, 2001) n=5
Silt (% of DW)	27 (2)	6.49 (2) to 12 (3)
Fine sand (% of DW)	46 (2)	21.91 (7) to 68 (3)
Coarse sand (% of DW)	28 (3)	27.24 (9.) to 73 (7)
Organic matter (% of DW)	6 (0)	1.83 (1) to 8 (1)
Pore water N (μ M)	324 (75)	0.58 (0) to 1 (0)
Pore water sulfide	not measured	17 (2) to 115 (81)

Figure captions

Figure 1. Mean of (a) biomass of ramet (b) biomass of leaves shoot⁻¹ (c) rhizome plus root to shoot ratio and (d) shoot production day⁻¹ of *Cymodocea rotundata* in sandy, medium and highly silted sediments. Different letters indicate significant differences between treatments ($p < 0.05$, Tukey HSD test). Error bars indicate + 1 standard deviation. (n=6)

Figure 2. Sulfide concentration in the pore water of a) control, sulfide-treated plants and unvegetated plots (Experiment 2), and b) of control, medium-sulfide and high-sulfide plots (Experiment 3). Different letters indicate significant differences between treatments ($p < 0.05$, Tukey HSD test).

Figure 3. Mean of a) shoot b) rhizome and c) root production and d) rhizome elongation rate of control and sulfide treated *Cymodocea rotundata*. Different letters indicate significant differences between treatments ($p < 0.05$, Tukey HSD test). Error bars indicate + 1 standard deviation. (n=3-4)

Figure 4. Mean of absolute (a) and relative (b) growth rate of the leaves of *Cymodocea rotundata* in control, medium-sulfide and high-sulfide plots in four time periods during Experiment 3. Error bars indicate + 1 standard deviation.

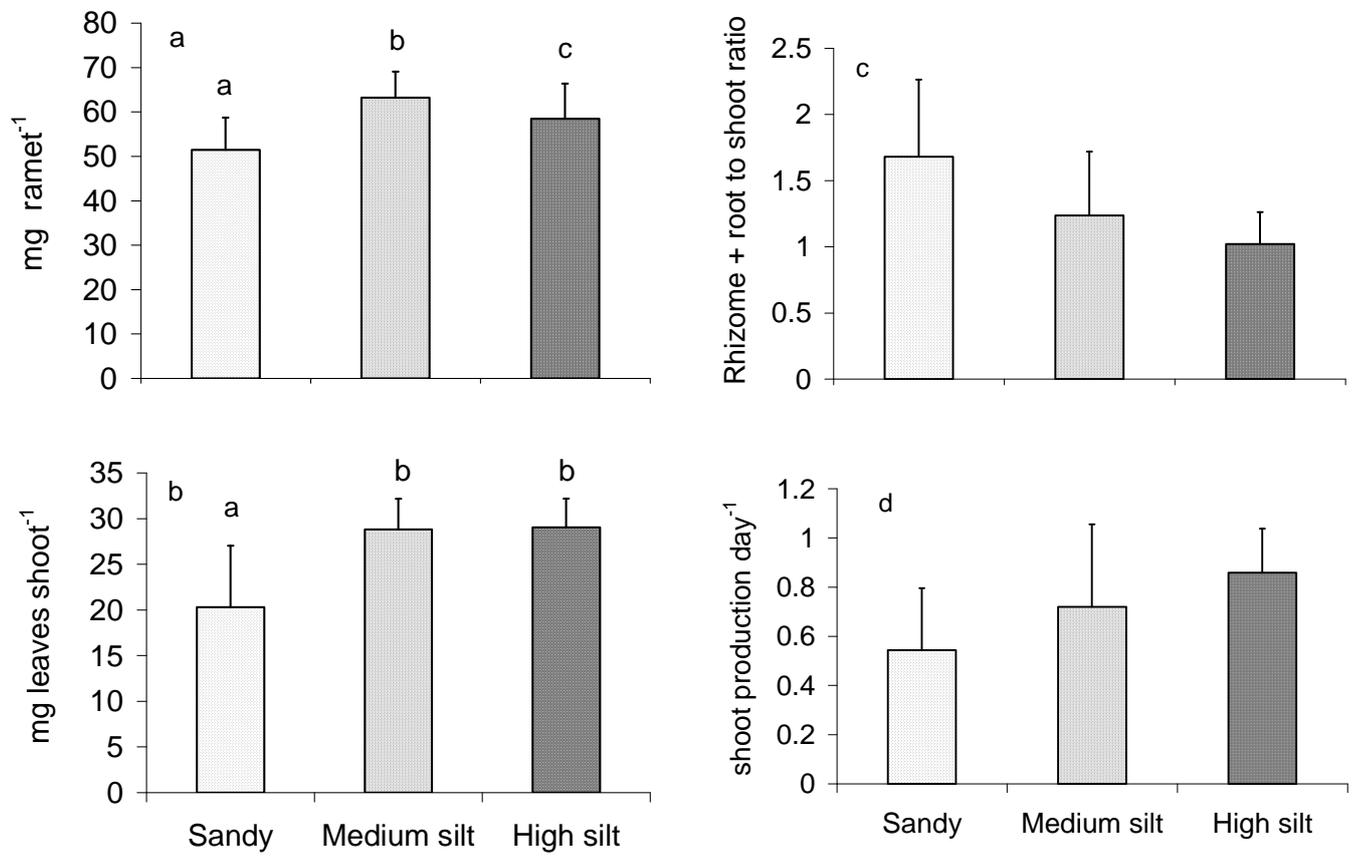


Figure 1. Halun et al.

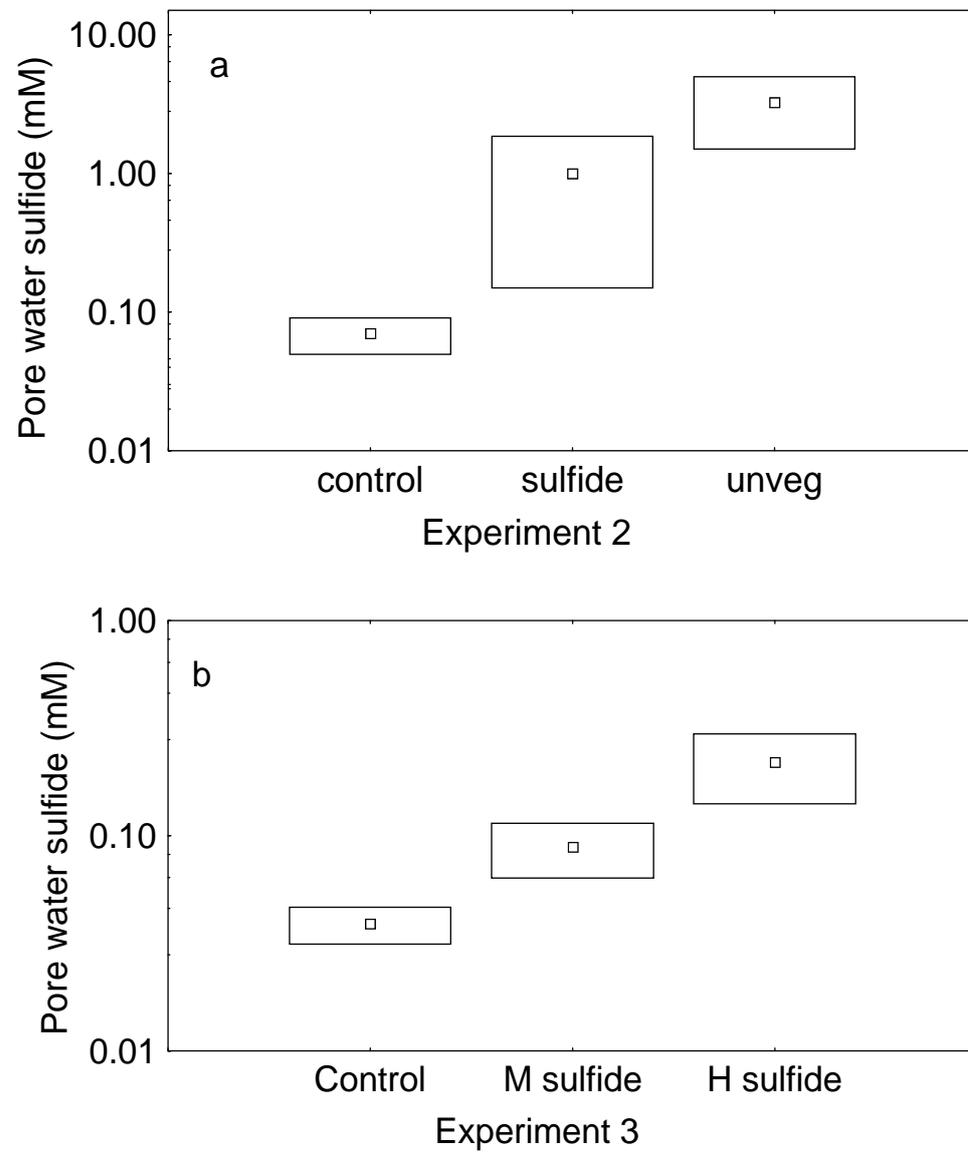


Figure 2. Halun et al.

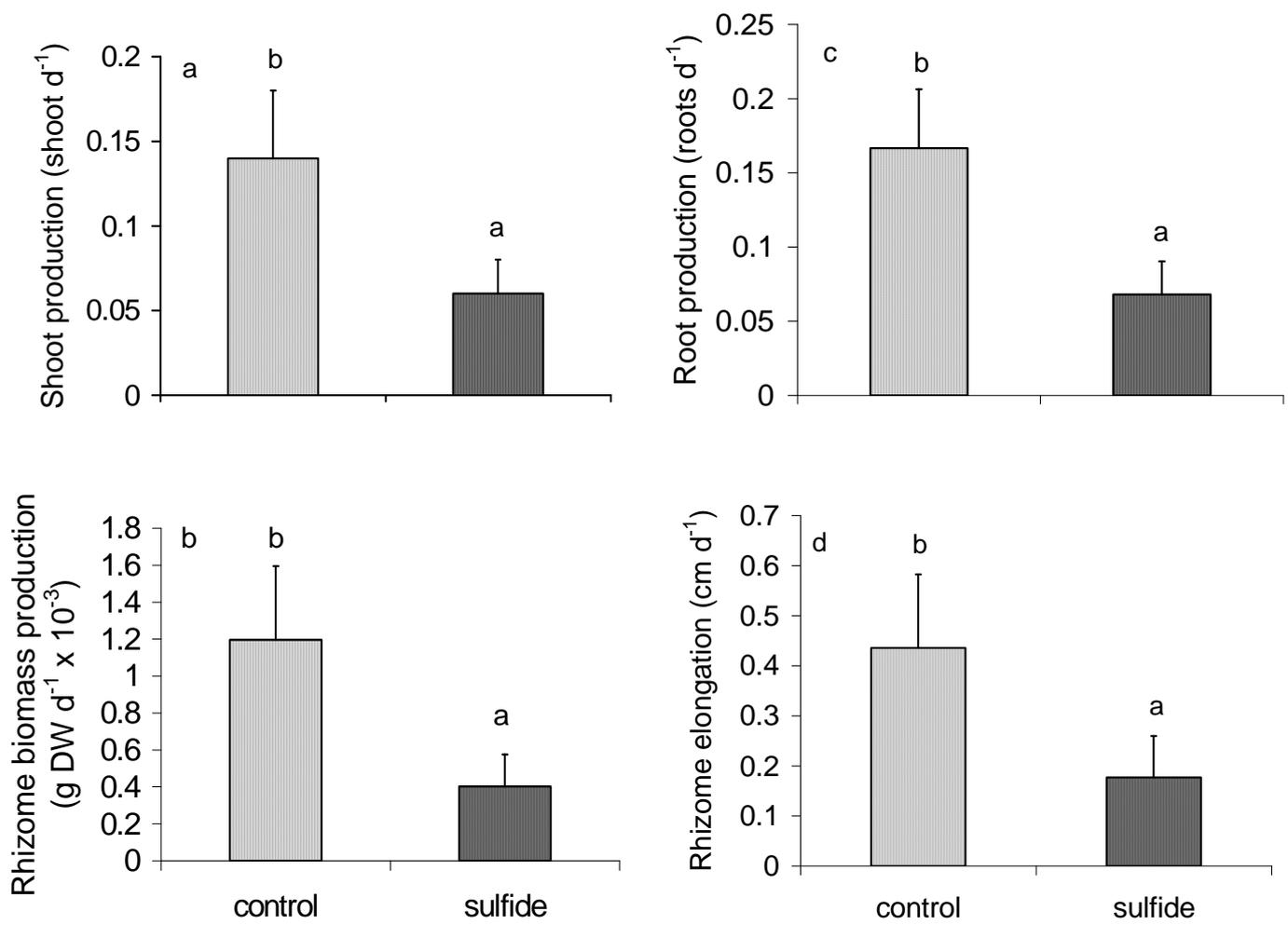


Figure 3. Halun et al.

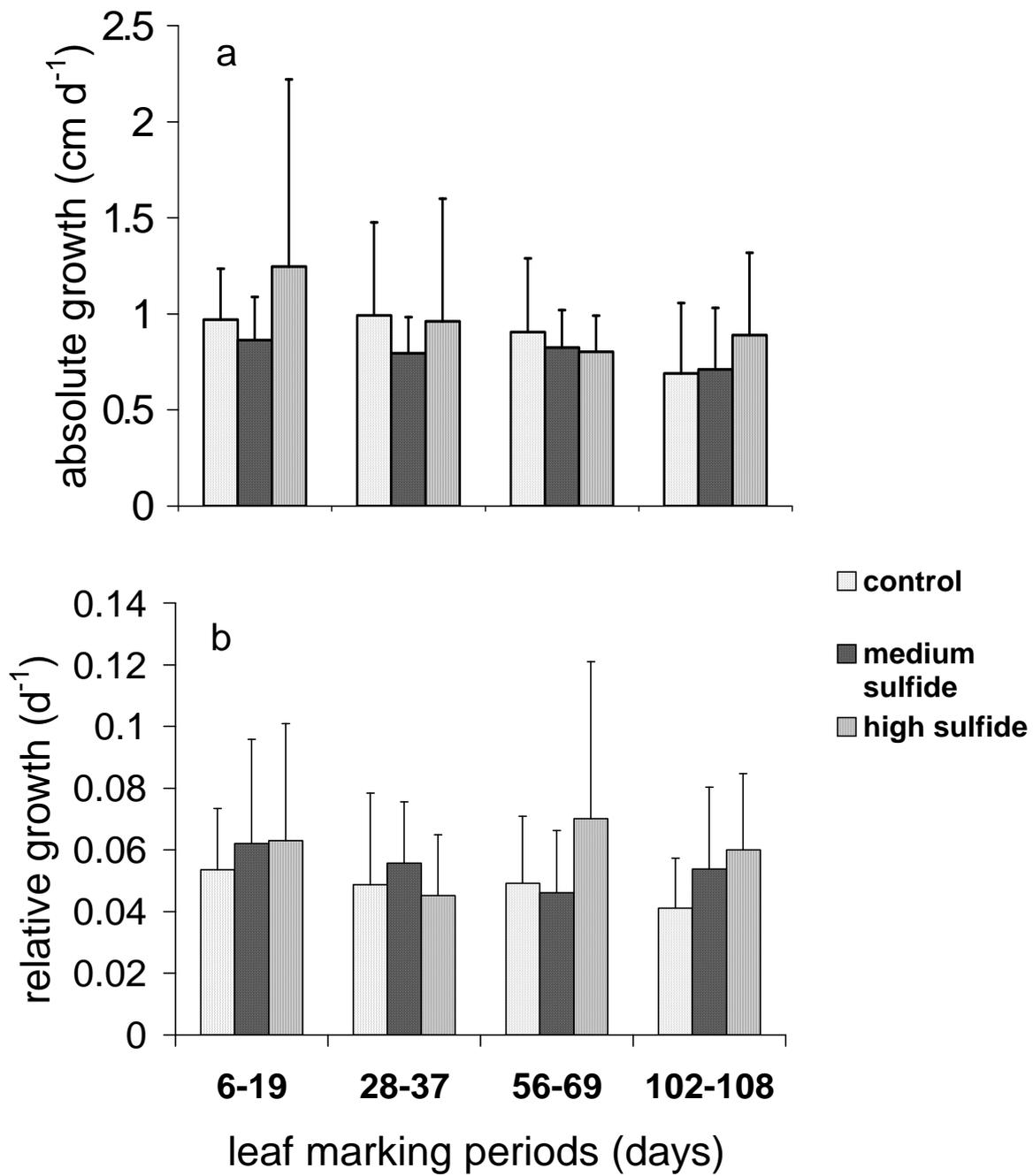


Figure 4. Halun et al.