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## Short-Term Sediment Burial Effects on the Seagrass Phyllospadix scouleri

by Carrie Craig, Sandy Wyllie-Echeverria, Emily Carrington, and Deborah Shafer

**BACKGROUND:** Seagrass meadows are highly productive and ecologically important ecosystems. Their leaves attenuate water flow, trap sediment from the water column; their roots stabilize and aerate the surrounding sediments. Seagrasses serve as nurseries and, in some cases, food for marine mammals, sea turtles, waterfowl and invertebrates (Hemminga and Duarte 2000; Kenworthy et al. 2006). Worldwide they are declining, in large part due to increasing pressure from anthropogenic impacts, such as nutrient enrichment, dredging, and shoreline modification (Short and Wyllie-Echeverria 1996; Orth et al. 2006).

Seagrasses in the genus *Phyllospadix*, also known as "surfgrasses," differ from others in that they inhabit exposed, rocky shore intertidal and subtidal environments (den Hartog 1970, Phillips and Meñez 1988). Like other seagrasses, *Phyllospadix spp*. play an important role in coastal ecosystems. *P. scouleri* and *P. torreyi* provide nursery habitat and food for the California spiny lobster, *Panulirus interruptus*, (Castaneda-Fernandez de Lara et al. 2005a, 2005b) and *P. scouleri* is critical foraging habitat for the endangered East Pacific green turtle, *Chelonia mydas* on the Pacific side of the Baja Peninsula (Lopez-Mendilaharsu et al. 2005). Recent work suggests that the surfgrass *P. iwatensis* provides structure to trap and retain allochthonous subsidies such as algal drifts and subtidal sea urchins (Hori 2006).

While human impacts to *Phyllospadix* spp. from sewage outfalls (Littler and Murray 1975) and oil spills (Foster et al. 1988) have been shown, the burial responses of *Phyllospadix* spp. have not been evaluated. Surfgrasses are likely to be impacted by beach nourishment and shoreline protection projects that place sand either directly or indirectly onto surf grass beds. Since the roots and rhizomes of *Phyllospadix* spp. attached to rocks are normally exposed, their responses to sediment burial may differ from other seagrasses whose roots and rhizomes are normally covered with sediments.

**PURPOSE:** This technical note summarizes the results of an experiment designed to investigate the short-term effects of sediment burial on the surfgrass *Phyllospadix scouleri* (Figure 1). *P. scouleri* inhabits rocky shorelines from Southeast Alaska to Baja California along the Northeast Pacific coast of North America (Wyllie-Echeverria and Ackerman 2003).



Figure 1. *P. scouleri* at Cattle Point, San Juan Island, Washington. *Photo: R. C. Phillips* 

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STUDY DESIGN AND METHODS: This study was designed to evaluate the survival and productivity of P. scouleri subjected to a range of burial depths from 3 to 25 cm. On 24 August 2007, 25 individual experimental units of P. scouleri were collected from tide-pools located in depths between 0.1 m and 1 m above mean lower low water at Mar Vista beach, on the semi-exposed western side of San Juan Island, Washington, USA (N 48°28'35.2", W 123°04'2.5"). An experimental unit is defined as a discrete assemblage of basal meristem and foliage leaves (referred to as the aboveground component) with attached roots and rhizomes (referred to as the belowground component for consistency with other seagrass studies). Shoot number within each experimental unit ranged from 8 to 50 individual shoots. Each experimental unit was carefully removed with a putty knife to minimize damage to roots and rhizomes. An attempt was made to collect samples of approximately the same size and with a minimal amount of visible flowering. Within 2 hr of collection, samples were transported to the laboratory and placed in a 1-m-diam outdoor mesocosm connected to a flow-through seawater system (Figure 2). Water in the tank was maintained at a depth of 40 cm. A HOBO temperature logger (TidbiT v2 Temp, UTBI-001) recorded water temperature in the mesocosm during the experiment. Water temperature in the tank fluctuated daily over the course of the experiment, ranging from 10.5 to 15.8°C (Figure 2) with a mean temperature of 11.6°C. This mean temperature was similar to late summer temperatures on a lower intertidal beach within 3 miles of the collection site (18 August - 28 September 2006: range =  $10-16.1^{\circ}C$ , mean temp =  $12.0^{\circ}C$ ).

Burial treatments were initiated 5 days later, on 29 August 2007, in order to allow experimental units time to adjust to the mesocosm environment. Each separate experimental unit was planted and buried upright in the sediment matrix in a 15-cm-diam plastic pot (Figure 3). Quikrete® play sand was used as proxy sediment for burial treatments because this sand is sterilized and free of contaminants. Using a Ro-Tap shaker and nest of sieves, average grain size was determined to be within the range of "coarse sand" (mean =  $1.64\Phi \pm 0.17$  (± standard error)), which is similar to a sediment grain size found in natural *P. scouleri* beds where samples were collected. Sand was soaked for 3 days in a flow-through seawater tank prior to use. Pots were cut according to the depth of the sediment burial treatment, perforated, and lined with permeable landscape cloth to increase



Figure 2. The mesocosm with all 25 experimental units.



Figure 3. Experimental units were buried in pots trimmed according to treatment (from left, nonburied, buried in 3 cm, 9 cm, 15 cm, and 25 cm of sediment).

water flow through the sediment. Experimental units were buried to a depth of 3, 9, 15, and 25 cm with five replicate pots for each burial depth treatment. The 3-cm and 9-cm treatments were chosen because other species have been reported to have critical thresholds for sedimentation near these depths (*Cymodocea nodosa*, 5 cm yr<sup>-1</sup>; *Halophila ovalis*, 2 cm yr<sup>-1</sup>; *Posidonia oceanica*, 5cm yr<sup>-1</sup>; *Zostera noltii*, 2 cm yr<sup>-1</sup> (Erftemeijer and Lewis 2006)). As a non-buried control, five pots held experimental units with only the roots and rhizomes surrounded by sediment. Each unit was randomly assigned a treatment and position within the tank. The burial lasted 15 days, a period that, although short, is comparable to a 12-day burial trial of *Zostera marina* that was able to characterize differences in productivity among burial treatments (Mills and Fonseca 2003).

# **RESPONSE VARIABLES: SURVIVAL, PRODUCTIVITY, AND METABOLIC RATE:** The total number of shoots, number of flowering shoots, and average meristem length (n=10) of each experimental unit was recorded before burial, to assess uniformity. Growth was assessed by inserting a needle just below the ligule of at least 10 shoots per sample before burial and measuring the distance between holes of inner, growing leaves and those of outer, more stationary leaves after burial (Zieman 1974). The number of new leaves produced during burial was also counted.

Light-saturated photosynthesis and dark respiration rates were measured for one leaf per experimental unit before and after burial with a Clark-type oxygen electrode unit (Hansatech Oxygraph DW3). The 10-ml chamber was filled with continuously circulated, stirred, and filtered seawater maintained at 11°C (Lauda Ecoline E100). This temperature was comparable to mean tank (11.6°C) and field (12.0°C) temperatures and was chosen in an effort to simulate normal metabolic rates. The leaf segments were cut approximately 4 cm long from the second-youngest leaf. They were kept for 15 minutes in a dark water bath before measurements began. Respiration rates were estimated as the rate of oxygen uptake during a 6-minute period in a darkened oxygen chamber; the rate of oxygen production was measured for 4 minutes while the plant was exposed to a saturating light intensity of 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (Kodak projector lamp with 300W Halogen photo optic bulb). All metabolic rates were normalized to the dry weight of the sample and are expressed in units of nmol O<sub>2</sub> g dry wt<sup>-1</sup> min<sup>-1</sup>.

Above- and belowground tissue biomass were determined at the end of the study period, by separating above- and belowground material, removing sediment and attached epiphytes, weighing each sample, drying the samples for 24 hr at  $60^{\circ}$ C, and weighing them again (Ott 1990). Biomass data were expressed in units of g dry weight m<sup>-2</sup>.

**DATA ANALYSIS:** Prior to analyses, data were checked for normality of distributions with the Shapiro-Wilk test and for homogeneity of variances with the O'Brien and Levene tests. Two response variables (e.g. change in shoot density and new leaf production) did not meet assumptions of normality, and were analyzed with the non-parametric Wilcoxon ranked sum test. If burial treatment had a significant effect on a response variable (p < 0.05), a post-hoc Tukey test was applied to determine which treatment levels were significantly different.

**RESULTS AND DISCUSSION:** Burial responses of seagrasses vary by species. Seagrasses with the di-meristematic form of leaf replacement (e.g., *Cymodocea nodosa, Thalassia testudinum* (Short and Duarte 2001) exhibited increased growth rates and leaf production rates when moderately buried, although shoot mortality lowered overall productivity (Marbà and Duarte 1994). However, *Zostera noltii*, a small-bodied species with a mono-meristematic form of leaf replacement (Short and

Duarte 2001), experienced high mortality rates at all burial depths (Cabaço and Santos 2007). Similarly, *Z. marina*, another species with the mono-meristematic form of leaf replacement, but large-bodied, experienced mortality of at least 50 percent when 4 cm of the aboveground shoot was buried (Mills and Fonseca 2003).

In this study, the maximum burial depth was selected to cover the plant meristem and a portion of the leaves above it. Meristematic tissues have high metabolic rates to support cell production and growth, but these regions do not have a well-developed photosynthetic apparatus and rely on oxygen transferred from the leaves (Pregnall 1984, Greve et al. 2003). Maintenance of aerobic conditions around the meristem and rhizomes is critical for uptake and translocation of nutrients (Smith et al. 1988).

**Shoot density and morphology.** Prior to burial, there was no significant difference between the average number of shoots per experimental unit in each treatment (p = 0.67) or the average proportion of shoots that were flowering per sample in each treatment (p = 0.33). The average distance between the intercalary meristem and the base of the *P. scouleri* in this study was  $12.5 \pm 0.4$  cm (n=246). There were significant differences in the average meristem length between treatments (p = 0.03), which ranged from 8.9 cm (9-cm burial treatment) to 15.6 cm (3-cm burial treatment).

At the conclusion of the experiment, there was a significant difference in shoot counts among treatments (Table 1). However, the changes in shoot counts for experimental units buried at depths of 3, 9, and 15 cm were not statistically different from the control (Figure 4). Only those experimental units buried to a depth of 25 cm exhibited a statistically significant decline in shoot count (Figure 4).

Although experimental units of *P. scouleri* experienced greater shoot mortality at deeper burial depths (Figure 4), shoots survived relatively well under intermediate burial levels when compared to other seagrass species. *Zostera noltii*, a small-bodied species, experienced total shoot loss after 2 weeks of burial by 8 cm of sediment (Cabaço and Santos 2007). The number of *Z. marina* shoots declined by 50 percent after 24 days of burial at 8 cm depth (Mills and Fonseca 2003). *Posidonia oceanica* experienced significant mortality at 5 cm of burial (Manzanera et al. 1995). Vermaat et al. (1997) report critical burial thresholds of 10-13 cm for *Syringodium isoetifolium, Cymodocea rotundata, Thalassia hemprichii, Cymodocea serrulata, Halodule uninervis, Halophila ovalis,* and *Enhalus acoroides* growing in Philippine waters. In the current experiment, shoot counts did not decrease significantly until buried by 25 cm of sediment (Figure 4).

**Shoot growth and new leaf production.** Mean shoot growth rate decreased significantly in all burial treatments; the decrease in shoot growth rate along a gradient of increasing burial depth was approximately linear (Figure 5). However, only those plants buried at depths of 15 and 25 cm grew at significantly lower rates than non-buried controls. The non-buried controls in this experiment grew an average of  $4.7 \pm 1.0$  mm shoot<sup>-1</sup> d<sup>-1</sup>, much less than the annual growth rates reported for *P. scouleri* in Baja California of  $17.2 \pm 1.9$  mm shoot<sup>-1</sup> d<sup>-1</sup> (Ramírez-García et al. 1998).

Table 1. Results of one-way ANOVAs to detect the effect of burial on response variables.					
Response Variable	Test Statistic	Probability			
Number of Shoots Exp. Unit <sup>-1</sup>					
Pre-burial		0.67			
Post-burial	H=10.6	0.03			
New leaf production (leaves/shoot <sup>-1</sup> /day <sup>-1</sup> )	H=1.008	0.73			
Growth rate (mm/day <sup>-1</sup> )	F=6.228	0.002			
Biomass (g dry weight m <sup>-2</sup> )					
Aboveground	F=1.267	0.316			
Belowground	F=1.829	0.163			
Above- to belowground	F=0.299	0.875			
Photosynthesis (µmol O <sup>2</sup> / gdw <sup>-1</sup> / min <sup>-1</sup> )					
Pre-burial	F=0.320	0.861			
Post-burial	F=1.591	0.217			
∆ Photosynthesis	F=1.591	0.217			
Respiration (µmol O <sup>2</sup> / gdw <sup>-1</sup> / min <sup>-1</sup> )					
Pre-burial	F=0.214	0.928			
Post-burial	F=1.424	0.264			
Δ Respiration	F=1.424	0.264			
N = 5 experimental units per treatment, with the exception of the post-burial photosynthesis and					

N = 5 experimental units per treatment, with the exception of the post-burial photosynthesis and respiration rates and change in photosynthesis and respiration rates, for which one sample failed in the 25-cm burial treatment, as well as for the growth measurements, as mentioned above.



Figure 4. Average percent change in shoot count per experimental unit subjected to various burial treatments. Positive values indicate an increase in shoot density while negative values indicate shoot mortality. (Error bars represent standard error. N = 5 experimental units per treatment. Values with the same letter are not statistically different).





After a period of 15 days, the effects of burial on leaf production could not be detected (Table 1). New leaf production in the non-buried controls (mean =  $0.013 \pm 0.008$  leaves shoot<sup>-1</sup> d<sup>-1</sup>, n = 33 shoots) was also lower than the annual rate ( $0.062 \pm 0.005$  leaves shoot<sup>-1</sup> d<sup>-1</sup>) reported by Ramírez-García et al. (1998). Northern waters tend to be colder, which could slow growth, and by September, the plants in this study were nearing the end of their growing season. Also, in the mesocosms, water flow was reduced and there was no intertidal submersion-exposure regime. A diatom bloom appeared in the tank approximately 7 days into the experiment, and probably also contributed to lower growth rates. The epiphytes covered about 50 percent of the leaves and coated the sides of the tank, and although an epiphytic bloom of approximately the same intensity was observed in the field, future experiments should consider the impact of epiphytes on growth response. The finding that growth is negatively correlated ( $r^2 = 0.96$ ) with burial depth is consistent with other studies (Marbá and Duarte 1994; Mills and Fonseca 2003), although some species have been shown to increase growth rates at low levels of burial (Duarte et al. 1997; Cabaço and Santos 2007). In this experiment, *P. scouleri* growth did not increase even at low levels of burial (Figure 5).

**Biomass.** There were also no significant effects of burial treatment on root biomass, shoot biomass, or the ratio of root-to-shoot biomass between treatments (Table 1). If energy is translocated between above- and belowground tissue in response to light limitation (Alcoverro et al. 1999; Brun et al. 2003; Cabaço and Santos 2007), this experiment may not have been long enough to detect this movement.

**Photosynthesis and respiration.** Burial reduces area available for photosynthesis, and thus reduces energy available for growth and to promote oxygen flow to roots and rhizomes. A reduction in leaf area, resulting from burial, may influence photosynthetic rate by forcing a reliance on tissue

that is both older and farther from the meristem as a source of sugar. Before burial, there were no significant differences among treatments in net photosynthetic rates or rates of dark respiration (Table 1). Post-burial rates of photosynthesis and respiration were also not significantly different among treatments (Table 1). Likewise, there was no significant effect of treatment on the change in rate of photosynthesis or respiration over the course of the experiment (Table 1).

**CONCLUSIONS:** *Phyllospadix spp.* have characteristics of late successional species; they have been shown to be dominant, long-lived, persistent, and slow to recover from disturbance (Dethier 1984, Turner 1985). In Oregon, more than three years were required for *P. scouleri* to recover from removal in 0.25-m<sup>2</sup> quadrats, as vegetative growth occurred slowly and seedling recruitment was low (Turner 1985). These studies demonstrate that the effects of activities that reduce shoot density may influence population stability for several years.

Surfgrasses are likely to be impacted by beach nourishment and shoreline protection projects that place sand either directly or indirectly onto surfgrass beds. Indirect placement can occur when sand placed onto beaches near the surfgrass beds subsequently moves onto the surfgrass beds, resulting in either partial or total burial of the beds. The results of this study suggest that short-term burial results in shoot mortality, decreased shoot counts, and reduced growth of *P. scouleri*. Changes in metabolic rates and biomass allocation could not be detected, although it is possible that the duration of burial was insufficient to cause measurable changes in these variables. A longer trial might capture responses in variables that integrate plant performance over longer periods. While photosynthesis, respiration, and biomass measurements could provide insight into plant responses at the physiological level, growth and survival may be the most valuable variables for ecologists and ecosystem managers interested in population level responses. It is possible that genetic differences may account for the high variability within treatments. The *P. scouleri* samples came from different tide pools and may have faced selection pressures for different metabolic rates or resource allocation strategies. Studies considering metabolic rates and biomass allocation might try to reduce within-treatment variability by controlling for potential genetic differences.

This experiment provided an opportunity to investigate short-term burial responses in a controlled environment; however, mesocosm conditions did not adequately mimic the rocky intertidal environment where *P. scouleri* lives. Most obvious is the absence of waves, current, and tidal fluctuations that influence sediment movement. Although these results are preliminary, they have implications for projects that may result in the burial of *P. scouleri*. The effects of dredging and disposal activities on seagrasses are complex, but the most likely form of direct impact involves either physical removal or burial of vegetation at dredging and disposal sites (Sabol et al. 2005, Erftemeijer and Lewis 2006). During heavy and sustained winter storms *P. scouleri* may undergo burial multiple times, and it is not known whether the effects of separate burial events are additive. Seasonal, age-related, or phenological differences may also dictate how resistant to burial a population might be. Future work should compare the potential impact of burial during different developmental stages such as flower maturation, pollination, and the setting of seeds over the growing season; add a field-based component; and design an experiment to account for genetic variation and local adaptation to sediment dynamics.

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