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Aquatic Botany 76 (2003) 329–337

**Aquatic
botany**

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Desiccation index: a measure of damage caused by adverse aerial exposure on intertidal eelgrass (*Zostera marina*) in an Oregon (USA) estuary

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Received 23 May 2002; received in revised form 1 April 2003; accepted 9 May 2003

Abstract

Eelgrass (*Zostera marina*) leaf necrosis resulting from intertidal aerial exposure is described. A desiccation index was developed and tested to quantitatively assess damage across intertidal *Z. marina* beds in Yaquina Bay, Newport, OR, USA. Results suggest that higher intertidal plants have more desiccation damage than those growing lower in the intertidal. This damage may partially explain why high intertidal plants tend to have shorter canopy heights as leaves tended to break at desiccation damage points.

Published by Elsevier B.V.

Keywords: Eelgrass; *Zostera marina*; Desiccation; Intertidal; Aerial exposure

1. Introduction

Tidal exposure and the resulting desiccation stress is probably the most important factor limiting the upper intertidal distribution of seagrass species (Koch, 2001). This relationship has been clearly shown in tropical seagrasses where exposures to daytime low water during spring tides resulted in seasonal losses of above-ground biomass (Vermaat et al., 1993; Erftermerjer and Herman, 1994; De Iongh et al., 1995; Stapel et al., 1997). In an extreme case, the upper margin of a *Zostera noltii* bed was described as “burned” following such an

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exposure (Van Lent et al., 1991). For the temperate zone eelgrass, *Zostera marina*, numerous authors have implied that desiccation is the probable cause of changes in seagrass abundance and morphology across the tidal gradient (Bayer, 1979; Jacobs, 1979; Kentula and McIntire, 1986; Keddy, 1987; Koch and Beer, 1996). However, none of the above studies directly measured desiccation damage on individual plants.

In Yaquina Bay following spring and summer low tide exposures, we have frequently observed upper intertidal *Z. marina* leaves with large non-pigmented necrotic patches. The nature and timing of this damage suggested that it might be the result of desiccation stress. Our goal was to determine if this necrosis in *Z. marina* was the result of desiccation stress and to evaluate its incidence across intertidal gradients in Yaquina Bay.

2. Methods

2.1. Study sites

Yaquina Bay encompasses an area of approximately 1582 ha of which 35% is intertidal and 65% is subtidal (Oregon Estuaries, 1973). The majority of intertidal seagrass is located in the lower estuary where *Z. marina* beds are found in three zones (Bayer, 1979; Boese and Robbins, unpublished data): (1) a subtidal and lower intertidal zone (established bed) consisting of dense numbers of perennial shoots located below 0.25 m, mean low low water (MLLW); (2) a transition zone (0.25–0.75 m MLLW), consisting of perennial patches and scattered annual shoots; and (3) an upper zone (0.75–1.5 m MLLW), with annual shoots only. As part of a long-term study of intertidal seagrass dynamics, six transects, three on steep (mean slope = 2.4%) and three on shallow (mean slope = 0.8%) bathymetric slopes were established. Each transect covered all three *Z. marina* zones.

2.2. July samples

For development of the desiccation index, a preliminary collection of 30 vegetative shoots was made in July, 2001. Ten shoots, collected by clipping at the sediment surface, were taken from one of the steeply sloped transects at each of three tidal positions: low intertidal (L), within the established bed (–0.5 to 0.0 m MLLW), near the upper margin of the established bed (M) (0.0 to + 0.25 m MLLW), and high in the transition-upper zone (H) (approximately +0.5 m MLLW).

In the laboratory, leaves were separated by age. In North East Pacific estuaries shoots of *Z. marina* typically have 3–5 leaves (Phillips, 1984). An additional leaf which is often separating from the sheath is usually present (Boese, personal observation). This leaf is always senescent and was discarded. The remaining younger leaves were excised at the top of the sheath, numbered from 1 to 5 according to age (1 being the youngest) and arranged in parallel on a plexiglass sheet. Epiphytes were then removed by wiping each leaf with a damp sponge and the cleaned leaves were assessed individually for desiccation damage.

Desiccation damage is characterized by small to large (10–500 mm²), non-uniform, non-pigmented areas with smooth margins (Fig. 1A and B). This type of damage is distinctly different from a wasting disease-like necrosis (Den Hartog, 1987) which is dark brown or

(A)



(B)

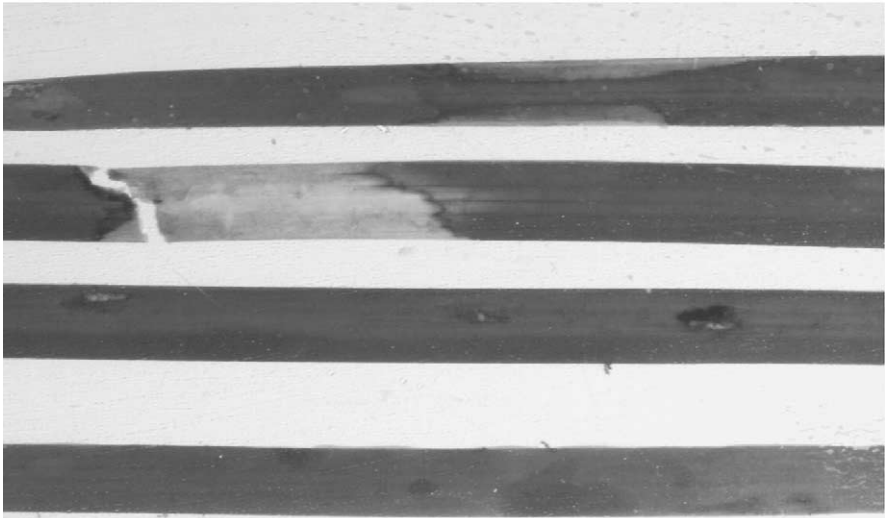


Fig. 1. (A) Typical patterns of desiccation damage on field collected *Z. marina* leaves. Damage areas are large non-pigmented areas with smooth margins shown on lowest two leaf sections. Upper leaf has no damage for comparison. (B) Desiccation damage on *Z. marina* leaves 14 days after leaf sections were dried using a heat gun. Note breakage in damaged area of the second leaf from the top which occurred during leaf placement for the photograph. For comparison, lower two leaf sections are undamaged.



Fig. 2. Desiccation index key adapted from Burdick et al. (1993) wasting disease index key. White areas in key represent desiccation damaged leaf sections.

black in coloration and tends to be exhibited in elongated streaks along the longitudinal leaf axis (Boese, personal observation). In addition, wasting disease-like necrosis is rare in Yaquina Bay during summer months.

For each leaf, desiccation damage was scored as a percentage ($\pm 1\%$) of the total leaf area using an adaption of Burdick et al. (1993) wasting index key which was modified to reflect desiccation damage morphology (Fig. 2). These evaluations were done separately by two individuals and their independent evaluations averaged to determine the desiccation index value for each leaf. For the July samples, the effects of leaf age and tidal position on desiccation damage were evaluated separately using one-way ANOVA with differences between means tested using Tukey's test for multiple pairwise comparisons.

2.3. August samples

In August, 2001, 230 *Z. marina* vegetative shoots were collected to determine the relationship of desiccation damage to tidal exposure and bathymetric slope (steep versus shallow). These shoots were collected across all six transects in the same manner as in July, with the exception that shoots were collected at four heights: S (below -0.5 m MLLW), L within the established bed (-0.5 to 0.0 m MLLW), M (0.0 to $+0.25$ m MLLW), and H (approximately $+0.5$ m MLLW). As in the July samples, ten shoots were collected at each tidal position within each transect with the exception of the highest tidal position where one of the transects had no vegetative shoots, resulting in an unbalanced experimental design. Shoots

were transported to the laboratory, rinsed in seawater then immediately sealed in plastic bags and stored (4 °C) for not more than 4 days before examination of desiccation damage.

Prior to excising leaves, the length of each collected plant (canopy height), number of leaves per shoot and the width of the widest leaf was measured. As in the July study senescent leaves were discarded. Based on the July results (see further), only the oldest remaining leaf (usually leaf 4) was evaluated for desiccation, however, in contrast to the July samples, leaves were evaluated for desiccation damage by three individuals. As in the July samples these three values were averaged to determine the desiccation index value for each leaf. Also noted was whether the leaf was broken and if the break appeared to have occurred at a desiccation point (i.e. no pigmentation at the break point). The effects of tidal height and bathymetric slope on plant metrics and desiccation index was assessed using two-way ANOVA with differences between means tested using Tukey's test.

2.4. Laboratory test

Because of concerns that the observed damage was due to some other factor than desiccation, a laboratory experiment was performed to demonstrate that drying leaves under controlled laboratory conditions could produce similar damage patterns to those observed in field collected leaves. Whole plants (with at least 5 cm of attached rhizome) were collected from a subtidal area of Yaquina Bay. In the laboratory, leaves were gently cleaned with a damp sponge and the leaves placed under damp paper towels with the exception of a 2 cm section of each leaf located approximately 10 cm above the sheath. This section was heated with a heat gun until the exposed section was visibly dried (leaf edges beginning to curl). Eight of these treated plants were then submersed in 40 l aquaria which were supplied with flow-through sea water (salinity >25‰, 12–15 °C) and under fluorescent grow-lights. These plants were maintained in an upright position by pushing the rhizome into a short section of PVC pipe which served as a weight. Plants were examined for damage 4, 7, and 14 days after treatment.

3. Results and discussion

3.1. Damage description

Desiccation damage on field-collected *Z. marina* leaves was usually concentrated on the distal third of the oldest leaves (Fig. 1A). This indicates that the damage had occurred at some time before the shoots were collected, as it would take time for a damaged area to lose all pigment and for the leaf to elongate. We have also observed that during some extreme low tides in the spring and summer, *Z. marina* leaves appeared to dry close to the sheath as that portion of the shoot was elevated above the sediment while the distal portions of the leaves were lying on the moist sediment surface where they presumably would not be as vulnerable. In these instances, dried but still pigmented sections of *Z. marina* leaves were observed that were approximately the same size and shape as the non-pigmented patches used to estimate desiccation damage. In some cases leaves which had not emerged from the sheath also appeared to be damaged.

Desiccating exposures were not limited to summer months as similar events have been observed during daylight winter low tides on cloudless and windy days. Similar damage morphology has also been observed on high intertidal collected plants following freezing conditions (Boese, personal observations). Thus, it is likely that desiccation-like damage may occur at any time of the year when shoots are exposed to adverse aerial conditions.

Drying leaves under controlled laboratory conditions resulted in damage that was similar to that observed in field-collected plants (Fig. 1B). In the laboratory experiment, heat-treated leaf sections began losing pigment four days after treatment with all pigment being lost from treated sections after 7 days. Shoots continued to grow after treatment: treated leaves elongated further and new leaves appeared, with the youngest damaged leaves exhibiting the most growth. After 14 days leaves were easily broken at damaged points as is illustrated in Fig. 1B.

3.2. Effect of tidal bathymetry

In the July samples there was a significant effect of tidal position on desiccation index (one-way ANOVA, $P < 0.001$), with apparent increases in damage with increasing tidal elevation (Fig. 3). In August, we found a similar significant tidal elevation effect (two-way ANOVA, $P < 0.001$) and damage again increased with tidal position (Fig. 4). There was no effect of bathymetric slope on desiccation index values (two-way ANOVA $P > 0.05$) and we also found no significant interaction between slope and tidal elevation.

For August desiccation index values there was considerable variation in how the three observers scored individual leaves. The within leaf mean coefficient of variation (CV) was 36% with the greatest variation occurring in leaves collected from the subtidal (CV = 42%), where desiccation damage was lowest. However, this observer variation was much less than the variation in leaves across all tidal positions (mean CV = 152%). Analysis of desiccation damage by three-way ANOVA using tidal position, slope and individual observer as factors was also attempted. Although the unbalanced designed precluded the ability to determine interactions, as with the previous two way-ANOVAs on mean desiccation index values, only

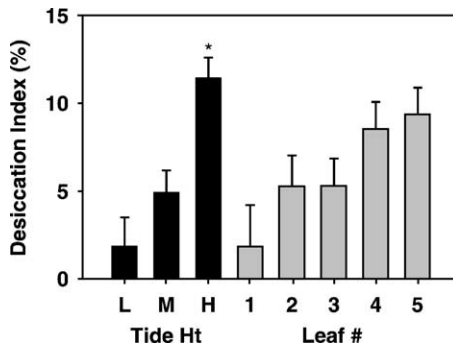


Fig. 3. July sampling results: effect of tidal position and leaf age on desiccation index. Values are mean desiccation index (%) + 1 S.E. L: lower intertidal; M: margin of perennial bed; H: transition zone. Leaf # 1–5 represent leaf ages from the youngest (1) to the oldest (5). (*) Significantly different from other values (Tukey's test, $P < 0.05$).

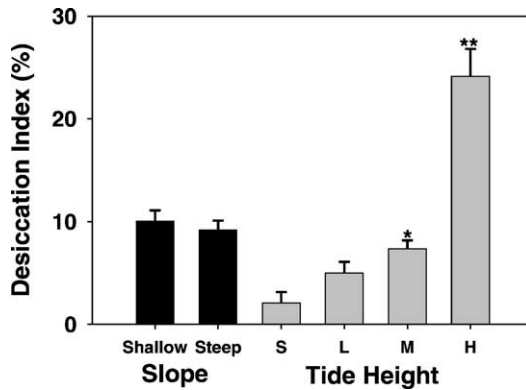


Fig. 4. August sampling results: effect of bathymetric slope and tidal position on desiccation index. Values are mean (%) + 1 S.E. S: subtidal; L: low intertidal; M: upper margin of perennial eelgrass bed; H: high intertidal. (**): Significantly different from all other values (Tukey's test, $P < 0.05$); (*) significantly different from S value.

tidal position had a significant effect ($P < 0.001$). Slope and observer did not significantly affect desiccation index values ($P > 0.05$).

The presence of any desiccation damage in subtidally collected shoots was a surprise. It is possible that these shoots were exposed to aerial conditions two weeks prior to our collections as the lowest tides of the month (approximately 0.3 m lower) occurred at that time. However, we cannot exclude the possibility of an additional factor causing similar necrosis.

As has been observed in previous studies (Bayer, 1979; Jacobs, 1979; Kentula and McIntire, 1986), canopy height and leaf width decreased with increasing tidal exposure (Table 1). In addition, canopy height was significantly greater on shallow bathymetric slopes (Table 1). These canopy height differences may in part be due to physical stresses. Higher intertidal plants are subjected to a longer duration of higher summer aerial temperatures and freezing winter temperatures than plants lower in the intertidal (Boese and Robbins, unpublished data). Steep slopes would not only tend to drain at higher rates than shallow slopes, they may also be subjected to higher wave and current energies which have been implicated in reduced leaf length (Koch, 2001). Leaf length is also correlated with the duration of water coverage (Jacobs, 1979; Kentula and McIntire, 1986) which depends on upon both tidal position and micro topographical features (depressions and seeps).

Desiccation damage also may contribute to shorter leaf length if damaged leaf sections tend to break more easily. While 60–70% of the oldest blades from the August collections were broken, in intertidal *Z. marina* (tidal position L, M, H), 70% of these leaves were broken at points in the leaf which had desiccation damage (Table 1). In contrast only a small and statistically different portion of these breakages were associated with desiccation damage points in S leaves (Table 1). In contrast to tidal position, there was no effect of bathymetric slope on the percentage of leaves that were broken at desiccation points (Table 1).

Based on the results of this study it is likely that desiccation during low tide exposures directly damages portions of exposed leaves, resulting in loss of photosynthetic pigments and eventual leaf breakage. If damage is severe (see lowest leaf Fig. 1A), pigmented portions of leaves above damaged areas may not be useful to the plant as a whole.

Table 1
Eelgrass measurements and observation from July and August samples

Tide height	Canopy height (cm)	Leaf width (mm)	Leaves per shoot	Leaves broken (%)	Broken at desiccation point (%)
July					
S	101.0 ± 2.7 c	8.3 ± 0.4 b	ND	50	ND
M	76.1 ± 3.6 b	8.3 ± 0.2 b	ND	80	ND
H	42.8 ± 7.8 a	4.6 ± 0.2 a	ND	60	ND
August					
S	155.6 ± 5.0 c	9.1 ± 0.2 c	3.7 ± 0.2 a	61.7 ± 6.2	13.3 ± 6.0 a
L	85.3 ± 5.0 ab	7.6 ± 0.2 b	3.9 ± 0.2 a	71.2 ± 6.3	54.2 ± 6.0 b
M	66.4 ± 3.8 b	6.9 ± 0.2 b	4.0 ± 0.1 a	61.0 ± 4.8	43.0 ± 4.6 b
H	43.9 ± 12.4 a	3.7 ± 0.1 a	5.7 ± 0.4 b	66.8 ± 15.6	42.0 ± 15.0 b
Slope					
Steep	74.3 ± 4.1 a	6.7 ± 0.2	4.6 ± 0.2 b	66.9 ± 5.2	36.1 ± 5.0
Shallow	101.3 ± 4.8 b	7.0 ± 0.2	4.0 ± 0.2 a	64.9 ± 6.1	40.2 ± 5.7

Values are means ± 1 S.E. which were compared using one-way ANOVA (July samples) and two-way ANOVA (August samples) using tidal position and bathymetric slope as factors. For the August samples, no significant interaction was found between tidal position and slope. Percentages of leaves broken and leaves broken at desiccation point values are for the oldest, non-senescent leaf (usually leaf # 4 or 5). ND: data not taken. Values with the same or no grouping letter (a, b, c) are not significantly different (Tukey's test, $P < 0.05$).

We found that using our modification of wasting disease index method to evaluate the extent of desiccation damage on *Z. marina* leaves was easy and rapid. This was especially true in the August samples as only the oldest, non-senescent leaf were evaluated. This simplified procedure was possible as the preliminary sample analysis showed that the oldest non-senescent leaves (leaf # 4 and 5) tended to have the most damage (Fig. 3).

Acknowledgements

Thanks to Luke Dennahan and Neal Morphiuss, both Saturday Academy interns who helped with portions of this study. Thanks also to Walt Nelson, Chris Andersen, Doug Bulthuis and Craig McFarlane for their initial reviews of the manuscript. I am also appreciative of the critical and extensive comments by J.E. Vermaat and an anonymous Aquatic Botany reviewer which greatly improved the paper's rigor and readability. This study has been funded wholly by the US Environmental Protection Agency. It has been subjected to the Agency's peer and administrative review, and it had been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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