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Comparison of photosynthetic characteristics of the seagrass congeners *Zostera marina* L. and *Zostera japonica* Ascher. & Graeb.

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ABSTRACT

On the Pacific coast of North America two seagrass species in the genus *Zostera* co-exist; the native species *Zostera marina*, and an introduced species, *Zostera japonica*. These two species typically occupy separate tidal elevations, with *Z. marina* occupying the lower intertidal and shallow subtidal zones, and *Z. japonica* occupying the mid- to upper intertidal zone. This study was designed to compare the photosynthetic characteristics of *Z. japonica* and *Z. marina* after exposure to high and low light. Nursery pots containing *Z. japonica* and *Z. marina* were grown intermixed in replicate mesocosm tanks at two different light levels (50 and 150 μmol photons m^{-2} s^{-1}). We measured photosynthetic parameters for *Z. japonica* and *Z. marina* leaf segments and whole plants (WP). *Z. japonica* leaf segment photosynthetic efficiency (α) was greater than that of *Z. marina* and based on the high photosynthetic rate, α and saturating irradiance, we suggest that *Z. japonica* is high light adapted. Whole plant (WP) photosynthetic rates were similar 123 ± 11 vs. 155 ± 21 μmol O_2 gDW^{-1} h^{-1} for *Z. marina* and *Z. japonica* respectively. However, the WP respiration rate of *Z. japonica* was 2 fold greater than *Z. marina*. Consequently, *Z. marina* would be expected to acquire and store more carbon than *Z. japonica*. We suggest that light limitation does not explain the observed disjunct vertical distribution of these two species and that other factors (e.g. rhizome growth, branching frequency and seed germination, etc.) likely play a large role in controlling *Z. japonica* colonization.

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1. Introduction

At least two seagrass species in the genus *Zostera* occur on the Pacific coast of North America: the native species *Zostera marina* L., and an introduced species, *Zostera japonica* Ascher. & Graeb. *Z. japonica* was first reported along the Pacific Coast of North America in 1957 (Hitchcock et al., 1969) and is thought to have been introduced along with oyster seed stock imported from Japan (Harrison and Bigley, 1982). In North America, these two species typically occupy separate zones, with *Z. marina* occupying the lower intertidal and shallow subtidal zones, and *Z. japonica* occupying the mid- to upper intertidal zone (Harrison, 1982a; Thom, 1990; Bulthuis, 1995; Britton-Simmons et al., 2010). However, in some areas, the vertical distribution of these species overlaps, and evidence indicates that density and biomass of both species are reduced in the presence of the other, leading to sparse mixed species stands (Harrison, 1982a; Bando, 2006). Consequently, concerns have been expressed regarding the potential for displacement of *Z. marina* by *Z. japonica*, and the impacts of this displacement on ecosystem structure and function (Bando, 2006).

Congener comparison suggests that *Z. japonica* exhibits morphological and life-history characteristics (e.g. high reproductive output, small size) that make it a successful colonizer of previously unoccupied mudflat (Ruesink et al., 2010). However, data have not been available to assess whether underlying physiological differences between the species can explain the observed zonation patterns. Since photophysiology underlies growth, production and distribution of any plant, photosynthetic parameters are critical to predicting potential spread of this non-native species in North America.

It has been suggested that *Z. japonica* is confined to the intertidal zone by light limitation, in both its native and introduced range. However, these conclusions were based on field observations of depth zonation (Huong et al., 2003) and experimental manipulations with growth rate as the primary metric (Harrison, 1982b). The objectives of this study were to evaluate the observed zonation patterns in relation to photosynthetic capacity.

It has been hypothesized that light limitation confines the distribution of *Z. japonica* to the shallow intertidal zones (Harrison, 1982b; Huong et al., 2003). This is consistent with a prevailing paradigm in seagrass ecology that light limitation controls the distribution of seagrasses, particularly at the deepest edges of the meadow (Ralph et al., 2007). Based on this hypothesis, we would expect *Z. japonica* to be less photosynthetically efficient and to have

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higher saturating irradiance than *Z. marina*. By growing *Z. japonica* and *Z. marina* intermixed at high and low light levels and subsequently measuring photosynthetic characteristics, we evaluate some of the photophysiological differences that may underlie the observed disjunct vertical distribution of these seagrasses in North America.

2. Methods

2.1. Plant collections and experimental treatments

Yaquina Bay is a tidally dominated, drowned river valley estuary located along the central Oregon coast, USA. Environmental conditions are summarized in previous publications (Kaldy, 2006; Lee and Brown, 2009; Shafer et al., 2011). Differences in plant stature and architecture of both above and below-ground tissues required the use of different collection and culture methods for the two species. *Z. japonica* from Yaquina Bay, is characterized by leaf lengths of ca. 15–20 cm, shoots densities of between 2000 and 10,000 shts m^{-2} (Kaldy, 2006) and thin, brittle rhizomes that are not conducive to bare-shoot transplanting. In contrast, *Z. marina* leaf lengths can exceed 130 cm with densities of 60–140 shts m^{-2} (Kaldy and Lee, 2007), and large robust rhizomes.

Z. japonica samples used in this experiment were collected (24 May 2010) haphazardly from the Idaho Flats portion of Yaquina Bay within a 50 m radius of 44.614167 N lat., 124.028417 W long. Using a hand trowel, 72 *Z. japonica* plugs containing intact plants with root material and associated sediments were harvested at low tide and placed into plastic nursery pots (10 cm × 10 cm × 10 cm). The sediment composition of the *Z. japonica* experimental population was 70–90% sand (Kaldy, 2006). Each *Z. japonica* nursery pot contained between 10 and 30 individual shoots.

Approximately 120 *Z. marina* shoots (without sediments) were harvested by hand from near the deep edge of the permanent bed at Idaho Point approximately 1.3 m below MLLW during low tide. Shoots were returned to the lab in coolers within 1 h of collection. Seventy-two shoots selected for use in the experiment were cleaned of epiphytes with a wet sponge; rhizomes were trimmed to a standard length of five nodes with a razor blade and leaves were trimmed to 50 cm. A single *Z. marina* shoot was transplanted into each nursery pot (13 cm diameter × 11 cm deep), filled with estuarine sand from Yaquina Bay and held overnight in flow-through seawater.

Each pot was labeled with a unique identifier code and randomly assigned to mesocosm tanks and treatments. The next day, 144 seagrass pots ($n = 72$ for each species) were transferred to six replicate mesocosms (1.5 m deep × 1.8 m long × 1.2 m wide), each supplied with light from a 1000 W metal halide grow light (Sunlight Supply Inc., Vancouver, WA, USA) centered over the tank on a 14:10 L:D cycle. In each mesocosm, two PVC shelves were held at a depth of 35 cm below the surface of the water. Instantaneous photon flux density (PFD) in the tanks was about 175–200 μmol photons m^{-2} s^{-1} just below the surface of the water. Plants were acclimated to these conditions for 10 d. After acclimation, one of the shelves in each tank was lowered to the bottom using ropes; this was considered the low light treatment (target PFD about 30 μmol photons m^{-2} s^{-1}). The other shelf remained at 35 cm depth and was considered the high light treatment (target PFD of >100 μmol photons m^{-2} s^{-1}). Light levels were chosen to be representative of the compensation irradiance and above the saturation irradiance for both *Z. marina* and *Z. japonica* (Touchette and Burkholder, 2000; Shafer et al., 2011). Thus each mesocosm tank had six replicates of each species growing under low and high light conditions (Fig. 1).

Mesocosm tanks were maintained at a target temperature of 14 ± 1 °C using a combination of flow through seawater (exchange rate was 200% d^{-1}), chillers, and heat exchangers. Temperature and salinity were monitored daily using a calibrated YSI 650 handheld meter (YSI Inc., Yellow Springs, OH, USA). Temperature was continuously monitored using Hobo tidbit temperature loggers (Onset, Pocasset, MA, USA) programmed to take measurements at 15 min intervals. Hobo HLI light loggers (Onset, Pocasset, MA, USA) were used in each mesocosm tank to verify the L:D cycle. Irradiance levels were verified in all tanks daily using a datalogger (LI-1400, LI-COR, Lincoln, NB, USA) with a spherical quantum sensor (LI-193SA, LI-COR, Lincoln, NB, USA). In three mesocosm tanks PFD was continuously monitored for the duration of the experiment at both depths using a LI-COR 1400 with underwater spherical quantum sensors (LI-193SA). Light levels were measured every minute; values were averaged every 15 min and stored. The 15 min averages were integrated and summed for hourly and daily estimates. During the initial 10 d acclimation period epiphytes were not removed. Once the experimental treatments began, tanks, light sensors, and plants were cleaned of epiphyte/periphyton accumulations every 3–5 days by manually wiping epiphytes from leaves and then filtering the tank with a small pump and filter. After holding plants at treatment conditions for 18–19 d, sub-samples of each species were randomly collected from both high and low light treatments from each tank for *P* vs. *E* curve determination.

2.2. Photosynthetic measurements

Photosynthesis–irradiance (*P* vs. *E*) relationships of *Z. marina* and *Z. japonica* plants cultured at high and low light levels were assessed in the laboratory using a Hansatech liquid-phase oxygen electrode system (Oxylab controller with DW3 chamber, Hansatech Instruments Ltd., Norfolk, England). *P* vs. *E* measurements for *Z. japonica* were made as described in Shafer et al. (2011). Minor modifications made for *Z. marina* include use of a single piece of leaf tissue harvested from the middle of the second leaf. For *Z. japonica*, 4–6 leaf segments taken from the middle of the second leaf from either a single or multiple shoots were used. Excised leaf segments were placed in filtered water at the same salinity and temperature (14 °C) and held in the dark for 30 min. The water jacket of the DW3 chamber was attached to a circulating water chiller to maintain chamber temperature at 14 °C. An external red LED light source (640 nm λ) was fitted to the chamber window (LH36/2R light array, Hansatech Instruments Ltd., Norfolk, England) and the light levels in the chamber were controlled via the Oxylab software. Light levels were verified using a LI-COR 1400 with a cosine corrected sensor (LI-190SA, LI-COR, Lincoln, NE, USA). The samples were kept in the darkened chamber for 5 min to allow for equilibration, and a 10 min measurement of dark respiration was made. Following respiration measurements, the samples were sequentially exposed to 5, 10, 15, 20, 60, 125, 300, and 800 μmol photons m^{-2} s^{-1}. The samples were exposed to each light level for 8 min to reach equilibrium rates of oxygen evolution (μmol ml^{-1} min^{-1}). Once the oxygen evolution measurements were completed, the tissue sample was removed from the chamber, measured for leaf area and dried to constant weight at 70 °C. A separate tissue section was harvested from the same leaf, measured for area, blotted dry to obtain fresh weight and processed for chlorophyll content using a DMSO extraction (Andersen et al., 1991; Shafer et al., 2011). Oxygen evolution rates were normalized to the dry weight (μmol O_2 gDW^{-1} h^{-1}) and chlorophyll content (μmol O_2 mg chl $a+b^{-1}$ h^{-1}) of the sample.

2.3. Photosynthetic curve-fitting

Photosynthetic parameters were calculated by curve fitting using the Smith–Talling function (Lederman and Tett, 1981;