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Effects of salinity on photosynthesis and respiration of the seagrass *Zostera japonica*: A comparison of two established populations in North America

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ABSTRACT

Photosynthetic responses were quantified for two *Zostera japonica* Aschers. and Graebn. populations from the northern and southern limits of distribution exposed to a range of salinities along the Pacific Coast of North America. Plants were collected from Padilla Bay, Washington (northern) and Coos Bay, Oregon, USA (southern) and cultured together in experimental tanks at 3 salinities (5, 20 and 35) under saturating irradiance for 3 weeks. Subsequently, photosynthesis–irradiance (*P* vs. *E* curves) relationships for leaf segments from the two populations were assessed using an oxygen electrode system. We found no evidence for diel rhythms in either light saturated photosynthesis (P_{max}) or dark respiration (R_d). For the Padilla Bay population, P_{max} ranged from 192 to 390 μmol O_2 g DW^{-1} h^{-1}; for the Coos Bay population P_{max} ranged from 226 to 774 μmol O_2 g DW^{-1} h^{-1}. Photosynthetic maxima of the Coos Bay plants occurred at a salinity of 20, whereas salinity had no effect on the photosynthetic maxima of the Padilla Bay plants. There were significant differences in leaf tissue R_d among salinity treatments but the two populations responded similarly to salinity. North American populations of *Z. japonica* are best adapted to intermediate salinities, displaying minimum R_d rates, lower compensation irradiance, higher saturation irradiance, and greater P_{max} rates at a salinity of 20. Additionally, the southern population may be better adapted to southward expansion along the Pacific Coast and changes associated with global climate change.

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1. Introduction

The seagrass *Zostera 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). For the last few decades, distribution of this species has been limited to British Columbia, Washington, and Oregon. Within this range, dramatic expansions have occurred in some areas (Posey, 1988; Baldwin and Lovvorn, 1994; Dumbauld and Wyllie-Echeverria, 2003). However, the recent discovery of new populations near Humboldt Bay, California, represents a southward range extension. Harrison and Bigley (1982) suggested that this species had only colonized a small fraction of the available suitable habitat, and that changes in the ecology of the intertidal flats were likely to result from continued spread of this species throughout its potential range. Concerns have been expressed regarding the potential for displacement of the native *Zostera marina* L. by *Z. japonica*, and the impacts of this displacement on native ecosystem structure and function (Bando, 2006). Management decisions require information regarding the potential for establishment and spread of introduced species, as well as their potential impacts to native ecosystem structure and function. In the case of *Z. japonica*, these decisions are complicated by the shortage of ecophysiological data available for this species either in its native range or on the American West Coast (Green and Short, 2003).

Although most seagrasses inhabit marine salinity regimes (Tyerman, 1982), several species are reported to be euryhaline (Den Hartog, 1970; Biebl and McRoy, 1971; Ralph, 1998). In addition to the expansion of *Z. japonica* along the coast, there has been an increase in the abundance and distribution across the salinity gradient within estuaries as well. Dudoit (2006) reports that in the South Slough National Estuarine Research Reserve (NERR) in Coos Bay Oregon, *Z. japonica* had moved 3.2 km upriver from the point of first introduction as reported by Posey (1988). Kaldy (2006) suggested that freshwater seeps may provide a desiccation refuge for *Z. japonica* in the high intertidal. Young et al. (2008) found that the distribution of *Z. japonica* in lower Yaquina Bay increased areal coverage by 400% between 1998 and 2007. In Yaquina Bay there was substantial spread of *Z. japonica* into many of the less saline slough areas of the lower estuary (Young et al., 2008). Between 2002 and 2009 Shafer and Kaldy (pers. obs.) have noticed that *Z.*

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japonica appeared to occur further upriver in Yaquina Bay. Taken together, this suggests that *Z. japonica* may be fairly euryhaline and tolerates a wide range of salinities which could affect its ability to colonize potential habitat. Although a wide range of factors control the occurrence and spread of non-native species (including seed viability and dispersal, germination success, predation pressure, etc.), an adequate positive carbon balance is critical for establishment and survival of any plant. Consequently, we focus our initial effort on evaluating how *Z. japonica* photosynthesis responds to large, chronic changes in salinity.

The photosynthetic capability of the seagrass congeners *Z. marina* (see review by Touchette and Burkholder, 2000) and *Z. noltii* Hornem. (Jiménez et al., 1987; Vermaat and Verhagen, 1996; Plus et al., 2005) have been well studied relative to *Z. japonica*. Several studies have identified diel endogenous rhythms in seagrass photosynthesis, with high rates during morning hours and depressed rates in the afternoon (Libes, 1986; Durako and Kunzelman, 2002; Silva and Santos, 2003). The presence of diel endogenous rhythms could lead to misinterpretation of data; however, the phenomenon has not been investigated in *Z. japonica*. Previous studies have evaluated the growth and morphological response of several *Zostera* congeners to chronic salinity changes (Kerr and Strother, 1985; Kamermans et al., 1999; Vermaat et al., 2000; Nejrup and Pedersen, 2008) and short-term photosynthetic responses under hypersaline (>35) conditions (Biebl and McRoy, 1971), but few have examined response to low salinity or longer-term (2–3 weeks) effects of high or low salinity (although see Ralph, 1998). Salinity ranges were on the order of 0–132 for *Z. marina* and *Z. muelleri*, while salinities of 15 and 35 were used for *Z. noltii*. There are few measurements of *Z. japonica* photosynthetic parameters in general and even fewer in relation to osmotic stress (Ogata and Matsui, 1965).

Huong et al. (2003) suggest that in Vietnam *Z. japonica* is stenohaline, occurring within the salinity range of 22–32. Based on observed expansions, we hypothesize that *Z. japonica* is more euryhaline than most seagrass species, withstanding salinity fluctuations from about 1 to 34. We use photosynthetic characteristics as metrics to evaluate its salinity tolerance. The goal of this study is to evaluate the physiological tolerances underlying the observed patterns of *Z. japonica* distribution, and predict the potential for expansion of this species to additional areas within its established range as well as outside the limits of its current range on the Pacific Coast of North America. The objectives of this study were to compare and contrast the photosynthetic responses of two *Z. japonica* populations located near the northern and southern limits of distribution for this species, across the range of salinities experienced within estuaries of the American Pacific Northwest. Experiments conducted at the boundaries of species' distribution are critical to understanding factors limiting the spread of introduced species (Byers et al., 2002).

2. Description of study areas

2.1. Padilla Bay, Washington

Padilla Bay is a relatively shallow, sheltered embayment located in northern Puget Sound, along the mainland coast of Washington. Tides are mixed semidiurnal, with a maximum tidal range of about 4 m (Bulthuis, 1995). Salinity ranges from 21 to 30, depending on freshwater inflow from the Fraser, Skagit, and Samish Rivers (Bulthuis, 1995). Padilla Bay has one of the largest seagrass areas in the Pacific Northwest (Bulthuis, 1995), representing about 16% of the total within the Puget Sound region (Wyllie-Echeverria and Ackerman, 2003). In 1989, Padilla Bay contained approximately 2900 ha of intertidal and subtidal *Z. marina* beds, 324 ha of intertidal *Z. japonica*, and 137 ha of *Ruppia maritima* (Bulthuis, 1995). *Z. japonica* occurs within an elevation range of +0.1 m to +0.8 m, relative to mean lower low water (MLLW) (Thom, 1990). *Z. japonica* samples used in this experiment were collected from the northern portion of Padilla Bay, on the south side of Samish Island (48.574657N lat., 122.538577W long.).

2.2. Coos Bay, Oregon

Coos Bay is a tidally dominated, deep draft, drowned river valley estuary located along the central Oregon coast. The system has mixed semidiurnal tides and a maximum tidal range of about 2 m (Lee and Brown, 2009). Salinity ranges between about 20 and 30 depending on freshwater inflow and site within the estuary (Lee and Brown, 2009). As of 2003, Coos Bay contained about 500 ha of *Z. marina* (Thom et al., 2003), *Z. japonica* is known to be present (Posey, 1988; Shafer, 2007) but its areal extent has not been summarized. *Z. japonica* specimens for this experiment were collected from a bed near Day Creek inlet, a tidal creek in the South Slough National Estuarine Research Reserve (NERR) at Coos Bay (43.316181N lat., 124.311290W long.). At the time of collection at low tide, salinity in the creek was measured at 0 using a refractometer. Thus, we expect that this population is subject to rapid daily fluctuations in salinity associated with tidal cycles.

3. Methods

3.1. Plant collections and experimental treatments

The effects of salinity on the photosynthesis–irradiance (*P* vs. *E*) relationships of these two populations of *Z. japonica* were assessed in the laboratory using a Hansatech liquid-phase oxygen electrode system (Oxylab controller with DW3 chamber, Hansatech Instruments Ltd., Norfolk, England) across a range of salinities that may be experienced by plants along the estuarine salinity gradient. Using a hand trowel, 10 cm sediment plugs containing intact plants with root material were harvested at low tide from intertidal beds of *Z. japonica*. Thirty plugs were harvested from Padilla Bay, Washington, transported to the laboratory facility at Newport, Oregon, and placed in white polycarbonate tanks (60 cm × 60 cm × 90 cm) within 8 h of collection. Each tank was supplied with >400 μmol photons m^{-2} s^{-1} from 1000 W metal halide lamps on a 14:10 L:D cycle. All three tanks were maintained at a temperature of 25 ± 2 °C using submersible aquarium heaters. Similarly, 30 plugs were collected near Day Creek Inlet in Coos Bay, Oregon and randomly assigned to treatment tanks within 4 h of collection. Each sample plug contained between 10 and 30 *Z. japonica* shoots. Samples were collected during the summer (June) when biomass was expected to be near its seasonal maximum (Thom, 1990; Kaldy, 2006). The photosynthetic temperature optimum for North American populations of *Z. japonica* is between 20 and 30 °C (Shafer et al., 2008) and a similar range of 18–23 °C was reported by Lee et al. (2005) in Korea. Plants were allowed to acclimate at room temperature (25 °C) for 24–48 h at a salinity of 32 before being adjusted to test salinities (5, 20, and 35). Culture media consisted of ambient seawater from Yaquina Bay; salinities were reduced using reverse osmosis water or increased using Red Sea Salts (Red Sea Fish Pharm Inc., Eliat, Israel). Reverse osmosis removes many of the impurities associated with tap water (ions, chlorine, etc.) but retains some nutrients. Initial nutrient concentrations were not measured; however, after 4 days media nitrogen concentrations ranged between <1 and 11 μM $NO_2 + NO_3$ and 1–11 μM NH_4. After 10 days all nutrient concentrations in all tanks were <2 μM. After holding plants at test salinities for 18–19 days, three sub-samples from each population were collected from each of the salinity treatments for *P* vs. *E* curves (details below). In order to account for

any diel variability, curves were run over the course of several days and sampled in a random sequence during the day (between the hours of 08:00 and 16:00). All treatments were continuously bubbled with air to maintain non-limiting CO_2 supplies in the culture media.

3.2. Photosynthetic measurements

Since photosynthetic rates within each leaf are known to vary from leaf tip to sheath (Enríquez et al., 2002), an effort was made to minimize this potential source of variance by selecting leaf sections from the mid-point of the second youngest leaf on each shoot. These leaf sections were in the range of 1.5–2.0 cm in length. Each sample was a composite of four to six leaf sections (from the same plant); care was taken to avoid visibly damaged tissue when selecting samples. Length and width measurements were taken for each piece of tissue using digital calipers. Leaf segments were placed in filtered water at the same treatment salinity and temperature (25 °C) and held in the dark for 30 min. Small sections (<3 cm^2) of 400 μm clear plastic mesh were used to hold multiple pieces of plant tissue in place side by side in order to avoid shading. We verified that the clear plastic mesh had no detectable impact on photon flux density reaching the leaf surface.

P vs. *E* curves were run using a Hansatech Oxylab™ liquid-phase oxygen electrode system with a DW3 chamber (Hansatech Instruments Ltd., Norfolk, England). This system uses a Clark-type electrode (Delieu and Walker, 1972) located in the floor of the water-jacketed DW3 (110 mm × 75 mm × 100 mm) reaction chamber. The volume of water in the chamber was 7 ml for each curve. Prior to each run (before introducing plant material), the oxygen concentration of the chamber media was reduced to 0.280 ± 0.010 μmol O_2 ml^{-1} by bubbling with N_2 gas. The water jacket of the DW3 chamber was attached to a circulating water chiller so that the temperature in the chamber was maintained at 25 °C. An external red LED light source (640 nm λ) was fitted to a chamber window (LH36/2R light array, Hansatech Instruments Limited, England) and the light levels in the chamber were controlled via the Oxylab software. Light treatments were verified using a LI-COR 1400 with a cosine corrected sensor (LI-COR, Lincoln Nebraska). The sample was placed in the chamber using the support loops on the chamber plunger such that the plant tissue was perpendicular to the light source. 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, 60, 125, 300, 750, and 1000 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 measurement was completed, the sample was removed from the chamber and further processed for chlorophyll content. 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^{-1} h^{-1}) of the sample. Photosynthetic parameters normalized to chlorophyll are not discussed here, but are tabulated for comparison to other studies and regression equations are provided for approximate conversions. Dry weight was estimated from the empirical relationship between dry weight and leaf area. Chlorophyll concentrations were determined on the same leaf segments used to determine *P* vs. *E* relationships. Leaf segments were extracted in 10 ml of 99.5% DMSO and incubated (65 °C) in the dark until pigments were fully leached (approximately 3 h) into the solvent (Andersen et al., 1991). Absorbance of the pigmented solvent was measured at 663, 649, and 470 nm using a Shimadzu UV-2101 spectrophotometer (Shimadzu, Kyoto, Japan).

3.3. Photosynthetic calculations

Photosynthetic parameters were calculated using the Smith–Talling function (Lederman and Tett, 1981; Henley, 1993). Data were fitted to this function using the Sigma Plot (version 11.0) statistical software package.

$$\text{Production} = P_{\max}\left(\frac{\alpha E}{\sqrt{P_{\max}^2 + (\alpha E)^2}}\right) + R_d$$

where $P_{\max}$ is the maximum rate of photosynthesis, R_d is the dark respiration rate, *E* is the photon flux density (μmol photons m^{-2} s^{-1}) and α (photosynthetic efficiency) is the initial slope of the photosynthetic curve with units of μmol O_2 gDW^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$. The compensation irradiance (I_c) represents the light level at which gross photosynthetic production equals respiration (net photosynthesis is zero; Bulthuis, 1987) and was determined by dividing the respiration rate by α (Henley, 1993). The saturation irradiance (I_k) was determined by dividing $P_{\max}$ by α (Touchette and Burkholder, 2000).

3.4. Data analysis

$P_{\max}$ and R_d values were tested for diel patterns (morning values vs. afternoon values) using a non-parametric, Kruskal–Wallis one-way ANOVA on ranks (SigmaPlot v. 11) because the data violated the assumptions of normality and homogeneity of variances required for parametric statistics. Photosynthetic parameters and tissue respiration rates were analyzed using a nested Analysis of Variance (ANOVA), with population nested within salinity (Sokal and Rohlf, 1995). The nested model does not have an interaction effect, thus we were unable to evaluate the potential for interaction between salinity and population. Bonferroni corrected Fisher's Least Significant Difference (LSD) tests were used to evaluate differences among salinity treatments and populations. For all ANOVA results, significance is reported as $p \leq 0.05$.

4. Results

4.1. Diel rhythms

Preliminary evaluations were made to test for the presence of diel rhythms in $P_{\max}$ and R_d (Fig. 1). One-way ANOVA on ranks indicates that there were no significant differences ($H = 1.043$, df = 1, $p = 0.307$) between median $P_{\max}$ measurements made in the morning and those made in the afternoon. Likewise, there were no significant differences in median R_d values between morning and afternoon ($H = 0.00725$, df = 1, $p = 0.932$). It should be noted that the morning sample size was smaller than the afternoon.

4.2. Photosynthetic characteristics

Both populations of *Z. japonica* exhibited typical photosynthesis–irradiance responses across the range of salinities investigated (Fig. 2). The Smith–Talling function fit the data well with R^2 values ranging between 0.91 and 0.99 (Table 1). The initial slope of the photosynthesis–irradiance curve (α) ranged from 2.2 to 21.4 (Table 1). No significant differences in the initial slope of the photosynthesis–irradiance curve could be detected between salinities or among populations within salinities (Table 2).

Both factors, population and salinity, exerted significant effects on light-saturated photosynthetic rates ($P_{\max}$) (Table 2). For the northern (Padilla Bay) population, $P_{\max}$ ranged from 192 to 390 μmol O_2 gDW^{-1} h^{-1}; for the southern (Coos Bay) population

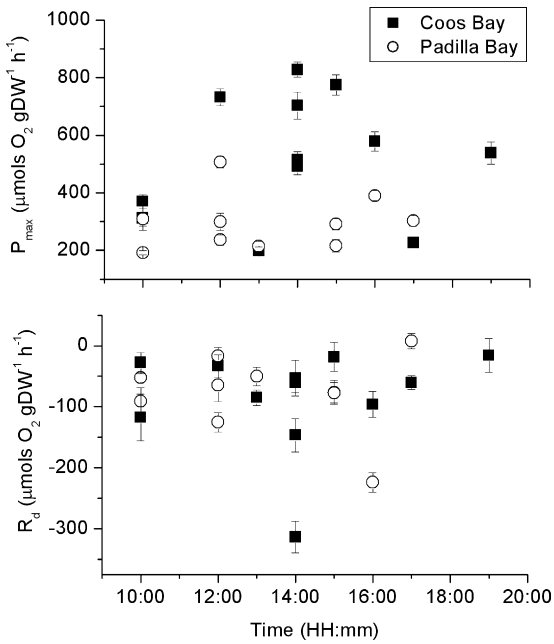

Fig. 1. *Zostera japonica* maximum rate of photosynthesis (P_{max}) and dark respiration rate (R_d) plotted against time of day. Error bars are standard error from curve fitting and in some cases are smaller than the symbol. Note differences in y-axis scales.

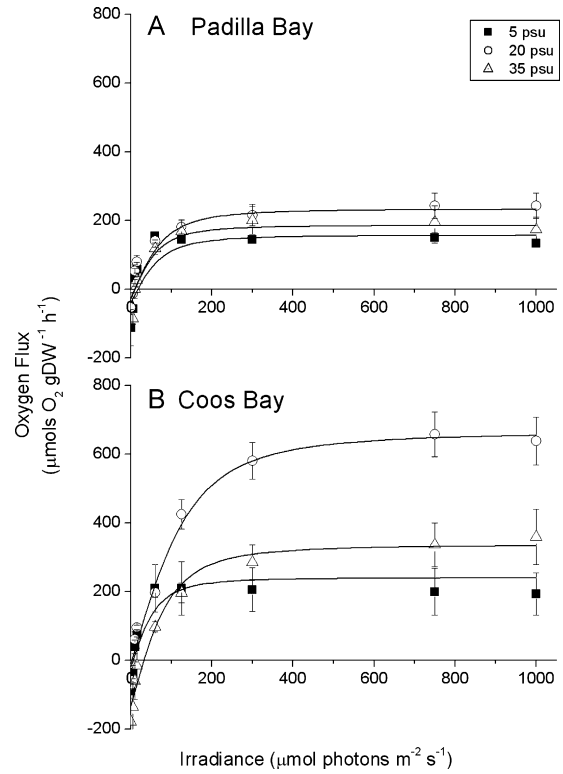

Fig. 2. The effect of salinity on the photosynthesis versus irradiance responses of *Zostera japonica* collected from Padilla Bay, Washington and Coos Bay, Oregon. Error bars indicate standard error ($n = 3$). Smith–Talling curves were fit iteratively; fit statistics are presented in Table 1.

P_{max} ranged from 226 to 774 µmol O_2 gDW^{-1} h^{-1}. For the Coos Bay population, light-saturated photosynthetic rates at a salinity of 5 were significantly lower than at 20 or 35, but light saturated photosynthetic rates at a salinity of 35 were not significantly different from those at a salinity of 20 (Table 3). No differences could be detected among salinity levels in the Padilla Bay population (Table 3).

Whereas saturation irradiance was significantly greater for the southern population than the northern population (Table 2), no significant differences could be detected due to salinity (Table 2). Compensation irradiance (I_c) was significantly affected by salinity (Table 2), and was generally lower at a salinity of 20 than at 5 or 35 (Table 3). The results of the Fisher's LSD multiple comparisons indicated that compensation irradiance at a salinity of 20 was significantly lower than at 5 (Table 3). No differences between populations within salinity could be detected (Table 2).

Table 1
Summary of photosynthetic parameters (±SE) generated by non-linear curve fitting using the Smith–Talling function for two populations of *Zostera japonica* measured at 25 °C and various salinities. Units of P_{max} and R_d for dry weight normalized samples are µmols O_2 gd w^{-1} h^{-1}, while units for α are µmols O_2 gd w^{-1} h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$. Photosynthetic parameters normalized to chlorophyll units are presented for comparison to other studies but are not discussed. Units of P_{max} and R_d for chlorophyll normalized samples are µmols O_2 mg Chl $a+b^{-1}$ h^{-1}, while units for α are µmols O_2 mg Chl $a+b^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$.

Population	Plant #	Salinity	Dry weight normalized			Chlorophyll $a+b$ normalized			
			P_{max}	R_d	α	P_{max}	R_d	α	R^2
Padilla	A048	5	192 (7)	−53 (7)	11 (1)	18 (1)	−5 (1)	1.0 (0)	0.9925
Padilla	A088	5	216 (22)	−76 (20)	17 (5)	15 (2)	−5 (1)	1.2 (0)	0.9487
Padilla	A080	5	390 (18)	−224 (16)	21 (3)	30 (1)	−17 (1)	1.7 (0)	0.9897
Padilla	A069	20	214 (17)	−50 (15)	8 (2)	36 (3)	−8 (3)	1.3 (0)	0.9707
Padilla	A050	20	302 (19)	8 (13)	2 (0)	34 (2)	1 (1)	0.2 (0)	0.9786
Padilla	A056	20	309 (24)	−91 (22)	17 (4)	37 (3)	−11 (3)	2.1 (0)	0.9696
Padilla	D066	35	291 (20)	−77 (17)	6 (1)	17 (1)	−5 (1)	0.3 (0)	0.9744
Padilla	A095	35	299 (30)	−65 (27)	8 (3)	19 (2)	−4 (2)	0.5 (0)	0.9492
Padilla	A083	35	237 (18)	−126 (16)	8 (2)	18 (1)	−10 (1)	0.6 (0)	0.9725
Coos	D094	5	226 (12)	−61 (11)	13 (2)	20 (1)	−5 (1)	1.2 (0)	0.9846
Coos	D088	5	492 (30)	−147 (27)	17 (4)	121 (7)	−36 (7)	4.2 (1)	0.9817
Coos	D096	5	312 (42)	−118 (38)	21 (7)	43 (6)	−16 (5)	2.8 (1)	0.9144
Coos	E096	20	774 (35)	−18 (24)	5 (1)	85 (4)	−2 (3)	0.6 (0)	0.9880
Coos	D069	20	538 (38)	−16 (27)	5 (1)	51 (4)	−2 (3)	0.5 (0)	0.9711
Coos	D085	20	732 (29)	−33 (19)	4 (1)	80 (3)	−4 (2)	0.4 (0)	0.9910
Coos	C097	35	370 (23)	−28 (17)	3 (1)	20 (1)	−2 (1)	0.2 (0)	0.9772
Coos	C092	35	578 (34)	−96 (21)	3 (1)	62 (4)	−10 (2)	0.3 (0)	0.9810
Coos	D067	35	514 (29)	−314 (26)	13 (3)	48 (3)	−29 (2)	1.2 (0)	0.9835

Biomass normalized parameters can be converted to chl normalized values using the following equations: $P_m = 0.125 \times -6.694$, $n = 18$, $r = 0.766$, $p \ll 0.0001$.
$R_d = 0.100 \times -0.601$, $n = 18$, $r = 0.814$, $p < 0.0001$.
$\alpha = 0.130 \times -0.187$, $n = 18$, $r = 0.790$, $p < 0.0001$.

Table 2
Results of nested ANOVAs evaluating the effects of salinity and population nested within salinity on the initial slope of the photosynthesis–irradiance curve (α), compensation irradiance (I_c), light-saturated photosynthetic rates (P_{max}), saturation irradiance (I_k), and dark respiration rates (R_d). Bold values are significant at $p < 0.05$.

Dependent	Factor	DF	SS	F	Prob > F
P_{max}	Salinity	2	131,394	3.87	**0.047**
	Pop (Sal)	3	400,500	7.88	**0.003**
R_d	Salinity	2	17,587	3.86	**0.048**
	Pop (Sal)	3	2637	0.386	0.726
α	Salinity	2	29.872	2.07	0.164
	Pop (Sal)	3	5.00	0.232	0.872
I_c	Salinity	2	216.55	4.026	**0.043**
	Pop (Sal)	3	21.73	0.269	0.846
I_k	Salinity	2	875.63	1.638	0.232
	Pop (Sal)	3	4449.4	5.549	**0.011**

4.3. Respiration rates

Differences in leaf tissue respiration rates among salinity treatments were significant (Table 2). The lowest rates of leaf tissue respiration were observed at a salinity of 20 (Table 3). Respiration rates at a salinity of 5 were significantly more negative than at higher salinity (Table 3). Whereas significant differences between populations, within each salinity, could not be detected (Table 2), leaf respiration rates in the Padilla Bay population were generally more negative than those in the Coos Bay population (Table 3).

Table 3
Summary of Bonferroni corrected Fisher's Least Significant Difference post hoc tests conducted for the nested ANOVA on photosynthetic parameters. Contrasts were performed for each population (salinity) nested combination; separate contrasts were performed for the main salinity effect. For each dependent variable, levels not connected by the same letter are significantly different at $p < 0.05$.

Dependent	Source	Level	LS mean ± SE	
P_{max}	Salinity	5	286 ± 50	b
		20	478 ± 53	a
		35	433 ± 53	ab
	Pop (Sal)	Padilla (5)	266 ± 75	c
		Coos (5)	307 ± 65	c
		Padilla (20)	275 ± 75	c
		Coos (20)	681 ± 75	d
		Padilla (35)	275 ± 75	c
		Coos (35)	592 ± 75	d
R_d	Salinity	5	−110 ± 18	b
		20	−36 ± 19	a
		35	−75 ± 19	ab
	Pop (Sal)	Padilla (5)	−117 ± 27	c
		Coos (5)	−102 ± 23	c
		Padilla (20)	−49 ± 27	cd
		Coos (20)	−22 ± 27	d
		Padilla (35)	−89 ± 27	cd
		Coos (35)	−61 ± 27	cd
I_c	Salinity	5	11 ± 2	a
		20	2.7 ± 2.1	b
		35	7.6 ± 2.1	ab
	Pop (Sal)	Padilla (5)	11 ± 3	d
		Coos (5)	10 ± 2	cd
		Padilla (20)	3.3 ± 3	cd
		Coos (20)	2 ± 3	c
		Padilla (35)	9.3 ± 3	cd
		Coos (35)	6 ± 3	cd
I_k	Salinity	5	28.9 ± 6.2	a
		20	43.7 ± 6.7	b
		35	42.3 ± 6.7	b
	Pop (Sal)	Padilla (5)	26.4 ± 9.4	d
		Coos (5)	31.3 ± 8.2	cd
		Padilla (20)	21.4 ± 9.4	d
		Coos (20)	66.1 ± 9.4	e
		Padilla (35)	26.9 ± 9.4	d
		Coos (35)	57.6 ± 9.4	ce

5. Discussion

We examined long-term photosynthetic responses to chronic lowering of salinity, such as might be encountered in estuaries or lagoons subject to extreme flooding conditions or altered hydrology. The Coos Bay (southern) *Z. japonica* population, collected in a tidal creek with freshwater inflows, was exposed to rapidly changing salinities (including zero) on a daily basis, whereas the Padilla Bay (northern) population was exposed to more stable salinity conditions ranging from 22 to 30. We expected that the stenohaline Padilla Bay population would be more sensitive to osmotic stress than the more euryhaline Coos Bay population.

Our data indicate that North American populations of *Z. japonica* photosynthetic characteristics are best adapted to intermediate salinities, displaying minimum respiration rates, lower compensation irradiance, higher saturation irradiance, and greater light-saturated photosynthetic rates at a salinity of 20. Thus, *Z. japonica* can also be considered a euryhaline species, tolerating long-term exposure to estuarine salinities in the range of 5–35. Some populations are also capable of tolerating short-term in situ exposure to zero salinity (e.g. Day Creek Inlet, Coos Bay, and Winant Slough, Yaquina Bay, Oregon). The lack of significant differences in photosynthetic efficiency, compensation irradiance, and dark respiration rates between the Padilla and Coos Bay populations suggests that these parameters are unaffected by latitudinal gradients or previous exposure to fluctuating salinity.

Seagrasses have been shown to exhibit a variety of mechanisms for acclimating to salinity fluctuations that range from changes in the cellular ion concentrations (organic osmolytes) to the elasticity of the cell wall (reviewed by Touchette, 2007). Cellular osmotic adjustments of ion concentrations occur in both vacuoles and the cytoplasm via a variety of mechanisms on time scales ranging from hours to days or weeks (Murphy et al., 2003; Touchette, 2007). In a time series experiment, *Z. marina* tended toward an osmotic equilibrium within 4 days in response to sustained salinity changes (Van Diggelen et al., 1987). Over the short-term, salinity response involves osmotic adjustments of inorganic ions and organic osmolytes such as proline, carbohydrates, and organic acids (Touchette, 2007). Most experiments on seagrass photosynthetic response to salinity tend to be short-term on the scale of hours to days. *R. maritima* exhibits decreased quantum yields indicating photosynthetic stress in response to short term salinity changes, but it can physiologically adjust after several days of chronic exposure (Murphy et al., 2003). Chronic salinity reduction over the course of several weeks should allow the *Z. japonica* plants to adapt to low salinity and reach a physiological equilibrium with respect to photosynthetic characteristics. We suggest that the Padilla Bay and Coos Bay populations may not have developed different tolerances to salinity variations despite inhabiting systems with very different salinity characteristics (stenohaline versus euryhaline). Thus, salinity may not be a strong selective force for this species.

Several studies have concluded that some seagrass species do exhibit diel patterns with high photosynthetic rates (or efficiencies) during the morning and lower rates during the afternoon (Libes, 1986; Durako and Kunzelman, 2002; Silva and Santos, 2003), we found no evidence of this in the two *Z. japonica* populations examined. The unrecognized presence of diel rhythms could

confound interpretation of seagrass photosynthetic parameters; testing for diel rhythms should be a routine part of photosynthetic investigations.

Although direct comparisons are sometimes difficult due to the variety of methods and metrics used, there appear to be no consistent patterns in seagrass photosynthetic responses across the salinity gradient. Touchette (2007) suggests that although both photosynthesis and respiration are often inhibited by extreme hypo or hyperosmotic conditions, the degree of inhibition is dependent on the acclimation period. We found that photosynthetic efficiency was relatively unaffected by long-term exposure to salinities ranging from 5 to 35. In contrast, photosynthetic efficiency of the seagrass *Halophila johnsonii* Eiseman was reported to increase linearly with increasing salinity, within the range of 0–40 (Torquemada et al., 2005). However, their study was designed to assess short-term salinity responses (1–20 h), whereas in our experimental design the plants were exposed to constant salinity treatments over a period of weeks. Likewise, saturation irradiance also appeared to be unaffected by differences in salinity.

The range of values for compensation irradiance (I_c) (3–33 μmol photons m^{-2} s^{-1}) and saturating irradiance (I_k) (13–199 μmol photons m^{-2} s^{-1}) observed for *Z. japonica* in this study were generally lower than those reported for other *Zostera* species (Touchette and Burkholder, 2000). This may be related to examination of tissue segments as opposed to whole plant incubations. *Z. japonica* compensation irradiance varied significantly within the range of salinities tested, with a minimum at a salinity of 20, and higher values at 5 and 35. In *H. johnsonii*, compensation irradiance was highest at zero salinity, and decreased with increasing salinity in the range of 0–50 (Torquemada et al., 2005).

Maximum light saturated photosynthetic rates for the southern *Z. japonica* population occurred at a salinity of 20. Similar results were reported for a Japanese population of *Z. japonica*; highest photosynthetic rates occurred at 3/4 strength and full-strength seawater, with markedly lower rates at both higher and lower salinities (Ogata and Matsui, 1965). *Z. marina* also exhibits maximum light saturated photosynthetic rates at a salinity of 20, with lower rates at both higher (25–35) and lower (5–10) salinities (Nejrup and Pedersen, 2008; Kaldy, unpublished data). Additionally, Kamermans et al. (1999) found that *Z. marina* production and PSII quantum efficiency were higher at a salinity of 22 than at 32. Some seagrass species (e.g. *Zostera muelleri* Irmisch ex Aschers. and *H. johnsonii*) exhibit photosynthetic maxima at higher salinities typical of oceanic seawater (Kerr and Strother, 1985; Torquemada et al., 2005). Other studies reported no relationship between *Halodule wrightii* Aschers. light saturated photosynthetic rates and salinity along an estuarine gradient (Dunton and Tomasko, 1994; Dunton, 1996).

The lowest respiration rates in *Z. japonica* were observed at a salinity of 20, with increased respiration rates at low (5) and high (35) salinities. Respiration rates of *Z. muelleri*, another intertidal species, were lowest at full-strength seawater, and approximately twice as great at 50% seawater salinity than at 100% seawater salinity (Kerr and Strother, 1985). In contrast, *Z. marina* respiration rates are reported to be relatively insensitive to changes in salinity ranging from zero (distilled water) to 2× and 3× seawater solutions (Biebl and McRoy, 1971; Hellblom and Björk, 1999; Kaldy, unpublished data).

Stands of *Z. japonica* located along the central and southern Oregon coast (Yaquina and Coos Bay) appear to be capable of more rapid growth and photosynthesis rates than northern populations (Padilla Bay, Washington; Shafer et al., 2008; this study). Southern stands are also more tolerant of warmer temperatures and become light-saturated at higher irradiances than the northern stand (Shafer et al., 2008; this study). Whether these differences results from variations in acclimation to local environmental conditions or have an underlying genetic basis is unknown. However, this body of evidence does suggest that southern stands may possess greater photosynthetic physiological tolerance, which may increase the potential for further expansion southward along the Pacific Coast and changes associated with global climate change.

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