

ASSESSING PHYSIOLOGICAL THRESHOLDS FOR EELGRASS
(*ZOSTERA MARINA* L.) SURVIVAL IN THE FACE
OF CLIMATE CHANGE

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ABSTRACT

Assessing physiological thresholds for eelgrass (*Zostera marina* L.)
survival in the face of climate change

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Seagrasses are well known for the important ecological roles they play in coastal marine waters worldwide. However, the severe rate of decline observed in seagrasses this century is expected to accelerate with climate change. Conservation efforts can be improved by quantifying physiological thresholds of seagrasses and using these estimates in modeling to forecast changes in distribution. This study examines the response of eelgrass (*Zostera marina* L.) across current temperatures to look for early warning signs of vulnerability and to evaluate the ways we determine critical thresholds for survival. Whole eelgrass ramets, collected from three beds in Morro Bay, California, were used to develop photosynthesis-irradiance (P-I) curves from 10-20°C. Productivity was not affected by changes in temperature when traditionally measured as the light-saturated photosynthetic rate to dark respiration rate (P:R) ratio. However, photosynthesis in light-limited conditions declined at higher temperatures, suggesting a decrease in productivity when coupled with the increased respiration rates observed at higher temperatures. Irradiance thresholds increased with temperature; critical irradiance was the most sensitive to increases in temperature due to the inclusion of overnight energy use, which also increases with temperature. Measurements of root and rhizome respiration, overnight respiration, and variation across eelgrass beds reveal that these are important components to consider when calculating survival thresholds to use in modeling. Differences in physiological responses across beds suggest that some eelgrass beds operate more efficiently than others in current conditions and are likely to be more resilient to the progressing stressors of climate change. Management of eelgrass in the face of climate change will require reliable distribution forecasts, and therefore accurate estimates of physiological thresholds, to guide mitigation and restoration efforts.

Keywords: *Zostera marina*, climate change, productivity, temperature, irradiance, photosynthesis, respiration, rhizomes, critical irradiance, Morro Bay

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TABLE OF CONTENTS

	Page
LIST OF TABLES	ix
LIST OF FIGURES	x
SECTION	
1. Introduction	1
2. Materials & Methods	9
2.1 Sample collection.....	9
2.2 Respirometry.....	10
2.3 Photosynthesis-irradiance (P-I) curves.....	13
2.4 Daily energy requirements.....	14
2.5 Root/rhizome respiration.....	16
3. Results	17
3.1 Eelgrass productivity and light requirements.....	17
3.1a Measuring productivity.....	17
3.1b Light thresholds and temperature.....	19
3.2 Measuring 24-hour energy budget.....	21
3.2a Day v. night respiration rates.....	21
3.2b Contribution of roots and rhizomes.....	22
3.3 Application of P-I curves.....	23
3.3a Shallow v. deep P-I parameters.....	23

3.3b	P-I parameters and morphology across beds.....	24
4.	Discussion.....	29
4.1	Overview.....	29
4.2	Eelgrass productivity and light requirements.....	30
4.2a	Measuring productivity.....	30
4.2b	Light thresholds and temperature.....	32
4.3	Measuring 24-hour energy budget.....	33
4.3a	Day v. night respiration rates.....	33
4.3b	Contribution of roots and rhizomes.....	34
4.4	Application of P-I curves.....	36
4.4a	Shallow v. deep P-I parameters.....	36
4.4b	P-I parameters and morphology across beds.....	37
4.5	Final remarks.....	39
	REFERENCES	41
	APPENDICES	
	A. Photosynthesis-Irradiance (P-I) Curves	47

LIST OF TABLES

Table	Page
1. Photosynthesis-irradiance (P-I) parameters across eelgrass beds.....	26

LIST OF FIGURES

Figure	Page
1. Generalized photosynthesis-irradiance curve (P-I) based on net photosynthesis.....	4
2. Location of three eelgrass beds in Morro Bay used for sample collection.....	10
3. The effect of temperature on light-saturated rate of photosynthesis (P _{max} ; a) and dark respiration (b).....	18
4. The effect of temperature on productivity (P:R) of eelgrass.....	19
5. Saturation irradiance, I _K (a) and compensation irradiance, I _C (b) across temperatures.....	20
6. Compensation irradiance (I _C) and critical irradiance (I _{C24}) versus temperature.....	21
7. Contribution to total biomass (a) and total respiration (b) by eelgrass tissue type.....	23
8. Productivity (P:R ratio) across eelgrass beds.....	27
9. Length of eelgrass blades by bed.....	28

1. INTRODUCTION

Seagrasses, marine angiosperms found in coastal ocean waters around the world, are important both ecologically and economically. Seagrass beds foster a diversity of primary producers, invertebrates, fish, and water fowl (Bell & Pollard 1989, Sanchez et al. 1996, Short et al. 1989, Thayer et al. 1975, 1984). Additionally, seagrasses provide a number of ecosystem services, such as sediment stabilization and provision of oxygen to the surrounding community (Nixon & Oviatt 1972, Short & Short 1984, Stevenson 1988). While the importance of seagrasses is highly recognized in the scientific community, 30% of mapped seagrass area worldwide has been lost over the last century and the rate of seagrass decline continues to accelerate (Waycott et al. 2009). Seagrass loss has been attributed to natural and anthropogenic (direct and indirect) causes, including climate change (Duarte 2002).

Several aspects of climate change appear to be affecting seagrass beds, including decreases in available light and warming ocean temperatures. Previous studies predict that these conditions will alter seagrass physiology, productivity, and distribution, but call for more research on the direct effects of climate-change induced conditions on seagrasses (Short & Neckles 1999). Calculating specific physiological thresholds for seagrasses survival is a first critical step before climate change models can be utilized to forecast changes in seagrass distribution.

Light is the most important factor limiting seagrass distribution and growth, especially at the deepest edges of beds (Bintz & Nixon 2001, Dennison & Alberte

1985, Ralph et al. 2007, Zimmerman et al. 1995). Decreases in time spent at or above irradiance thresholds, for example due to increases in water column turbidity, have been tied to decreases in abundance and distribution of seagrasses, including eelgrass *Zostera marina* (Dennison & Alberte 1982, Dennison & Alberte 1985, Herzka & Dunton 1998, Zimmerman et al. 1994), the predominant seagrass on the West and East Coasts of the United States (Green & Short 2003).

In addition to anthropogenic increases in turbidity and eutrophication from agriculture and development (eg. Short et al. 1996), climate change is expected to reduce light available to seagrass through two mechanisms. The first is a function of projected sea level rise, which is expected to increase water depths, thus causing changes in tidal variation, altering water movement, and increasing seawater intrusion into estuaries, all of which result in higher light attenuation by the water column (Short & Neckles 1999). The second aspect of climate change that will affect the light available to seagrass beds is the increasing frequency of extreme weather events including storms, precipitation, and flooding. These events will cause higher eutrophication, phytoplankton growth, and turbidity of coastal waters, which in turn will limit light available for seagrass beds (Short & Neckles 1999).

In addition to reduction in light availability, climate change may affect seagrass productivity via an increase in ocean temperature. Temperature affects productivity directly, via metabolic rate, as well as indirectly, through an interaction with light requirements. Climate change is predicted to increase sea

surface temperatures worldwide. Changes in ocean temperature have already been observed over the past century; between the 1950s and 1990s the top 300m of the water column of the world ocean increased by an average of 0.31°C (Levitus et al. 2000). Previous studies have found that light requirements for eelgrass to maintain a given photosynthetic rate increase in higher temperatures (Wetzel & Penhale 1983, Murray & Wetzel 1987, Moore et al. 1997, Moore 2004). Therefore, elevated temperatures may make present light conditions insufficient for eelgrass to maintain current levels of productivity if minimum time necessary at saturating irradiances is no longer met. Differences in environmental light and temperature combinations have been found to be responsible for differences in growth and recovery rates of seagrasses. After restoration efforts in 2005, Moore et al. (2012) observed greater expansion rates of eelgrass in the coastal bays of Virginia than in the Chesapeake Bay (66% versus 2%) due to relatively lower temperatures combined with higher light levels than in the Chesapeake. Increased sea surface temperatures coupled with reduced light availability will create two sources of light stress for seagrasses (Wetzel & Penhale 1983, Murray & Wetzel 1987, Moore et al. 1997, Moore 2004).

Because of the aforementioned services and sensitivity to natural and anthropogenic disturbances, understanding the response of seagrasses to climate change is critical for the conservation of these species. Quantification of the physiological parameters necessary for seagrass growth and survival can be used to inform climate change models and forecast changes in seagrass

distribution. One way photosynthetic parameters of seagrasses are commonly quantified is by using photosynthetic shoots or portions of shoots to generate photosynthesis-irradiance (P-I) curves (Fig. 1) (eg. Dennison 1987, Dennison & Alberte 1986, Herzka & Dunton 1997, Marsh et al. 1986, Zimmerman et al. 1989). P-I parameters can be used to assess the physiological responses of seagrasses to a variety of environmental conditions, which in turn can be applied to models forecasting eelgrass distribution under various climate change scenarios.

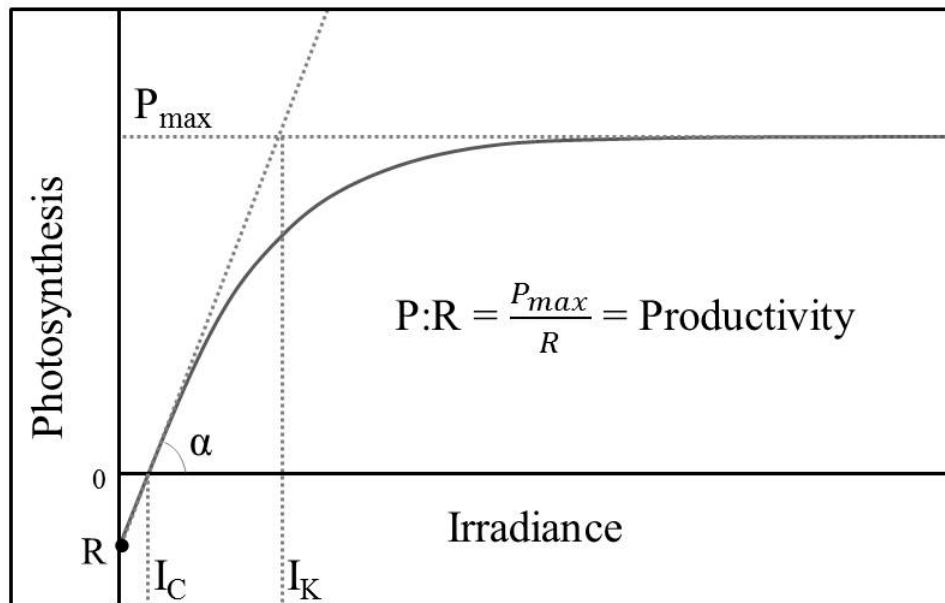


Figure 1. Generalized photosynthesis-irradiance curve (P-I) based on net photosynthesis. Parameters that can be identified using P-I curves include: P_{max} (light-saturated photosynthetic rate), R (dark respiration rate), $P:R$ (the ratio of P_{max} to R , a proxy for productivity), α (the initial slope, representing the light-limited rate of photosynthesis), I_K (saturation irradiance, the irradiance at the intersection of α and P_{max}), and I_C (compensation irradiance, the irradiance at which photosynthetic rate equals respiration rate).

Several issues arise when using P-I curves to inform distribution models. For one, many lab-derived P-I curves have been criticized for overestimating net photosynthesis by measuring oxygen evolution and consumption of shoots alone (Dunton & Tomasko 1994, Fourqurean & Zieman 1991). In recent years, seagrass biologists have acknowledged the need to include root and rhizome tissue to properly calculate whole plant carbon budget and photosynthetic light requirements (Dunton & Tomasko 1994, Hemminga 1998, Herzka & Dunton 1998, Koch & Beer 1996, Kraemar & Alberte 1993, Lee et al. 2007, Zimmerman et al. 1989, 1995).

Zostera marina, the subject of this study, is the most cosmopolitan of the seagrasses, found on temperate coasts of every continent except Antarctica (Green & Short 2003). When measuring photosynthesis alone, the optimum temperature for *Z. marina*, based on a worldwide average, is 23.3°C (Lee et al. 2007). However, the average optimum temperature for *Z. marina* overall growth is only 15.3°C (Lee et al. 2007). This inconsistency is due to the differential effects of temperature on factors other than photosynthesis, including nutrient uptake, leaf senescence, and respiration (Bulthuis 1987, Herzka & Dunton 1997, Lee et al. 2007, Lee & Dunton 1999, Marsh et al. 1986). The use of P-I curves in assessing the effects of temperature on whole *Z. marina* plants allows total respiration to be considered when quantifying irradiance thresholds across a range of temperatures.

Second, estimates of seagrass light requirements are often based on the compensation irradiance (I_c), which does not take overnight respiration into

account. In addition to capturing the immediate response of seagrasses to their environment, P-I curves can also be used to calculate irradiance thresholds necessary for long-term growth, such as the critical irradiance (I_{C24}). I_{C24} is defined here as the average irradiance needed during the daylight hours for energy produced by photosynthesis to equal energy consumed by respiration over a 24-hour period.

Irradiance thresholds that do incorporate daily respiration often assume consistent respiration rates over a 24-hour period (e.g., Dennison & Alberte 1995, Marsh et al. 1986). Several studies have provided evidence that growth rates and oxygen metabolism are slower overnight than during the day (Kemp et al. 1987) and are under endogenous control (Williams & Dennison 1990). Quantitative knowledge of the differences between day and night respiration rates may be useful for improving distribution model accuracy, especially when determining irradiance thresholds for long-term survival, such as I_{C24} .

Inclusion of whole plants and evaluation of diel respiration patterns in seagrasses may improve the accuracy of distribution predictions based on P-I curves. Still, further knowledge of the variation in seagrass response to temperature across small geographic scales is needed to determine how broadly P-I curves can appropriately be applied to model changes in distribution.

Increased temperatures may affect seagrass productivity directly by increasing metabolic rates. Generally, both maximum photosynthetic rate (P_{max}) and respiration rate increase with temperature (Lee et al. 2007). However, respiration increases more drastically than photosynthesis at progressively

higher temperatures, often leading to decreases in productivity (Bulthuis 1983, Dennison 1987, Herzka & Dunton 1997, Marsh et al. 1986, Masini & Manning 1997, Moore et al. 1997, Perez & Romero 1992).

The impacts of rising temperatures on seagrass productivity and light requirements depend on several factors. Temperate seagrass species have a wider, and often lower, range of optimal growth temperatures than tropical seagrasses (11.5°C-26°C versus 23°C-32°C, respectively) (Lee et al. 2007). Species within the same latitudes may also exhibit differences in response to temperature (Collier et al. 2011, Masini & Manning 1997, Perez & Romero 1992). For example, Collier et al. (2011) observed opposite effects of growth temperature on two species of Great Barrier Reef seagrasses when grown at 27°C and 33°C; *Halodule uninervis* production increased 10-fold at the higher temperature, whereas *Zostera muelleri* production decreased 10-fold at the higher temperature.

Intraspecific variation in photosynthetic parameters has been observed across adjacent seagrass beds occupying different depths. Studies comparing shallow and deep beds have provided evidence that deeper beds have higher light-limited rates of photosynthesis (α) and lower saturation irradiance (I_K) to reach maximum photosynthetic rate (Masini & Manning 1997). Even *Z. marina* occupying different depths within the same bed have demonstrated differences in photosynthetic characteristics in a temperature-controlled lab setting, with shallow eelgrass having significantly higher light-saturated rate of photosynthesis (P_{max}) and dark respiration rates than deeper eelgrass (Dennison & Alberte 1982,

Dennison & Alberte 1986). Comparisons of the physiological thresholds of eelgrass across beds and depth gradients are necessary to determine how broadly P-I curves can be applied for conservation purposes.

To add to the previous body of literature, we conducted a study designed to address issues with accurately measuring the response of seagrass to changing light and temperature levels. The objective of this study was to gain a quantitative understanding of the effect of current temperatures on productivity and light requirements of *Z. marina* and how these factors vary across beds within a limited geographic area. A novel respirometry system was used in an outdoor laboratory to develop P-I curves for eelgrass across the range of temperatures currently observed in Morro Bay, a small California estuary. Irradiance thresholds and productivity levels were compared across eelgrass beds and temperatures. An accurate quantification of these parameters required the assessment of factors that affect overall eelgrass energy budget, but have often been overlooked, including the contribution of rhizomes to total plant respiration and the comparison of day and night respiration rates. Therefore, we hypothesized that: 1) as temperature increases within the range observed in Morro Bay, *Zostera marina* productivity will decrease and light requirements will increase, 2) roots and rhizomes will be responsible for a relevant portion of total plant respiration, 3) day and night respiration rates will not be equal, and, 4) at any given temperature, baseline levels of productivity and light requirements will vary across beds and depths.

2. MATERIALS AND METHODS

2.1 *Sample collection*

Eelgrass samples were collected between August and November of 2011 from three beds located near the mouth, mid area, and back of Morro Bay Estuary, California (Fig. 2). The mouth bed experiences relatively consistent salinity, turbidity, and temperature, due to its proximity to ocean waters (N35°22.320' W120°51.628'). Samples were collected at two locations within the mouth bed to account for depth gradients; the “shallow” area (spanning 0-4' depth at the 0' tide) and the “deep” area (spanning 6-16' depth at the 0' tide). The mid bay bed experiences variability in salinity, turbidity, and temperature due to seasonal freshwater input from Chorro and Los Osos Creeks, runoff from the watershed, and proximity to disturbances, such as boat traffic (N35°20.844' W120°50.688'). The mid bay bed is fairly uniform in depth (1-2' depth at the 0' tide). The back bay bed is subjected to similar conditions as the mid bed, but is often exposed at low tide (0-0.5' depth at the 0' tide; N35°19.821' W120°50.988').

Whole eelgrass ramets were collected by hand via wading or diving, with great care taken to keep roots intact. Ramets were stored in raw seawater while transported to the Cal Poly Center for Coastal Marine Sciences (CCMS) pier in Avila Beach, California, about 20 miles south of Morro Bay. At the CCMS pier, ramets were stored for one to six days in flow-through filtered seawater tanks at ambient temperatures prior to use.

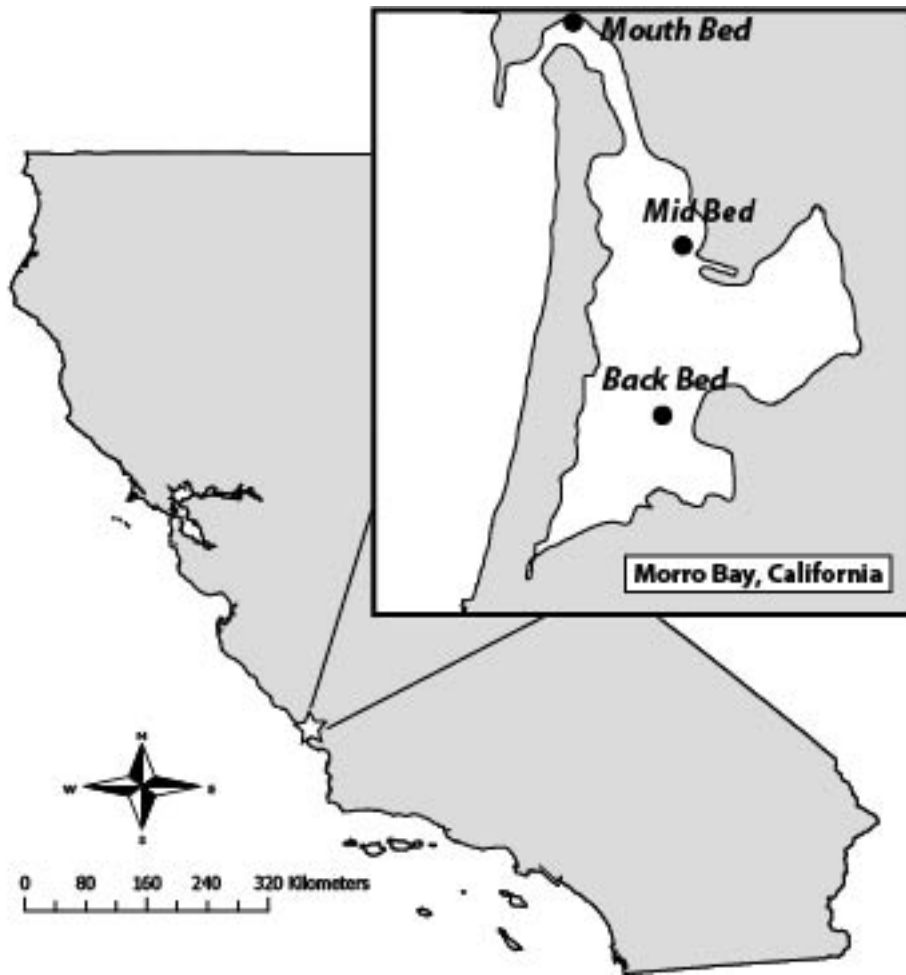


Figure 2. Location of three eelgrass beds in Morro Bay used for sample collection. The Mouth Bed (N35°22.320' W120°51.628'), the Mid Bed (N35°20.844' W120°50.688'), and the Back Bed (N35°19.821' W120°50.988'). Modified from Needles & Wendt 2012.

2.2 Respirometry

A novel respirometer was designed and constructed for use in an outdoor lab setting at the CCMS pier, as well as *in situ*. The respirometer consisted of a 3.72L UV-transparent acrylic cylinder attached to an external platform at the base of the chamber. The platform housed three pumps, two solenoid valves, an Aanderaa 3835 oxygen optode (connected to a Satlantic STOR-X Submersible

Data Logger), and a flow rate sensor, all connected in a circuit to the acrylic chamber via ¼" diameter clear Tygon laboratory tubing. Additionally, the system was connected to a chiller, consisting of an aluminum coil resting in the water bath of a LAUDA cooling unit (Ecoline Staredition E100 Immersion Thermostat Circular Water Bath and RE100 Cooling Thermostat).

Before respirometry, physical data on each eelgrass sample were recorded, including length of longest blade per ramet and percentage of blades that were broken, discolored, or both broken and discolored. One-way ANOVAs were run on each physical trait using eelgrass bed as the predictor. Epiphytes and sediments were gently removed from the ramets by hand under running filtered seawater. The sample was loosely bundled together and weighted with two or three large metal nuts for insertion into the respirometer. Once respirometry was complete, the sample was divided into photosynthetic and non-photosynthetic parts and dried in an Isotemp Muffle Furnace (Fisher Scientific) at 60°C until a consistent weight was achieved (approximately 24 hours).

Each eelgrass sample was maintained at a constant temperature within the respirometer while subjected to several light and one dark treatment (30-60 minutes/treatment), yielding an individual photosynthesis-irradiance curve for each sample. The temperature range used in this study (~10-20°C) represents the typical span observed in Morro Bay throughout the year (based on 2009 water quality data from the San Luis Obispo Science & Ecosystem Alliance). Light treatments were applied by placing one of seven covers, made of one to seven layers of neutral density screening, over the respirometer. The same

seven covers were used for the duration of the study. Prior to respirometry, initial measurements were taken with two LI-193 spherical quantum sensors, one inside the respirometer and one in the bucket, to quantify the percent ambient light reaching the sensor under each treatment, as well as light attenuation from the respirometer itself (0%, 1.4%, 1.9%, 3.7%, 5.6%, 8.7%, 17.9%, 25.9% of ambient light reached the sensor during each of the seven treatments and 52.7% of ambient light reached the sensor in the respirometer alone). The dark treatment was applied by covering the respirometer with three layers of heavy duty contractors' bags. Ambient irradiance was measured every second using a LI-193 spherical quantum sensor attached to a LI-1400 data logger that recorded an integrated 15 second average. The sensor was placed in a bucket of seawater adjacent to the chamber to avoid shading of the sensor by the eelgrass. Light measurements were converted according to the treatment used to determine the actual amount of light reaching the inside of the chamber. Oxygen and temperature measurements were taken every second by the optode and integrated into five second averages.

Net photosynthesis or respiration rate was calculated by measuring the slope of the change in oxygen concentration over time within the chamber for each treatment. There was a lag time of approximately ten minutes for the effect of the treatment on the oxygen concentration to be observed; the lag time was excluded from the slope calculations. Average irradiance was calculated for each treatment, while average temperature was calculated for the entire sample across all treatments to determine average temperature for each P-I curve.

2.3 Photosynthesis—irradiance (P-I) curves

Respirometry was performed on samples from each of the three eelgrass beds ($n_{\text{mouth}}=20$; $n_{\text{mid}}=12$; $n_{\text{back}}=12$; $n_{\text{total}}=44$). P-I curves were generated for each sample by plotting average irradiance ($\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$) against net photosynthesis or respiration ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ g biomass}^{-1} \text{ h}^{-1}$) for each treatment. P-I curves were fit to the data points from each sample using the following asymptotic equation:

$$f(x) = \gamma + \frac{1}{A + \beta/x}$$

where $A = 1/P_{\text{max}}$; β = the curvature of the function, directly related to the initial slope; and γ = dark respiration (y at $x=0$). The initial slope for each P-I curve was determined by calculating the slope of tangent(β) at $y=0$. The compensation irradiance (I_C) is defined as the light level at which net photosynthesis and respiration rates are equal and was calculated as x when $y=0$ using the asymptotic equation. I_C was substituted into the derivative function for x to calculate initial slope at $y=0$:

$$f(x) = \gamma + \frac{1}{A + \beta/x}$$

$$\text{If } f(I_C) = 0, \text{ then } I_C = -\frac{\beta\gamma}{A\gamma+1}$$

$$f'(I_C) = \frac{\beta}{(A I_C + \beta)^2}$$

The saturation irradiance (I_K), was calculated as the light level at which tangent(β) intercepts P_{max} and is an indication of how much light is necessary to

saturate the photosystems. As a proxy for productivity, net P_{\max} to dark respiration rate (P:R) ratios were calculated for each curve.

Only samples with at least three successfully applied treatments were fit to the asymptotic equation and used in further analysis. A total of 31 replicates yielded usable P-I curves ($n_{\text{mouth}}=13$, $n_{\text{mid}}=8$, $n_{\text{back}}=10$), spanning the temperature range of 10-20°C.

A two-way ANOVA was run on each P-I parameter (P_{\max} , dark respiration, P:R ratios, α , I_K , and I_C), using bed and temperature as categorical and continuous factors, respectively, and testing for an interaction between factors ($\alpha=0.05$). Prior to analysis, several of the P-I parameters were transformed to stabilize variance (α to $\log_{10}(\alpha)$, $\text{tangent}(\beta)$ to $\log_{10}(\text{tangent}(\beta))$, I_C to $\sqrt{I_C}$, and γ to $\log_{10}(-\gamma)$).

P-I curves for shallow ($n=9$) and deep ($n=4$) areas of the mouth bed were compared using a two-way ANOVA for P_{\max} , dark respiration, P:R ratios, α , I_K , and I_C using temperature and area in bed as continuous and categorical factors, respectively, and testing for an interaction between factors ($\alpha=0.05$).

2.4 Daily energy requirements.

For each bed, three or more samples were used to compare day and night dark respiration rates under consistent temperatures ($n=12$). After daytime respirometry was complete, the sample was maintained at temperature in the chamber and held overnight. The rate of overnight respiration was determined by calculating the slope of the linear portion of the change in oxygen concentration

from the time it was dark until the chamber was nearly depleted of oxygen, usually around midnight. A general linear model was run to test the effects of bed, time of respiration, temperature, and interactions between any of the factors on respiration rates of samples run during the day compared to different samples run at night ($\alpha=0.05$). Additionally, a paired t-test was run to compare day respiration rates to night respiration rates within individual samples ($\alpha=0.05$).

The critical irradiance (I_{C24}) was determined based on 24-hour energy usage. In 2011, Morro Bay night length ranged from 10-14 hours throughout the year (U.S. Naval Observatory, Naval Oceanography Portal); therefore, critical irradiances based on a median 12 hour night were compared. I_{C24} was calculated for each sample and night length by dividing 24-hour energy use by hours of daylight (12) to determine the necessary rate of photosynthesis to meet 24-hour energy demands and the associated irradiance on each P-I curve. Although overnight respiration rates were measured in this study, I_{C24} estimates were calculated using only daytime respiration rates so that I_{C24} could be determined for all 31 samples for which P-I curves were generated.

A two-way ANOVA, using bed and temperature as categorical and continuous factors, respectively, was run for I_{C24} . A two-way ANOVA was run to evaluate whether or not there was a true difference between I_C and I_{C24} and compare how they responded to temperature. Irradiance threshold (I_C or I_{C24}), temperature, and the interaction of the two were used as predictors ($\alpha=0.05$).

2.5 Root/rhizome respiration

For each of the three eelgrass beds, at least three samples were used to compare whole ramet dark respiration to root/rhizome dark respiration at a consistent temperature, resulting in 8 pairs for comparison ($n_{\text{mouth}}=3$, $n_{\text{mid}}=2$, $n_{\text{back}}=3$). After the daytime measurements for the P-I curve were complete, the sample was removed from the chamber and separated into photosynthetic and non-photosynthetic parts. The non-photosynthetic portion (rhizomes and roots) were loosely bundled together and reinserted into the chamber. Root/rhizome respiration was measured in the dark for an hour. Dark respiration rates for ramets and roots/rhizomes of the same sample (run at one temperature) were normalized by dry biomass and compared using a mixed-effects ANOVA, with eelgrass sample as a random effect and eelgrass bed and tissue type as fixed effects.

Respiration rates for the photosynthetic portion of ramets (shoots and blades) were calculated using the following equation:

$$R_{\text{shoot}} = \frac{(R_{\text{ramet}} * B_{\text{ramet}}) - (R_{\text{rhizome}} * B_{\text{rhizome}})}{B_{\text{shoot}}}$$

where R=dark respiration rate ($\mu\text{mol O}_2 \text{ L}^{-1} \text{ h}^{-1} \text{ g}^{-1}$) and B=dry biomass (g). To calculate percent contribution of each tissue type to total respiration, the tissue-specific respiration rate was multiplied by the biomass of the tissue for each sample.

3. RESULTS

3.1 *Eelgrass productivity and light requirements*

3.1a *Measuring productivity*

Productivity, as measured by P:R ratio, was not significantly affected by temperature. Looking at the components of the P:R ratio individually, light-saturated photosynthetic rate (P_{max}) had a general trend of increasing with temperature, but was not significant ($p=0.0664k$, Fig 3a). Dark respiration rate, however, did increase with temperature as expected ($p=0.002$; Fig 3b). The magnitude of the increase in respiration rate with temperature was not large enough to have an observable effect on the P:R ratio (Fig. 4).

The rate of light-limited photosynthesis (α) significantly decreased with increasing temperature ($p=0.0472$), indicating that increases in temperature cause a decrease in photosynthetic rate when light levels are below saturating.

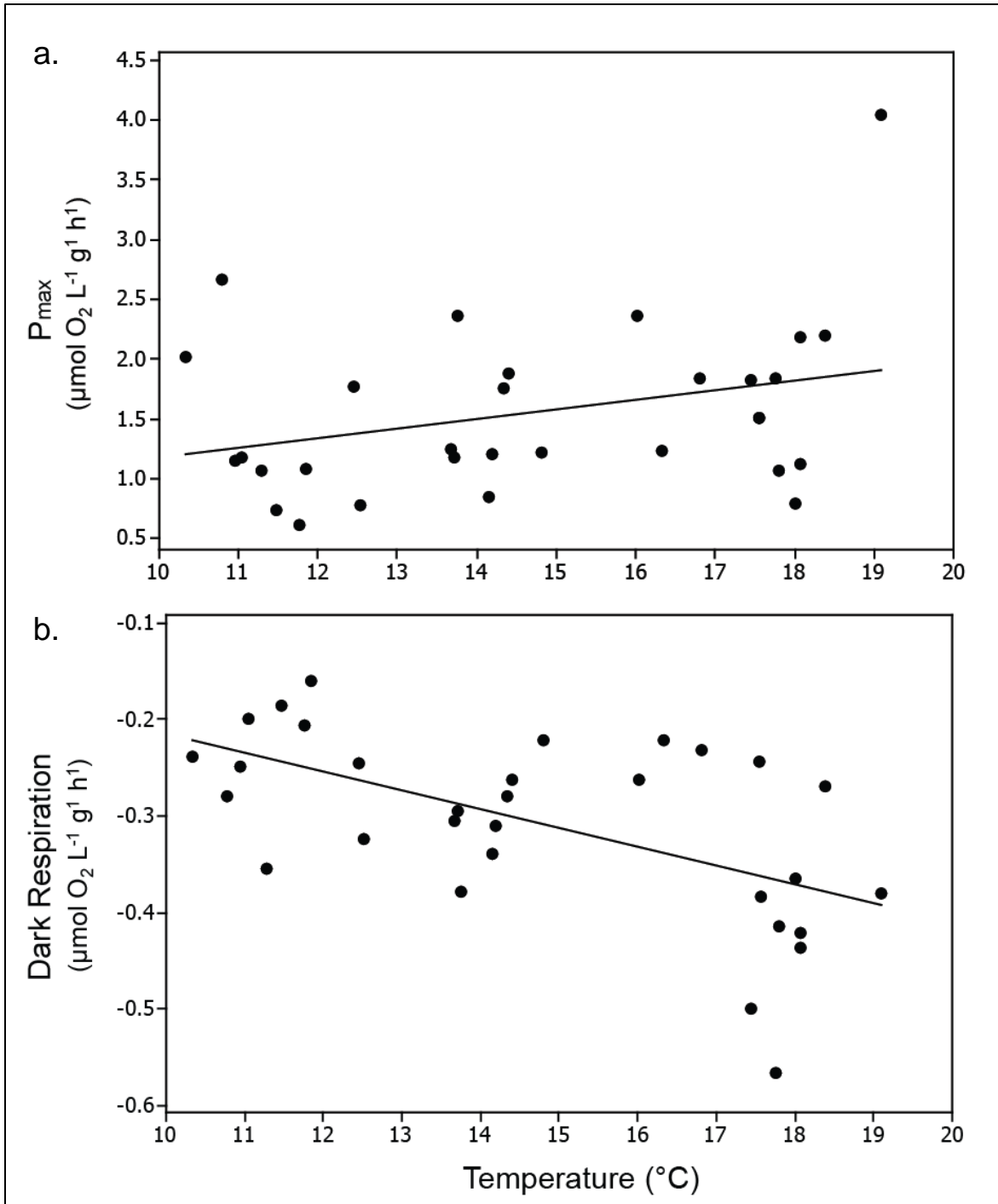


Figure 3. The effect of temperature on light-saturated rate of photosynthesis (P_{max} ; a) and dark respiration (b). P_{max} had a general trend of increasing as temperature increased ($p=0.0664$), while dark respiration increased significantly with temperature ($p=0.002$).

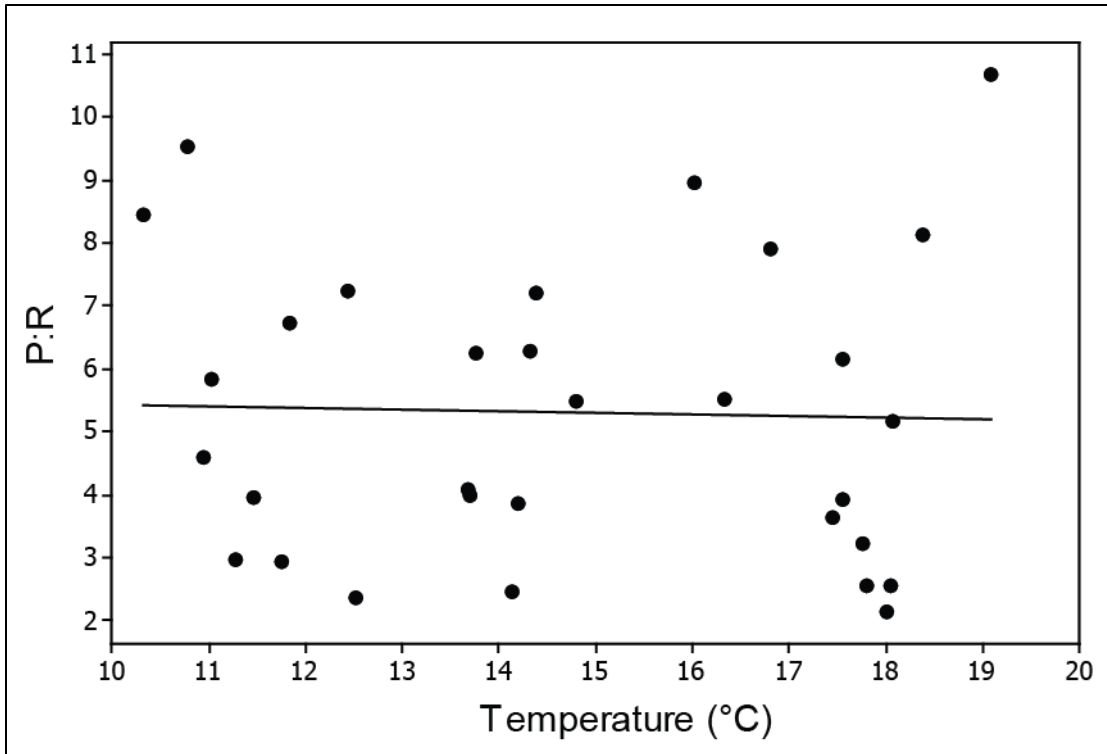


Figure 4. The effect of temperature on productivity (P:R) of eelgrass. P:R ratios were calculated as the net light-saturated photosynthetic rate (P_{max}) divided by the dark respiration rate for each eelgrass sample. P:R ratios did not differ significantly across temperatures.

3.1b Light thresholds and temperature

The light needed to reach a given photosynthetic rate also increased with increasing temperatures. More light was needed to reach both saturating (I_K $p=0.0235$; Fig. 5a) and compensating (I_C $p=0.0071$; Fig. 5b) irradiances as temperature increased.

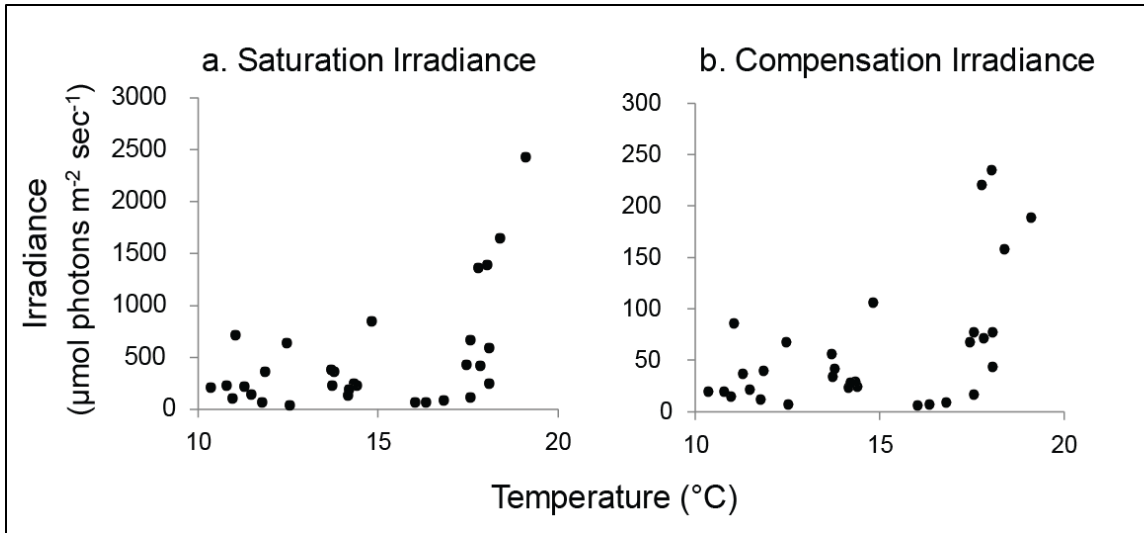


Figure 5. Saturation irradiance, I_K (a) and compensation irradiance, I_C (b) across temperatures. I_K ($p=0.0235$) and I_C ($p=0.0071$) increased significantly with increasing temperatures according to the two-way ANOVA (predictors: bed, temperature, bed*temperature; $\alpha=0.05$).

I_{C24} increased with increasing temperatures ($p=0.0036$). Average I_C and I_{C24} were significantly different from one another ($p<0.0001$) as expected. Increases in temperature caused increases in light requirements for both I_C and I_{C24} ($p=0.0069$), but temperature had a much more dramatic effect on I_{C24} than I_C ($p=0.0077$; Fig. 6).

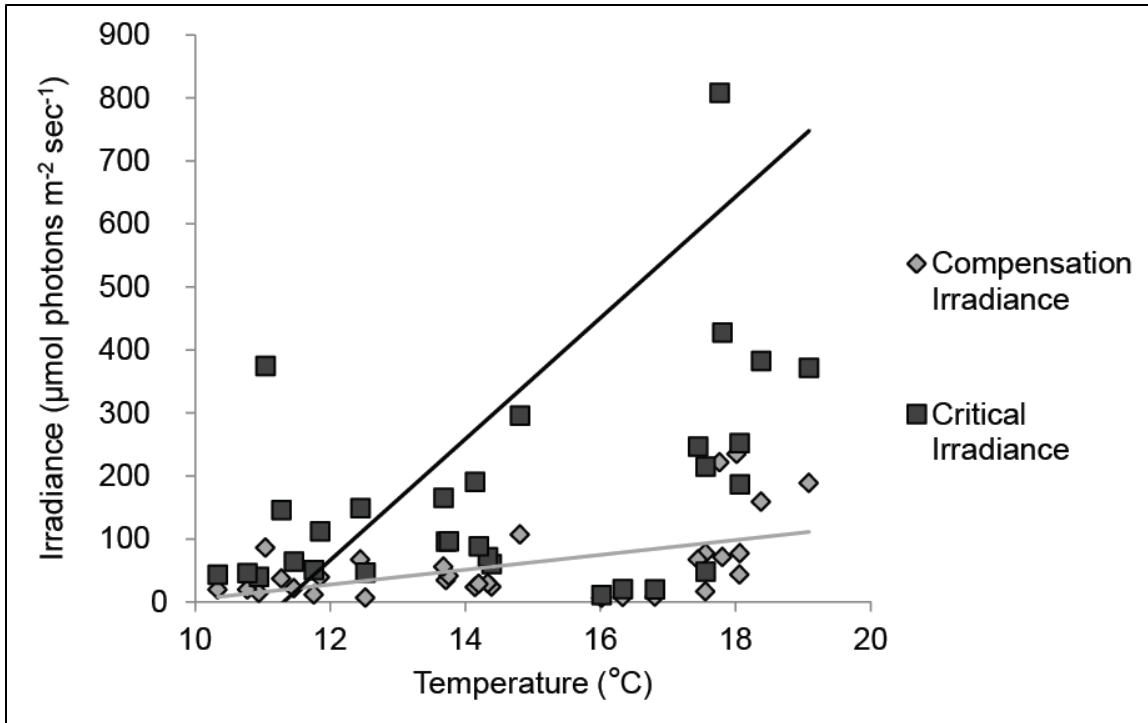


Figure 6. Compensation irradiance (I_c) and critical irradiance (I_{c24}) versus temperature. I_c and I_{c24} were significantly different from one another on average ($p < 0.0001$), and I_{c24} increased with temperature much more dramatically than I_c ($p = 0.0077$) according to the two-way ANOVA (predictors: light requirement (I_c or I_{c24}), temperature, light requirement*temperature; $\alpha = 0.05$).

3.2 Measuring 24-hour energy budget

3.2a Day v. night respiration rates

Day and night respiration rates of eelgrass samples were not the same. The general linear model comparing respiration rate of samples measured during the day and overnight showed an effect of temperature ($df=1$, $F=12.46$, $p=0.0041$) as expected, as well as time of day ($df=1$, $F=4.73$, $p=0.0505$) on respiration rate. There was also weak evidence that bed had an effect on respiration rate ($df=2$, $F= 3.44$, $p=0.0660$). The effect of bed on respiration was

observed at a significant level in the analysis of the P-I curves by bed (Table 1), but is likely not significant in the day v. night respiration rate analysis due to the small sample size used for overnight measurements.

The paired t-test showed that night respiration (mean rate $-0.2530 \mu\text{mol O}_2 \text{ L}^{-1} \text{ g dry biomass}^{-1} \text{ hour}^{-1}$) was significantly lower than day respiration (mean rate $-0.3344 \mu\text{mol O}_2 \text{ L}^{-1} \text{ g dry biomass}^{-1} \text{ hour}^{-1}$) by 25% (mean difference $0.0813 \mu\text{mol O}_2 \text{ L}^{-1} \text{ g dry biomass}^{-1} \text{ hour}^{-1}$, $p=0.037$).

3.2b Contribution of roots and rhizomes

The underground, non-photosynthetic portions of the eelgrass ramet contributed significantly to the overall respiration of the plant. On average, the roots and rhizomes represented 17.1% ($\pm\text{SE } 1.1\%$) of the dry biomass of the sample. However, roots and rhizomes were responsible for an average of nearly 40% ($\pm\text{SE } 3.1\%$) of total plant respiration (Fig. 7). Calculating respiration rate of tissues per unit of dry biomass, rhizomes and roots respired over three times the rate of the shoots and leaves, $-0.78 (\pm\text{SE } 0.12)$ and $-0.24 (\pm\text{SE } 0.03) \mu\text{mol O}_2 \text{ L}^{-1} \text{ g biomass}^{-1} \text{ h}^{-1}$, respectively.

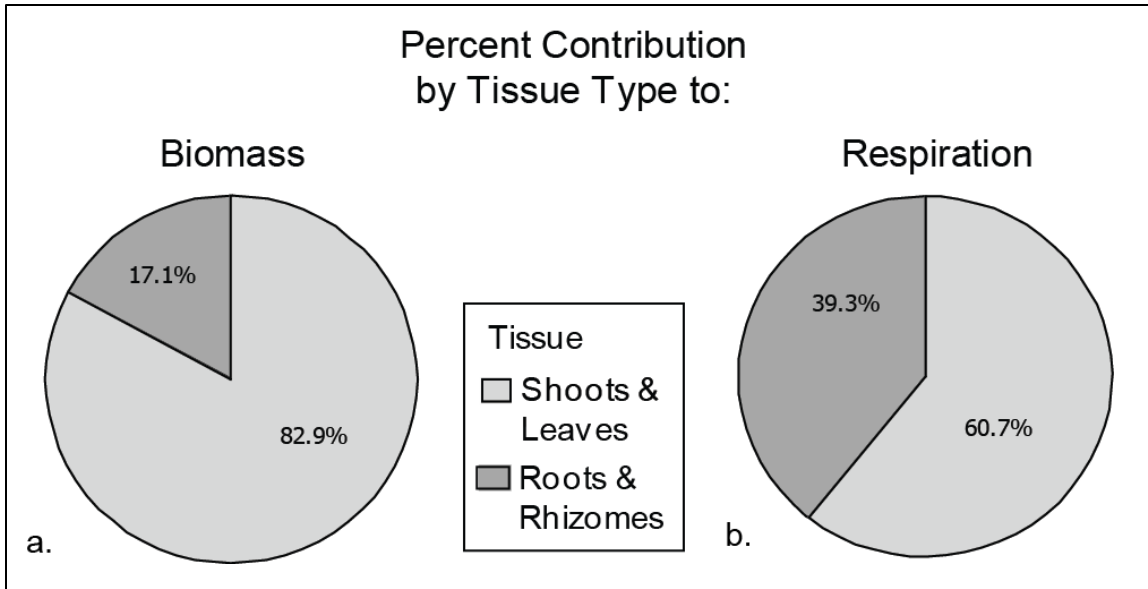


Figure 7. Contribution to total biomass (a) and total respiration (b) by eelgrass tissue type. Roots and rhizomes represented 17.1% (\pm SE 1.1%; n=31) of dry biomass of the samples, yet were responsible for 39.3% (\pm SE 3.1%; n=8) of the total respiration.

3.3 Application of P-I curves

3.3a Shallow v. deep P-I parameters

Within the mouth bed, depth had only mild effects on P-I parameters. There was slight evidence of an interaction between depth and temperature on respiration in the mouth bed ($p=0.0751$). This indicates that there may be a difference in how the eelgrass from the two depths responded to temperature, with respiration in the deep bed increasing more dramatically with temperature than the shallow bed.

There was also mild evidence that α may differ across depths in the mouth bed ($p=0.0802$). The observed trend indicates that eelgrass at the shallow portion

of the bed may have a higher α than that of the deeper portion of the bed (least squares means 13.79×10^{-3} and 4.96×10^{-3} , respectively).

P:R ratios, as well as their components (net P_{\max} and dark respiration) showed no differences between shallow and deep areas within the mouth bed. Neither I_K nor I_C varied between depths at the mouth bed.

3.3b P-I parameters and morphology across beds

Nearly all P-I parameters varied significantly across eelgrass beds, except for light requirements (Table 1). Productivity (P:R ratio; $p=0.0155$), as well as the individual components P_{\max} ($p=0.0251$) and dark respiration rate ($p=0.0481$), varied significantly by bed. Least squares means were calculated for P:R ratios to compare average productivity without the effects of temperature. Average productivity appears to be much higher in the mouth bed than the mid bed, with the average productivity of the back bed falling somewhere in between (Fig. 8).

Light-limited photosynthetic rate (α) varied across beds ($p=0.0137$), with the highest mean α at the mouth bed, making it most efficient at photosynthesizing at lower irradiances

Light thresholds were virtually the same across beds. I_K did not vary by bed, however there was slight evidence that I_C may differ across beds ($p=0.0714$; Table 1). There was no interaction between bed and temperature in any of the models, indicating that there was no difference in the response to temperature across beds.

l_{C24} varied by bed ($p=0.0095$). There was an interaction between temperature and bed ($p=0.0341$), indicating that the extent of the effect of temperature on l_{C24} varies across beds.

Morphological traits of eelgrass samples collected from Morro Bay were compared across beds. Significant differences were observed for blade length and blade damage across eelgrass beds. The average length of the longest blade per ramet differed by eelgrass bed ($p<0.001$; Fig. 9). Additionally, the percent of blades in a sample that were broken ($p=0.022$) or discolored ($p=0.041$) varied significantly across beds. There was a mean of 18.0% (\pm SE 3.4) broken blades for the mouth bed, 23.6% (\pm SE 4.7) for the mid, and 35.1% (\pm SE 4.7) for the back bed. Mean percent discolored blades were 4.2% (\pm SE 1.0%) for the mouth, 5.7% (\pm SE 1.4%) for the mid, and 0.7% (\pm SE 1.4%) for the back bed. There was no difference in the percent of individual blades that were both broken and discolored across eelgrass bed.

Parameter (95% CI)	Eelgrass Bed			F ratio	p value
	Mouth Bed	Mid Bed	Back Bed		
P:R	5.78 (4.59, 7.28)	3.22 (2.40, 4.33)	5.19 (3.99, 6.76)	4.94	0.016*
P _{max}	1.76 (1.43, 2.16)	1.08 (0.83, 1.41)	1.32 (1.04, 1.67)	4.29	0.025*
R	-0.3 (-0.27, -0.34)	-0.33 (-0.29, -0.39)	-0.25 (-0.22, -0.29)	3.44	0.048*
α	10.0x10 ⁻³ (6.5x10 ⁻³ , 15.2x10 ⁻³)	4.7x10 ⁻³ (2.8x10 ⁻³ , 8.2x10 ⁻³)	3.6x10 ⁻³ (2.2x10 ⁻³ , 5.9x10 ⁻³)	5.12	0.014*
I _k	240 (140, 413)	379 (190, 757)	215 (116, 399)	-	-
I _c	25 (16, 39)	47 (26, 85)	55 (33, 94)	2.94	0.071
I _{C24}	65 (39, 108)	243 (127, 446)	167 (93, 299)	5.64	0.010*

Table 1. Photosynthesis-irradiance (P-I) parameters across eelgrass beds. Parameters were calculated for P-I curves from individual eelgrass samples then least squares means were calculated for each eelgrass bed to remove the effects of temperature (n=31; df=2). P:R ratios were calculated as the net light-saturated photosynthetic rate (P_{max}; $\mu\text{mol O}_2 \text{L}^{-1} \text{g dry biomass}^{-1} \text{hour}^{-1}$) divided by the dark respiration rate (R; $\mu\text{mol O}_2 \text{L}^{-1} \text{g dry biomass}^{-1} \text{hour}^{-1}$) for each sample. Initial slope (α) is measured as increase in photosynthetic rate per photon ($\mu\text{mol O}_2 \text{L}^{-1} \text{g dry biomass}^{-1} \text{hour}^{-1} / \mu\text{mol photons m}^{-2} \text{s}^{-1}$). Light requirements (saturation irradiance (I_k), compensation irradiance (I_c), and critical irradiance (I_{C24})) are represented as $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. I_{C24} was calculated based on energy needs for a typical night in Morro Bay (12 hours of darkness).

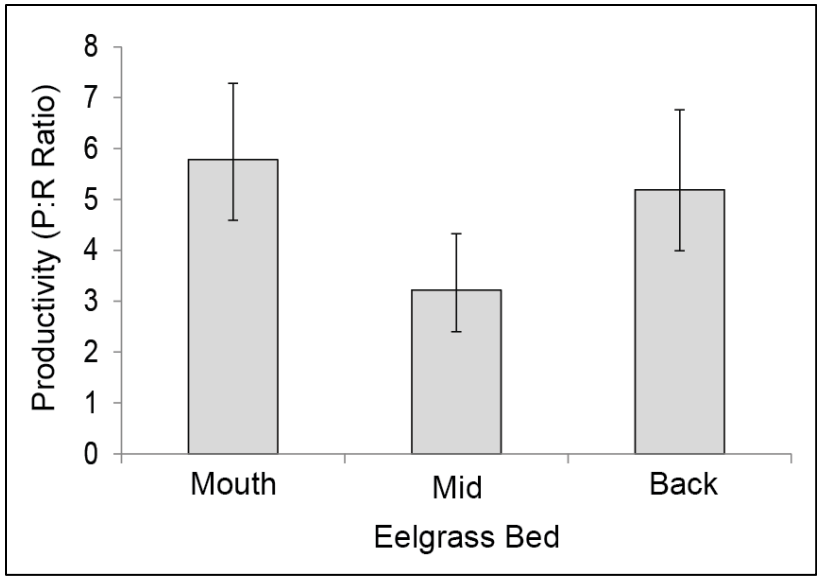


Figure 8. Productivity (P:R ratio) across eelgrass beds. P:R ratios were calculated as the net light-saturated photosynthetic rate (P_{max}) divided by the dark respiration rate (R) for each sample. Least squares means of P:R ratios were calculated for each eelgrass bed to remove the effect of temperature. P:R ratio varied significantly across beds ($p=0.0155$). Bars represent 95% confidence intervals.



Figure 9. Length of eelgrass blades by bed. Values are based on the average length of the longest blade per ramet for each sample then averaged for each bed. The average length of the longest blade per ramet (dots) significantly differed across beds ($p=0.0014$). Bars represent standard error.

4. DISCUSSION

4.1 Overview

Seagrass distribution has dwindled over the past century (Waycott et al. 2009) and its range is expected to shift further with the accelerating stressors of climate change (Short & Neckles 1999). Conservation efforts to mitigate the effects of climate change on seagrasses can be better informed by identifying initial warning signs of seagrass decline (Hemminga & Duarte 2000) and forecasting changes in distribution through modeling. Reliable modeling requires accurate knowledge of the physiological response of seagrass across current temperatures. Based on the response of eelgrass across the current temperature range in Morro Bay, we found 1) eelgrass productivity and light requirements are already negatively affected by high temperatures, 2) measurements of productivity and light requirements should be expanded to account for the concomitant environmental changes associated with climate change (i.e. increased temperature and light limitation), 3) the contribution of overnight respiration and root/rhizome respiration are important for calculating entire plant 24-hour energy budget, and 4) P-I curves cannot be applied universally, but provide insight into the resilience of eelgrass beds to climate change, relative to one another.

4.2 Eelgrass productivity and light requirements

4.2a Measuring productivity

Zostera marina in Morro Bay appears to live within its optimal growth temperature range based on measurements of productivity (P:R ratio) in saturating light conditions (P_{max}). No change in P:R was observed across the current temperature range. However, respiration increased with temperature, supporting previous observations that respiration increases more dramatically with temperature than P_{max} . (Bulthuis 1983, Dennison 1987, Herzka & Dunton 1997, Marsh et al. 1986, Masini & Manning 1997, Moore et al. 1997, Perez & Romero 1992).

Around the world, average optimal growth temperatures are ~15-20°C, above which productivity begins to decrease due to the dramatic effect of temperature on respiration (Marsh et al. 1986). For example, eelgrass from two populations were successfully maintained by Evans (1983) at 15°C, but died within four weeks when grown at 25°C. If eelgrass in Morro Bay follows the trend of other populations throughout the world, an increase of even 1°C may push *Z. marina* out of its optimal temperature range and inhibit growth.

The optimum temperature for photosynthesis and growth is commonly based on measurements taken in saturating light conditions. Because of the predicted decrease in available light, it is important to consider how temperature may affect growth rates in less favorable light conditions. In terrestrial plants (Pisek 1973), as well as seagrasses (Bulthuis 1987), photosynthetic rate peaks at lower temperatures when plants are in low rather than high light environments.

In our study, the decrease in light-limited photosynthetic rate combined with the increase in respiration rate at higher temperatures suggests that eelgrass in Morro Bay that is not able to reach saturation irradiance is already experiencing decreased productivity in high temperatures.

Because the light-limited photosynthetic rate is associated with the light reaction of photosynthesis, it is considered directly proportional to irradiance (Bulthuis 1987, Platt & Jassby 1976); however, if seagrasses are exposed to temperatures above their physiological tolerance range, α decreases (Bulthuis 1987) due to the loss of structural integrity of the photosynthetic apparatus (Berry & Bjorkman 1980). Evidence from previous studies indicates that α of *Z. marina* decreases anywhere between 19°C and 35-40°C (Bulthuis 1983 & 1987, Evans et al. 1986, Marsh et al. 1986, Orth & Moore 1986, Olesen & Sand-Jensen 1993). Marsh et al. (1986) observed a maximum rate of light-limited photosynthesis at 0°C and a minimum rate at 35°C, with relatively no change between 5-30°C. The decrease in α observed in our study suggests that the light-limited photosynthetic rate is more sensitive to temperature than the light-saturated photosynthetic rate, and is already decreasing at higher temperatures within the current range. We suggest that productivity be calculated based on realistic, light-limited conditions, rather than the traditional method of calculating productivity based on light-saturated conditions.

4.2b Light thresholds and temperature

Climate change is expected to decrease available light through increasing sea level and increases in turbidity. On top of the physical light limitations imposed by climate change, the increase in ocean temperature will also cause light requirements of eelgrass to increase, as evidenced by the increase in both I_K and I_C with increased temperature.

The values for I_C and I_K are comparatively higher here than in previous studies on eelgrass, which ranged from 1-85 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and 7-450 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively (Lee et al. 2007). This difference in magnitude is likely due to the methods employed. Where previous studies measured seagrass light thresholds based on leaf segments alone, we used whole ramets (leaves, shoots, roots, and rhizomes) in our calculations of I_C and I_K , a method known to cause a five-fold increase in I_C (Dunton and Tomasko 1994).

Additionally, the higher magnitude of light thresholds measured in our study may be due to the more representative in situ conditions we used—natural outdoor light and filtered seawater pumped directly from ambient ocean waters—and by the way we measured the light environment. During experimental trials, eelgrass samples were bundled together and likely experienced mild self-shading; the light sensor was not adjacent to the samples so recorded light values only represent the light reduction caused deliberately by each treatment. Because this method was consistent across trials, we are confident that the trends in light thresholds are representative of in situ eelgrass response.

Although compensation irradiance (I_C) has commonly been considered the minimum amount of light necessary for seagrass survival, our results suggest that critical irradiance (I_{C24}) may be a better proxy for light requirements in higher temperatures. Both compensation irradiance and critical irradiance increased with increasing temperature, indicating that more light is required to maintain a positive carbon balance in higher temps (Bulthuis 1987). However, it is clear that critical irradiance is much more sensitive to increases in temperature (Fig. 6). Because critical irradiance is based on 24-hour energy needs, the increase in overnight respiration caused by increased temperature is reflected in the amount of light needed to balance the increase in energy use.

4.3 Measuring 24-hour energy budget

4.3a Day v. night respiration rates

Our data suggest that modeling future eelgrass distribution will require us to be able to make reliable estimates of plant energy budgets. One important consideration in calculating 24-hour energy use is the comparison of night and day respiration rates. Respiration rates at night were on average 75% that of day time rates, meaning energy budget estimates that assume consistent 24-hour respiration rates based on a only day measurements may be overestimating energy costs. Due to our small sample size, further measurements are needed to make statements regarding the quantitative difference between day and night respiration rates.

Few studies have compared day and night respiration rates of seagrasses. Growth measurements of *Halophila decipiens* revealed higher growth rates during the day than at night, regardless of light environment, indicating that growth pattern is endogenously controlled (Williams & Dennison 1990). Growth measurements of *Z. marina* also revealed lower growth rates at night, and were correlated to growth rates during the prior day, indicating that energy produced during daylight hours is used for day-, as well as night-, time growth (Kemp et al. 1987). More research is needed to quantify differences in day and night respiration rates of seagrasses and to assess the variation of these diel patterns among and within populations. Quantification of night time respiration rates will improve estimates of daily energy and light requirements used to determine the potential for long-term survival.

4.3b Contribution of roots and rhizomes

Another important consideration in establishing the response of eelgrass beds to climate change is incorporating root and rhizome respiration rates into estimates of 24-hour energy budgets. As predicted, roots and rhizomes contributed significantly to overall plant respiration; however, the respiration rate (per unit dry biomass) of roots/rhizomes in this study was much higher than expected. Representing only 17% of the biomass of the plant, yet responsible for nearly 40% of total plant respiration, roots and rhizomes respired at a rate over three times higher (per unit biomass) than shoots and leaves.

Though little data is available on root and rhizome respiration rates of seagrasses, shoots and leaves usually respire at a rate ~2-5 times higher (per unit biomass) than underground tissues, depending on the species (Hemminga 1998). Eelgrass shoot/leaf respiration has been reported as 3x that of roots/rhizomes (Kraemer & Alberte 1993).

Various methods have been employed to measure seagrass root/rhizome respiration and may partially explain the present inconsistencies. Caffrey and Kemp (1991) measured eelgrass respiration by placing intact ramets in divided chambers and measuring changes in water column O₂ surrounding only the roots/rhizomes. During normal functioning, photosynthetically derived O₂ from leaves is passed down to below ground tissues, via specialized structures (Hemminga 1998, Zimmerman et al. 1995). During photosynthesis, 10% of the oxygen produced is released from the roots and rhizomes (Caffrey and Kemp 1991); the amount of O₂ from the leaves that is used for root/rhizome respiration is unknown. By separating roots and rhizomes from photosynthetic tissues we measured all the O₂ used for respiration (from the water column) rather than providing the shoots and leaves as an immeasurable source of O₂. Therefore, previous studies may have underestimated root and rhizome respiration, depending on the method used.

The magnitude of root/rhizome respiration measured in our study may be inflated due to microbial aerobic respiration. Because of the lack of oxygen diffusion into the sediment from the water column (Hemminga 1998), oxygen-dependent microbes dwell on and around the subterranean tissues of eelgrass.

The gentle rinsing of roots and rhizomes before respirometry may not have been sufficient to remove all microbes. The inclusion of these microbes may have artificially increased the uptake of O₂ that was attributed to respiration of the roots/rhizomes.

The contribution of roots and rhizomes to whole plant respiration is dependent on the ratio of photosynthetic to non-photosynthetic tissue, known as the shoot: root (s: r) ratio (Hemminga 1998). Allocation of belowground tissue varies greatly by geographic region (Kraemer & Alberte 1993). Percent total biomass for eelgrass roots/rhizomes has been reported as 57% in Denmark (Sand-Jensen 1975), 20% in Monterey Bay, Ca. (Kraemer & Alberte 1993), and 10% in Elkhorn Slough, Ca. (Britting et al. unpubl., as cited in Kraemer & Alberte 1993). Because s:r ratios vary within species depending on the light environment (Hillman et al. 1989) and because our sample composition was consistent (17.1% roots/rhizomes, $\pm 1.1\%$), we are confident that the tissue distribution of samples was representative of the Morro Bay eelgrass population.

4.4 Application of P-I curves

4.4a Shallow v. deep P-I parameters

The difference between eelgrass growing in shallow v. deep areas is thought to be analogous to the difference between sun v. shade growing terrestrial plants—plants receiving higher light levels have higher photosynthetic and respiration rates (Dennison & Alberte 1982). However, we found no differences between P-I parameters at different depths. The lack of the effect of

depth seen here is likely due to the fact that both “shallow” and “deep” samples spanned an intermediate depth range, and likely received comparable light exposure due to the high water clarity in this area of the bay.

4.4b P-I parameters and morphology across beds

Though we found no differences in P-I parameters at different depths, there were clear differences in basal P-I parameters across eelgrass beds, with the mouth bed demonstrating the highest averages for P_{max} , P:R ratio, α , and lowest average I_{C24} (Table 1). This suggests that the mouth bed is in the best condition and will be the most resilient to the progressing stressors of climate change.

Though the cause of the differences between beds is unclear, genotypic and phenotypic variation can produce differences in photosynthetic response to temperature in terrestrial plants (Berry & Bjorkman 1980). In eelgrass, genotypic variation in growth rates (Evans 1983), as well as optimum temperatures for photosynthesis and dark respiration (Biebl & McRoy 1971) have been observed for different ecotypes (eg. subtidal v. intertidal populations). Genetic analysis and “common garden” experiments are needed to determine if bed differences in Morro Bay can be attributed to genotypic differences.

Variation in light-limited photosynthetic rate (α) across beds may be explained by depth. Deeper beds often have higher α than shallow beds (Masini & Manning 1997); higher light-limited photosynthetic rates are a form of photoacclimation for deeper (often light-limited) beds to increase carbon

production with less light (Lee et al. 2007). Although we did not see this pattern within the mouth bed, it is represented across beds. The variability in ambient environmental conditions in the bay makes it difficult to determine the exact cause of physiological differences across beds. Depth is often correlated with other water quality variables; deep areas are usually away from nutrient sources (resulting in less phytoplankton growth and more light), are typically close to or along coasts (providing water and sediment renewal via wave exposure), and have less sediment re-suspension and turbidity than shallower areas (Greve & Krause-Jensen 2005). All of these correlations between water quality and depth apply to eelgrass beds in Morro Bay, making it difficult to single out depth as the cause for physiological differences across beds.

Differences in light requirements across beds were not apparent until 24-hour energy demands were taken into account. Duarte et al. (2007) observed that seagrasses in turbid environments have higher light requirements than their clear-watered counterparts. The mouth bed, which had the lowest average critical irradiance (I_{C24}), indeed has the clearest conditions of the three, while the mid and back bed experience more turbid conditions due to their close proximity to input from Chorro and Los Osos Creeks. The difference in I_{C24} across beds further supports the notion that it is a more sensitive indicator of irradiance needs than I_C and should be used to determine light thresholds for eelgrass survival.

Morphological differences also provide insight about the relative condition of eelgrass beds. The mid bay, in addition to demonstrating the highest critical irradiance, had the longest blades of the three beds. Longer eelgrass blades

(combined with overall lower biomass and shoot density) are indicative of reduced light environments (Behm & Boumans 2002, Short et al. 1995, Olesen & Sand-Jensen 1993). In low light, eelgrass plants allocate energy to elongate leaves, rather than produce new ones, to reach shallower depths where more light is available (Short et al. 1995). The mouth bed had shortest blades, and the lowest percentage of broken blades, indicating that their short stature is the result of natural growth length, rather than the result of damage from boat traffic or other mechanical disturbances. Our data suggest that eelgrass in the mid bed is morphologically adapted to a light-limited environment and is already experiencing stressful conditions.

It is important to quantify P-I parameters for individual eelgrass beds, even within small geographic areas, including Morro Bay. The physiological and morphological differences across eelgrass beds suggest that some beds function more efficiently than others and will respond better to the stressors of climate change. Identification of resilient beds for transplant to areas favorable for growth in future conditions will be useful to mitigate for unavoidable eelgrass losses.

4.5 Final remarks

The continued persistence of seagrasses over the coming century is heavily dependent on our actions, both in contributing to and mitigating for the effects of climate change. Models used to forecast changes in eelgrass distribution can be improved with realistic estimates of physiological thresholds for survival. These estimates can be better quantified by expanding the ways we

measure productivity and survival, and by incorporating whole ramets, nightly respiration rates, and variation across beds in these measurements. The response of eelgrass across the current temperature range suggests that light-limited productivity and light requirements are already negatively affected by high temperatures. Now, and in the coming years, it is important that we adjust our methods to account for the combined effects of light, temperature, and other variables that may interact to have compounding impacts on eelgrass physiology. Furthermore, P-I curves are valuable tools for comparing the relative performance of eelgrass beds and can aid in the planning and execution of targeted restoration efforts.

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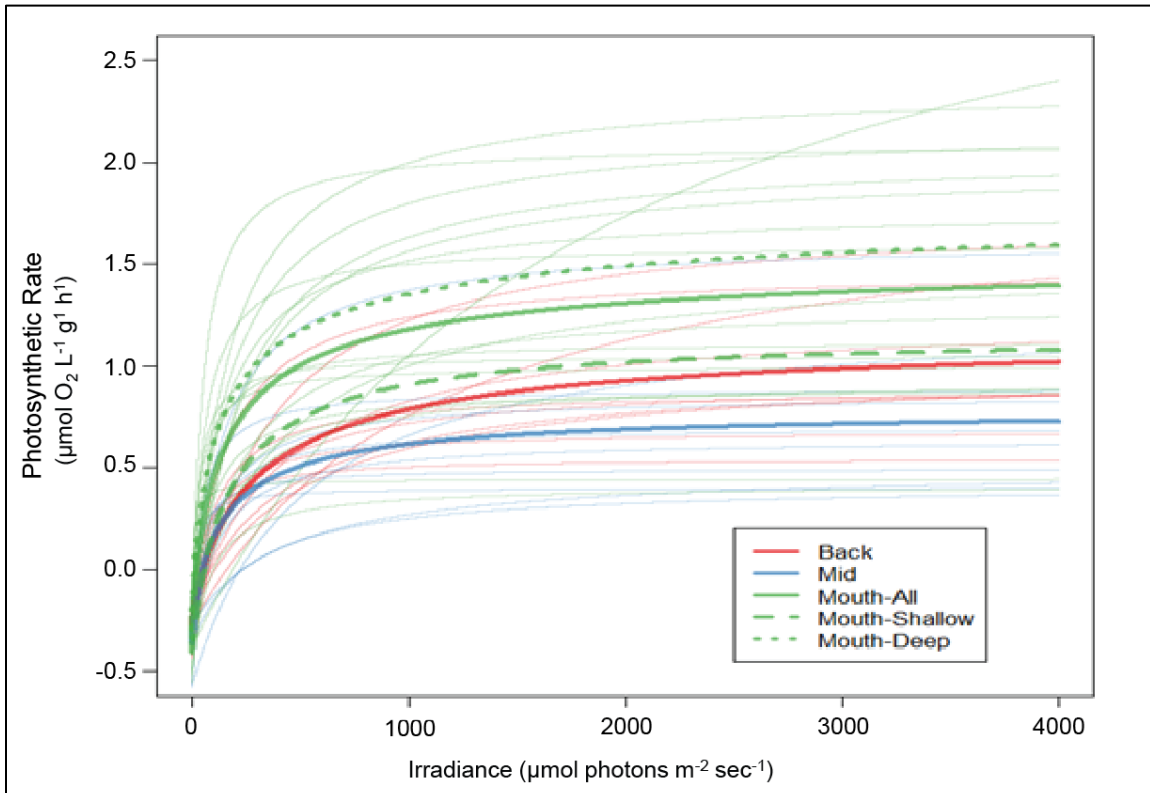
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APPENDIX A. Photosynthesis-Irradiance (P-I) Curves



One P-I curve was generated for each eelgrass sample at a particular temperature between 10°C and 20°C ($n=31$). Point-wise averages were calculated from individual curves to fit a summary curve for each eelgrass bed (color-coded thicker lines; $n_{\text{mouth}}=13$, $n_{\text{mid}}=8$, $n_{\text{back}}=10$) and depth (green dashed lines; $n_{\text{shallow}}=9$, $n_{\text{deep}}=4$).