



*Padilla Bay*

National Estuarine Research Reserve

Reprint Series No. 22  
Reprinted November 1995

**THE EFFECT OF EXPOSURE TO ENVIRONMENTAL  
NORMOXIA AND HYPOXIA ON PHOTOSYNTHETIC  
RATE AND CHLOROPHYLL CONCENTRATION IN  
INTERTIDAL ZOSTERA MARINA LEAVES**

Sharon R. Riggs

May 1995

Publication No. SWR-95-77

The Padilla Bay National Estuarine Research Reserve is one of the reserves in the National Estuarine Research Reserve System. One of the purposes of the Reserve is to facilitate research and monitoring at Padilla Bay to provide information for the conservation and management of the nation's estuaries, in particular greater Puget Sound and other estuaries in the Pacific Northwest. The Padilla Bay National Estuarine Research Reserve assists the dissemination of this information from research and monitoring by publishing a Reprint Series and a Technical Report Series.

The **Reprint Series** includes research grant reports, out of print agency reports and student reports dealing with the Padilla Bay estuary. Reports are reprinted without revision or editing. Final reports for research grants and Masters Theses should be treated as unpublished data and should not be cited without permission of the author(s).

The **Technical Report Series** includes articles, reports of research projects, data reports, bibliographies and reviews dealing with the Padilla Bay estuary.

Communications concerning receipt or exchange of Technical Reports or Reprints or submission of manuscripts should be directed to the Research Coordinator at Padilla Bay National Estuarine Research Reserve. Communications concerning the content of reports and reprints should be directed to the author(s).

Padilla Bay National Estuarine Research Reserve  
10441 Bayview-Edison Road  
Mount Vernon WA 98273-9668  
(360)428-1558

Padilla Bay National Estuarine Research Reserve is managed by the Shorelands and Environmental Assistance Program, Washington State Department of Ecology, in cooperation with the Estuarine Reserves Division, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce. The preparation of this document was financially aided through a grant to the Washington State Department of Ecology with funds obtained from NOAA/Office of Ocean and Coastal Resource Management, and appropriated for Section 306 or 315 of the Coastal Zone Management Act of 1972, as amended.



Reprint Series No. 22  
Reprinted November 1995

**THE EFFECT OF EXPOSURE TO ENVIRONMENTAL  
NORMOXIA AND HYPOXIA ON PHOTOSYNTHETIC RATE  
AND CHLOROPHYLL CONCENTRATION IN INTERTIDAL  
ZOSTERA MARINA LEAVES**

**Sharon R. Riggs**

**May 1995**

**Publication No. SWR-95-77**

**THE EFFECT OF EXPOSURE TO ENVIRONMENTAL NORMOXIA AND  
HYPOXIA ON PHOTOSYNTHETIC RATE AND CHLOROPHYLL  
CONCENTRATION IN INTERTIDAL *ZOSTERA MARINA* LEAVES**

A Thesis

Presented to

The Faculty of

Western Washington University

---

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Sharon R. Riggs

May 1995

**Bibliographic citation:** The effect of exposure to environmental normoxia and hypoxia on photosynthetic rate and chlorophyll concentration in intertidal *Zostera marina* leaves. Master's Thesis. Western Washington University, Bellingham, Washington. 74 pp. Padilla Bay National Estuarine Research Reserve Reprint No. 22, Reprinted November, 1995.

A reprint of the Washington State Department of Ecology pursuant to National Oceanic and Atmospheric Administration Award No. NA270R216. The views expressed herein are those of the author and do not necessarily reflect the view of NOAA, the Washington State Department of Ecology or any of their subagencies. Copies of this reprint are available from the Padilla Bay National Estuarine Research Reserve.

The Washington State Department of Ecology is an Equal Opportunity and Affirmative Action employer. If you have special accommodation needs, please contact the Padilla Bay Reserve at (360)428-1558 or (360)757-1549.

**THE EFFECT OF EXPOSURE TO ENVIRONMENTAL NORMOXIA  
AND HYPOXIA ON PHOTOSYNTHETIC RATE AND CHLOROPHYLL  
CONCENTRATION IN INTERTIDAL ZOSTERA MARINA LEAVES**

BY

SHARON R. RIGGS

Accepted in Partial Completion  
of the Requirements for the Degree  
Master of Science



---

Moheb A. Ghali, Dean of the Graduate School

ADVISORY COMMITTEE



---

Chair, Dr. David Schneider



---

Dr. Rich Fonda



---

Dr. Gisele Muller-Parker



---

Dr. Douglas Bulthuis

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of this thesis is allowable only for scholarly purposes. It is understood, however, that any copying or publication of this thesis for commercial purposes, or for financial gain, shall not be allowed without written permission.

Signature

*Aharon R. Riggs*

Date

5/8/95



THE EFFECT OF EXPOSURE TO ENVIRONMENTAL NORMOXIA  
AND HYPOXIA ON PHOTOSYNTHETIC RATE AND CHLOROPHYLL  
CONCENTRATION IN INTERTIDAL *ZOSTERA MARINA* LEAVES

by  
Sharon R. Riggs

**ABSTRACT.** Exposure of intertidal *Zostera marina* leaves to dark environmental normoxia or hypoxia significantly reduced photosynthetic rates but had little effect on chlorophyll concentrations in leaf tissue. A 2 x 3 factorial experiment was conducted at each of three temperatures: 10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C. Photosynthetic rates were determined using a radioisotope method and leaf chlorophyll was extracted and measured spectrophotometrically.

Leaves exposed to normoxia in the dark at 10<sup>o</sup>C and at 30<sup>o</sup>C had significantly greater mean photosynthetic rates than leaves exposed to hypoxia in the dark. At 20<sup>o</sup>C there was a significant interaction between the factors oxygen and time. Time had a significant effect on photosynthetic rates for hypoxic treatments but was not significant for normoxic treatments. The photosynthetic rate means for leaves exposed to hypoxia were significantly lower than normoxic means for each time tested (24, 36, and 48 hours).

At 10<sup>o</sup>C and 20<sup>o</sup>C, mean chlorophyll concentrations in leaves exposed to normoxia or hypoxia in the dark were not significantly different. At 30<sup>o</sup>C mean chlorophyll *a* concentration in *Z. marina* leaves was significantly higher in leaves exposed to normoxia in the dark than for leaves exposed to hypoxia in the dark.

These results indicate that *Zostera marina* growing in estuaries that are prone to ponding or eutrophication may be impacted if conditions exist that encourage hypoxic conditions. Those conditions would include temperatures of 30<sup>o</sup>C or higher, decomposition of macroalgae in ponded sites, or prolonged exposure to the dark at 10<sup>o</sup>, 20<sup>o</sup>C, or 30<sup>o</sup>C.

## **ACKNOWLEDGEMENTS**

I would like to thank the members of my committee for their encouragement and support. My thanks go to my committee chair, Dr. David Schneider, for his time reading rough drafts and for his suggested improvements, Dr. Gisele Muller-Parker for her invaluable help with the radioisotope work, Dr. Rich Fonda for reviewing my statistical model and offering suggestions for multiple range comparisons, and my colleague, Dr. Douglas Bulthuis.

I would like to thank Dr. Bulthuis for the major portion of funding for this research which was through a grant (NA270R216-0, National Oceanic and Atmospheric Administration) awarded to him to study the effects of light reduction on seagrasses in Padilla Bay, Washington. My heartfelt thanks go to my supervisor at Padilla Bay, Terry Stevens, for his patience and understanding while I pursued this degree.

Thanks also go to Dr. Brian Bingham for helping me better understand the statistical tests; to Travis Shaw and Sherri Rodgers for their moral support; and to Gene McKeen, Shannon Point Marine Center, and Doug Doolittle, Biology, for helping me locate supplies and equipment. Clint Burgess at the Instrument Center built the light table for this study and did it in record time.

## TABLE OF CONTENTS

INTRODUCTION.....	1
Objectives.....	4
METHODS AND MATERIALS	
Photosynthetic rates and chlorophyll concentrations.....	6
<i>Zostera marina</i> collection.....	6
Experimental design.....	6
Laboratory procedures and calculations.....	9
Statistical analysis.....	16
P-I curves.....	17
<i>Zostera marina</i> collection and laboratory procedures.....	19
Data analysis.....	20
RESULTS.....	21
10°C Experiment.....	21
Photosynthetic rates.....	21
Chlorophyll concentrations.....	26
20°C Experiment.....	26
Photosynthetic rates.....	28
Chlorophyll concentrations.....	40
30°C Experiment.....	40
Photosynthetic rates.....	40
Chlorophyll concentrations.....	50
Photosynthesis vs. Irradiance Curves.....	59
DISCUSSION.....	64
Photosynthetic rates and chlorophylls.....	64
P-I curves.....	69
LITERATURE CITED.....	71



## LIST OF FIGURES

<b>Figure 1.</b> Location of collection site (X) for intertidal <i>Zostera marina</i> plants in Padilla Bay, Washington.....	7
<b>Figure 2.</b> Portions of <i>Zostera marina</i> leaf used for photosynthetic rate experiments and for chlorophyll analysis.....	13
<b>Figure 3.</b> The P-I curve characteristics measured for this study were initial slope ( $\alpha$ ), $P_{max}$ and $I_k$ . The y-intercept is zero, or the light compensation point ( $I_c$ ), where respiration equals gross photosynthesis and net photosynthesis is zero. ....	18
<b>Figure 4.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 10°C on mean photosynthetic rates in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=8, $\pm$ s.e.).....	22
<b>Figure 5.</b> Comparison of photosynthetic rates for intertidal <i>Zostera marina</i> leaves exposed to normoxic water in the light for one hour at 10°C and <i>Z. marina</i> leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours. Photosynthetic rates for leaves exposed to normoxic or hypoxic water in the dark were significantly less than for leaves exposed to normoxic water in the light.....	24
<b>Figure 6.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 10°C on mean chlorophyll <i>a</i> concentrations in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, $\pm$ s.e.) .....	27
<b>Figure 7.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 10°C on mean chlorophyll <i>b</i> concentrations in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, $\pm$ s.e.) .....	28
<b>Figure 8.</b> Comparison of chlorophyll <i>a</i> concentrations for intertidal <i>Zostera marina</i> leaves exposed to normoxic water in the light for one hour at 10°C and <i>Z. marina</i> leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours.....	29
<b>Figure 9.</b> Comparison of chlorophyll <i>b</i> concentrations for intertidal <i>Zostera marina</i> leaves exposed to normoxic water in the light for one hour at 10°C and <i>Z. marina</i> leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours. Chlorophyll <i>b</i> concentrations in leaves exposed to normoxic water in the light were significantly less than chlorophyll <i>b</i> concentrations in leaves exposed to hypoxic water for 71.5 hours but were not significantly different from all other treatments.....	30
<b>Figure 10.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 10°C on mean chlorophyll <i>a:b</i> ratios in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, $\pm$ s.e.).....	31

**Figure 11.** Profile of the main effects of normoxic and hypoxic treatments over time at 20°C on mean photosynthetic rates in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=8, ± s.e.)..... 34

**Figure 12.** Comparison of photosynthetic rates for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to dark normoxic or hypoxic water for 24, 36, and 48 hours. Photosynthetic rates for leaves exposed to dark normoxic water for 24 and 48 hours were not significantly different from leaves exposed to normoxic water in the light. All other treatment means were significantly less than those of the leaves exposed to normoxia in the light..... 38

**Figure 13.** Profile of the main effects of normoxic and hypoxic treatments over time at 20°C on mean chlorophyll *a* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.) .....41

**Figure 14.** Profile of the main effects of normoxic and hypoxic treatments over time at 20°C on mean chlorophyll *b* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.) .....42

**Figure 15.** Comparison of chlorophyll *a* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 24, 36, and 48 hours. Mean chlorophyll *a* concentration in leaves exposed to normoxia for one hour in the light were not significantly different from all other treatments..... 43

**Figure 16.** Comparison of chlorophyll *b* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 24, 36, and 48 hours. Chlorophyll *b* concentrations in leaves exposed to normoxic water in the light were not significantly different from all other treatment means ..... 44

**Figure 17.** Profile of the main effects of normoxic and hypoxic treatments over time at 20°C on mean chlorophyll *a:b* ratios in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.).....45

**Figure 18.** Profile of the main effects of normoxic and hypoxic treatments over time at 30°C on mean photosynthetic rates in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=8, ± s.e.).....47

**Figure 19.** Comparison of photosynthetic rates for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 30°C and *Z. marina* leaves exposed to dark normoxic or hypoxic water for 3.5, 8, and 12 hours. Photosynthetic rates for leaves exposed to dark normoxic or hypoxic water were significantly less than for leaves exposed to normoxic water in the light.....51

<b>Figure 20.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 30°C on mean chlorophyll <i>a</i> concentrations in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9, ± s.e.).....	53
<b>Figure 21.</b> Profile of the main effects of normoxic and hypoxic treatments over time at 30°C on mean chlorophyll <i>b</i> concentrations in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9, ± s.e.).....	55
<b>Figure 22.</b> Comparison of chlorophyll <i>a</i> concentrations for intertidal <i>Zostera marina</i> leaves exposed to normoxic water in the light for one hour at 30°C and <i>Z. marina</i> leaves exposed to normoxic or hypoxic water in the dark for 3.5, 8, and 12 hours. Mean chlorophyll <i>a</i> concentrations in leaves exposed to normoxia for one hour in the light were not significantly different from all other treatments.....	56
<b>Figure 23.</b> Comparison of chlorophyll <i>b</i> concentrations for intertidal <i>Zostera marina</i> leaves exposed to normoxic water in the light for one hour at 30°C and <i>Z. marina</i> leaves exposed to normoxic or hypoxic water in the dark for 3.5, 8, and 12 hours. Chlorophyll <i>b</i> concentrations in leaves exposed to normoxic water in the light were not significantly different from all other treatment means.....	57
<b>Figure 24.</b> Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 30°C on mean chlorophyll <i>a:b</i> ratios in intertidal <i>Zostera marina</i> leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9, ± s.e.).....	58
<b>Figure 25.</b> P-I curve for intertidal <i>Zostera marina</i> leaves exposed to normoxia in the light for one hour at 10°C. Each point represents mean photosynthetic rate (means, ± s.e.).....	60
<b>Figure 20.</b> P-I curve for intertidal <i>Zostera marina</i> leaves exposed to normoxia in the light for one hour at 20°C. Each point represents mean photosynthetic rate (means, ± s.e.).....	61
<b>Figure 21.</b> P-I curve for intertidal <i>Zostera marina</i> leaves exposed to normoxia in the light for one hour at 30°C. Each point represents mean photosynthetic rate (means, ± s.e.).....	62



## LIST OF TABLES

<b>Table 1.</b> Collection dates and times for intertidal <i>Zostera marina</i> plants collected from Padilla Bay, Washington. The last column indicates the plants were collected for exposure to normoxic and hypoxic water in the dark for the designated length of exposure (hours) at the designated temperature (°C). P-I = plants collected for P-I curve determinations .....	8
<b>Table 2.</b> Dissolved oxygen content of seawater (mg·L <sup>-1</sup> ) in BOD bottles pre-treatment and post-treatment. All treatments took place in the dark. Pre-treatment means, n=5, n=4*. Post-treatment means, n=9. Normoxia (> 2 mg·L <sup>-1</sup> dissolved oxygen). Hypoxia (> 0 mg·L <sup>-1</sup> dissolved oxygen but ≤ 2 mg·L <sup>-1</sup> ). ND = no data. Saturation data from Strickland and Parsons (1972), 30.5 ‰ salinity: 10°C: 9.28 mg·L <sup>-1</sup> ; 20°C: 7.64 mg·L <sup>-1</sup> ; 30°C: 6.35 mg·L <sup>-1</sup> .....	12
<b>Table 3.</b> Analysis of variance for photosynthetic rates in <i>Zostera marina</i> leaves exposed to normoxia or hypoxia at 10°C for 48, 71.5, and 97 hours (α = 0.05).....	23
<b>Table 4.</b> Mean photosynthetic rates of intertidal <i>Zostera marina</i> leaves at 10°C (n = 8, ± s.e.). Normoxic (> 2 mg·L <sup>-1</sup> dissolved oxygen) treatments were in the dark. Hypoxic (> 0 mg·L <sup>-1</sup> dissolved oxygen but ≤ 2 mg·L <sup>-1</sup> ) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light.....	25
<b>Table 5.</b> Analysis of variance for chlorophyll a:b ratios in <i>Zostera marina</i> leaves exposed to normoxia or hypoxia in the dark at 10°C for 48, 71.5, and 97 hours (α = 0.05). There were no significant differences among treatment means for chlorophylls a or b.....	32
<b>Table 6.</b> Results of Tukey's multiple range comparison test for chlorophyll a:b ratio over time at 10°C for intertidal <i>Zostera marina</i> leaves collected from Padilla Bay, Washington. Treatments connected with an underline are not significantly different (α = 0.05). Treatment duration was 48, 71.5, and 97 hours.....	33
<b>Table 7.</b> Analysis of variance for photosynthetic rates in <i>Zostera marina</i> leaves exposed to normoxia or hypoxia at 20°C for 24, 36, and 48 hours (α = 0.05).....	35
<b>Table 8.</b> Results of Tukey's multiple range comparison test for photosynthetic rate (μg C·cm <sup>-2</sup> ·hr <sup>-1</sup> ) at 20°C for <i>Zostera marina</i> leaves collected from Padilla Bay, Washington. Treatments connected by an underline are not significantly different (α = 0.05). Treatment duration was 24, 36, and 48 hours.....	37
<b>Table 9.</b> Mean photosynthetic rates of intertidal <i>Zostera marina</i> leaves at 20°C (n=8, ± s.e.). Normoxic (> 2 mg·L <sup>-1</sup> dissolved oxygen) treatments were in the dark. Hypoxic (> 0 mg·L <sup>-1</sup> dissolved oxygen but ≤ 2 mg·L <sup>-1</sup> ) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light.....	39

**Table 10.** Analysis of variance for chlorophyll *a:b* ratio in *Zostera marina* leaves exposed to normoxia or hypoxia at 20°C for 24, 36, and 48 hours ( $\alpha = 0.05$ ). There were no significant differences among treatment means for chlorophylls *a* or *b*..... 46

**Table 11.** Analysis of variance for photosynthetic rates for *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 30°C for 3.5, 8, and 12 hours ( $\alpha = 0.05$ ) ..... 48

**Table 12.** Results of Tukey's multiple range comparison test for photosynthetic rates ( $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) over time at 30°C for *Zostera marina* leaves collected from Padilla Bay, Washington, and exposed to dark normoxic and hypoxic treatments. Treatments connected with an underline are not significantly different ( $\alpha = 0.05$ ). Treatment duration was 3.5, 8, and 12 hours..... 49

**Table 13.** Mean photosynthetic rates of intertidal *Zostera marina* leaves at 30°C ( $n=8$ ,  $\pm$  s.e.). Normoxic ( $> 2 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen) treatments were in the dark. Hypoxic ( $> 0 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen but  $\leq 2 \text{ mg}\cdot\text{L}^{-1}$ ) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light ..... 52

**Table 14.** Analysis of variance for chlorophyll *a* for *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 30°C for 3.5, 8, and 12 hours ( $\alpha = 0.05$ ). There were no significant differences among treatment means for chlorophyll *b* or chlorophyll *a:b* ratios..... 54

**Table 15.** P-I curve characteristics for intertidal *Zostera marina* leaves exposed to normoxia for one hour in the light. Least squares regression was performed on mean ( $n=8$ ) initial slopes.  $P_{\text{max}}$  values are mean ( $n=8$ ).  $P_{\text{max}}$  was not reached at 30°C.  $I_k = P_{\text{max}}/\alpha$ , where  $\alpha$  = initial slope..... 63

## INTRODUCTION

Hypoxia and anoxia occur in estuarine sediments (McLusky, 1974; Day et al., 1989; ) and waters (Cooper and Brush, 1993). The effect of environmental anoxia on photosynthesis in seagrasses (*Thalassia testudinum* and *Halophila decipiens*) has been the focus of only one published study (Hammer, 1972). No studies to date have addressed the effects of environmental hypoxia on photosynthesis in seagrasses, particularly *Zostera marina* L. However, studies have examined the effects of hypoxia and anoxia on *Z. marina* respiration (McRoy, 1966), root and rhizome metabolism (Smith et al., 1988), seed germination (Moore et al., 1993), and seedling growth (Churchill, 1992).

Environmental anoxia is a complete lack of oxygen. Environmental hypoxia in the water column is reported as  $\leq 2 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen (Dortch et al., 1994; Rabalais et al., 1994) while normoxia is defined as  $> 2 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen (Rabalais et al., 1994). Low oxygen concentrations in estuarine waters can result from oxygen depletion by bacterial respiration during decomposition of an excess of plant growth which is often caused by the input of large amounts of nutrients (Fahy et al., 1975; Harlin and Thorne-Miller, 1981). When plants are not photosynthesizing (i.e. in the dark), bacterial and plant respiration may further lower oxygen concentrations in the water column. Anoxia has been reported in *Zostera* beds at night in the Netherlands (Broekhuysen, 1935) and may occur regularly in shallow *Z. marina* beds in Alaska (McRoy, 1966). *Zostera* growing under *Enteromorpha radiata* mats shows symptoms typical of decay due to long-term exposure to anaerobic conditions (i.e. upper leaves have normal green appearance while leaves and stalks are flaccid and yellow) (Den Hartog, 1994).

Higher plants can generally survive without oxygen for a limited period of time, especially if they have organs protected from gas exchange (e.g. rhizomes), but extended anoxia eventually results in death (Crawford et al., 1989; Brändle, 1991). Metabolic adaptations of plants to anoxia include: maintenance of internal aeration, diversification of the end products of glycolysis, storage of large amounts of food reserves, and coupling of metabolic pathways (Crawford, 1978; Brändle, 1991).

Fairly extensive work has been done on the effects of environmental and cellular hypoxia and anoxia on commercially important terrestrial plants such as peas, rice, and wheat. Pea seedlings die under anoxic conditions at different temperatures and at internal ethanol concentrations exceeding 60  $\mu\text{M}$  (Barclay and Crawford, 1981). Effects of anoxia and hypoxia in rice include decreased synthesis of mitochondrial proteins (Couee et al., 1992), inhibition of normal chloroplast photomorphogenesis in coleoptiles of light-germinated rice seedlings (Kordan and Ashraf, 1990), and changes in levels of enzymes involved in glycolysis in rice seedlings (Mertens et al., 1990). Effects of anoxia in rice and wheat seedlings include changes in intracellular pH, glucose-6-phosphate level, and metabolic rate (Menegus et al., 1989; Menegus et al., 1991). An interaction between anoxia, temperature, and pH is reported for wheat seedlings (Waters et al., 1991a) as is the influence of hypoxic exposure prior to anoxia on tolerance to anoxia, alcoholic fermentation, and sugar levels (Waters et al., 1991b).

The effects of anoxia have also been studied with respect to freshwater wetland plants. Rhizomes of anoxia-intolerant plant species (e.g. *Glyceria maxima*, *Juncus effusus* and *Iris germanica*) exhibit increased catalase activities when returned to air after periods of prolonged anoxia while levels of catalase remain fairly constant in anoxia-tolerant species (e.g. *Schoenoplectus lacustris*, *Acorus calamus* and

*Iris pseudacorus*) under the same conditions. Post-anoxic oxidation of anaerobically accumulated ethanol may result in a surge of acetaldehyde production, which exerts a toxic effect on the recovering tissues (Monk et al., 1987). The ability of the rhizomes of freshwater species such as *Phragmites australis*, *Schoenoplectus lacustris* and *Typha latifolia* to survive prolonged periods of anoxia allows these species to survive in habitats where anoxia-intolerant species cannot (Crawford et al., 1989). Some of the characteristics observed in freshwater wetland plants in response to anoxia may be true for seagrasses as well.

Two factors which affect photosynthesis in all plants, including seagrasses, are temperature and light. *Zostera marina* can survive a temperature range of -1.5<sup>o</sup> to 30<sup>o</sup>C (Lüning and Freshwater, 1988) and optimum gross photosynthesis occurs between 22<sup>o</sup> and 30<sup>o</sup>C while light-saturated net photosynthesis decreases at 35<sup>o</sup>C (Biebl and McRoy, 1971; Penhale, 1977; Bulthuis, 1987). Temperature may affect photosynthesis by influencing metabolism and growth (Sutcliffe, 1977). It may also act as a physiological check on enzyme controlled processes such as carbon fixation and respiration (Den Hartog, 1970). When modelled by computer, small changes in light and temperature (or their combined interaction) result in the decreased productivity of *Z. marina* and eventual loss of the community (Wetzel and Neckles, 1986). High summer temperatures can act synergistically with light reduction to: increase respiration, adversely affect enzymes involved in general metabolism, and weaken eelgrass plants for invasion by disease (Burkholder et al., 1992).

Because low oxygen and temperature may influence the production and/or activity of enzymes, the potential exists for chlorophyll to be affected by hypoxia or high temperatures which may, in turn, affect a plant's ability to photosynthesize. Vascular plants contain both chlorophylls *a* and *b*. Chlorophyll *b* serves to broaden

the spectrum of light absorption for photosynthesis and usually makes up about one-fourth of the total chlorophyll content (Weier et al., 1974; Raven et al., 1981).

*Zostera marina* rooted under macroalgal mats in shallow areas in the summer may be subjected to both high water temperatures and dark, hypoxic conditions. This study examines whether exposure of intertidal *Z. marina* leaves to dark environmental normoxia or hypoxia affects apparent gross photosynthesis and the concentration of chlorophylls in leaf tissue and whether these effects are influenced by length of exposure. If photosynthetic rates in *Z. marina* leaves are negatively affected by dark environmental hypoxia (such as might occur under macroalgal mats or at night) then changes in the chlorophyll content may reflect possible damage to the photosynthetic apparatus. If photosynthesis in *Z. marina* leaves is negatively affected by environmental hypoxia then, in areas where environmental hypoxia occurs regularly, a decrease in *Z. marina* productivity would be expected.

### Objectives

The objectives of this study were:

- 1) to determine the effect of dark, environmental hypoxia or normoxia for different time periods at 10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C on photosynthetic capacity and chlorophyll content in leaves of *Z. marina*;
- 2) to identify exposure times to hypoxic water in the dark at each temperature for intertidal *Z. marina* leaves after which they cannot photosynthesize, after a period of recovery, at levels similar to leaves exposed to normoxic water and light;
- 3) to chart P-I curves at 10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C for *Z. marina* leaves exposed to light normoxic conditions to determine if photosynthetic rates at the highest

- irradiance achieved at each temperature were, in fact, light saturated; and
- 4) to compare photosynthetic rates at the highest irradiance achieved at each temperature for *Z. marina* plants exposed to light normoxic conditions and *Z. marina* leaves exposed to dark, environmental normoxia or hypoxia at the same temperatures.

## **METHODS AND MATERIALS**

### **Photosynthetic rates and chlorophyll concentrations**

#### ***Zostera marina* collection**

Intertidal *Zostera marina* plants were collected on September 6, 12, and 18, 1993, from a *Z. marina* meadow that was accessible at low tide by walking or at high tide by snorkeling or SCUBA. The site was located northwest of the Bayview State Park beach parking area on the eastern shore of Padilla Bay (Fig. 1). A buoy was randomly placed in a relatively homogeneous *Z. marina* site and random compass points and distances from the buoy were chosen for quadrat locations before going into the field. All plants within a 0.0625 m<sup>2</sup> quadrat were gathered by hand (including a 2.5 - 5 cm portion of rhizome) until at least twice the number needed was collected for each experiment. Plants were held outdoors in an ice chest and kept cool with ice packs under natural daylight until processed for the experiment (Table 1) at Shannon Point Marine Center in Anacortes, Washington.

#### **Experimental design**

A completely randomized 2 x 3 factorial experimental design was used for this study. The two main factors were oxygen level and time held at those oxygen levels. The factor oxygen contained two levels (hypoxic, > 0 mg·L<sup>-1</sup> dissolved oxygen but ≤ 2 mg·L<sup>-1</sup> and normoxic, > 2 mg·L<sup>-1</sup> dissolved oxygen) and the factor time contained three levels which varied with experimental temperature. The lengths of time held in treatment and recovery times were based on Hammer (1972) and preliminary data I generated. The times varied between temperatures as the preliminary data indicated that more time was needed to see a decrease in photosynthetic rate at the

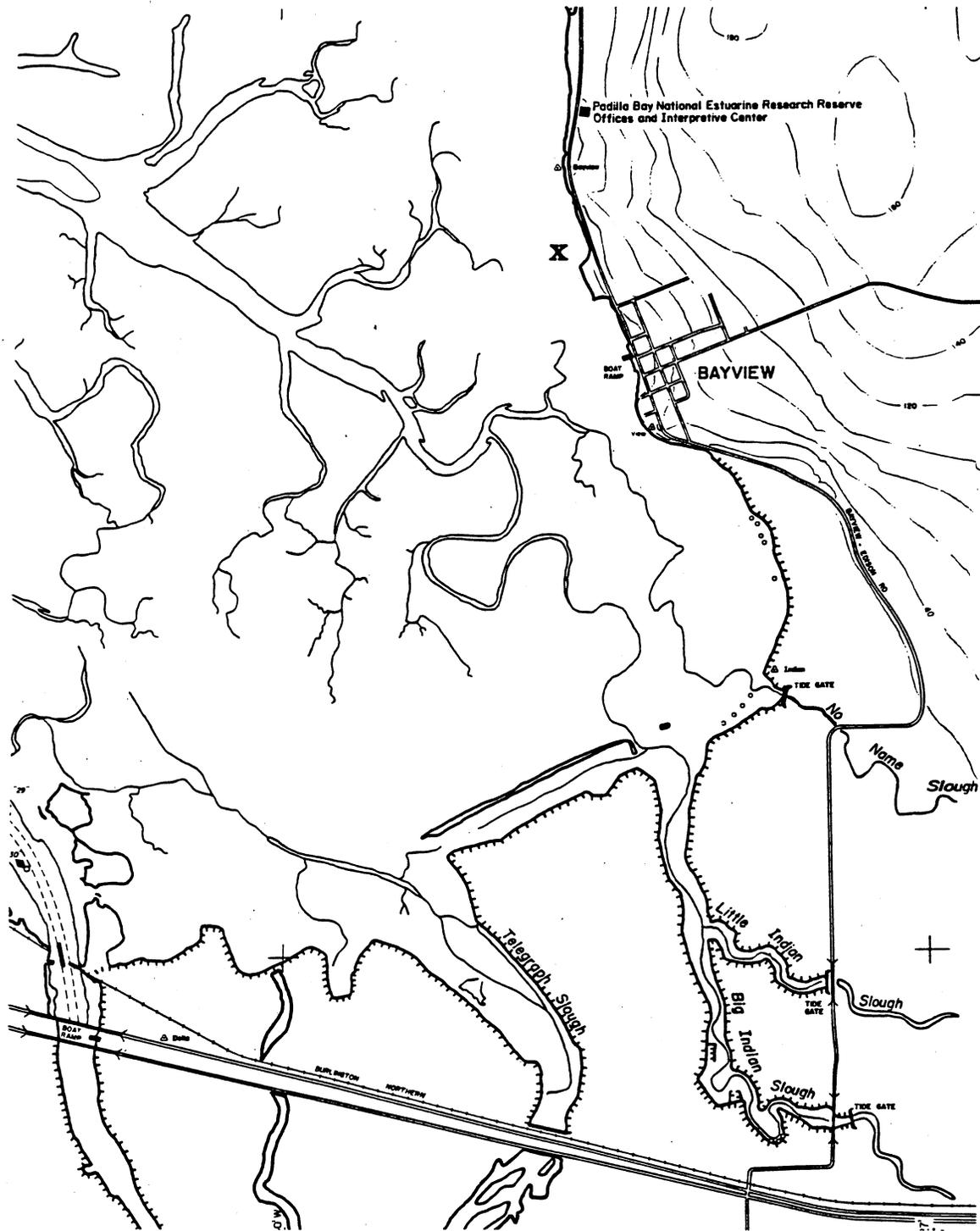


Figure 1. Location of collection site (X) for intertidal *Zostera marina* plants in Padilla Bay, Washington.

Table 1. Collection dates and times for intertidal *Zostera marina* plants collected from Padilla Bay, Washington. The last column indicates the plants were collected for exposure to normoxic and hypoxic water in the dark for the designated length of exposure (hours) at the designated temperature (°C). P-I = plants collected for P-I curve determinations.

<u>Collection</u>		<u>Plants Placed in</u>		<u>Hours Plants</u> <u>Held Prior to</u> <u>Treatment</u>	<u>Experiment</u> <u>Plants were</u> <u>Collected for:</u>
<u>Date</u>	<u>Time</u>	<u>Date</u>	<u>Time</u>		
9/6/93	1130	9/6	1800	6.5	36 h at 20°C
		9/7	1000	22.5	48 h at 20°C
		9/7	1130	24.0	24 h at 20°C
9/8/93	1630	9/9	0730	15.0	P-I curve at 20°C
9/12/93	1630	9/13	0700	14.5	48 h at 10°C
		9/13	0900	16.5	71.5 h at 10°C
		9/13	1057	18.5	97 h at 10°C
		9/14	0700	38.5	P-I curve at 10°C
9/18/93	1330	9/19	1800	28.5	12 h at 30°C
		9/20	1600	50.5	P-I curve at 30°C
		9/20	2135	56.0	8 h at 30°C
		9/21	1045	69.0	3.5 h at 30°C

lower temperatures. Two of the temperatures chosen for these experiments (10<sup>o</sup> and 20<sup>o</sup>C) were based on actual water column measurements for Padilla Bay in spring and summer (Cassidy and McKeen, 1986). Although the highest actual water temperature recorded to date for a pond in Padilla Bay during a summertime low tide is 28<sup>o</sup>C (Dr. Douglas Bulthuis, Padilla Bay National Estuarine Research Reserve, personal communication), the 30<sup>o</sup>C temperature was chosen because *Z. marina* is known to survive and photosynthesize at this temperature (Marsh et al., 1986). The dependent variables measured in *Z. marina* leaves after exposure to normoxia or hypoxia over time were photosynthetic rates and chlorophyll concentrations.

The three experiments were designed as follows:

10<sup>o</sup>C: *Zostera marina* leaves were held in the dark in normoxic water or in hypoxic water for 48, 71.5, and 97 hours with a 90-minute recovery period under light (~150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

20<sup>o</sup>C: *Zostera marina* leaves were held in the dark in normoxic water or in hypoxic water for 24, 36, and 48 hours with a 90-minute recovery period under light (~150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

30<sup>o</sup>C: *Zostera marina* leaves were held in the dark in normoxic water or hypoxic water for 3.5, 8, and 12 hours with a 150-minute recovery period under light (~150  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ).

For the photosynthesis experiments, n = 8 (plus one dark bottle to correct for bacterial uptake of <sup>14</sup>C) and for chlorophyll analysis, n = 9.

### **Laboratory procedures and calculations**

The laboratory procedures were conducted at Shannon Point Marine Center in Anacortes, Washington. Biological Oxygen Demand (BOD) bottles (300 ml) were

filled with filtered seawater (5  $\mu\text{m}$ , 30.5 ‰) and nitrogen gas (99.9% pure) was bubbled in each bottle for 1.5 - 2 hours or until the oxygen, measured with an oxygen meter (YSI Model 57) and BOD probe, was  $<1 \text{ mg}\cdot\text{L}^{-1}$ . Nitrogen gas was distributed to the bottles through plastic tubing and airstones. Any seawater displaced from the bottles by bubbling was replaced. Nitrogen was bubbled through 9 bottles for the hypoxic treatments. Air was bubbled through 9 bottles for the normoxic treatments.

The second youngest leaf was clipped from each *Z. marina* plant because it was fully pigmented and had little or no epiphytic cover (Bulthuis, 1983; Enriquez et al., 1992). Only healthy leaves were used. One leaf was placed in each BOD bottle.

The BOD bottles containing leaves in hypoxic water were stoppered and placed in a plastic tray, then wrapped in three black plastic bags to achieve total darkness, and sealed. The bottles were placed in an environmental chamber (Nor-Lake Scientific) at the experimental temperature (10<sup>o</sup>, 20<sup>o</sup>, or 30<sup>o</sup>C). The BOD bottles containing leaves in normoxic water were left unstoppered, placed in a plastic tray and wrapped in three black plastic bags to achieve total darkness and placed in the same environmental chamber. A wire frame held the plastic bags away from the open bottle necks so air was free to circulate. Air was pumped into the normoxic treatment bags and an outlet provided air flow through the bags.

After the designated treatment times (Table 1), the bottles were removed from the environmental chamber and 1 ml syringe samples of water were immediately taken from each treatment bottle (for the 10<sup>o</sup> and 30<sup>o</sup>C experiments) and approximately 1 ml of each sample was injected into the flow-through chamber of an oxygen electrode connected to a precision oxygen meter (Strathkelvin Instruments Oxygen Meter, Model 781) to measure oxygen concentration. A Lauda K<sup>-2</sup>/R

Circulator (Brinkman Instruments) controlled the temperature of the water jacket around the oxygen electrode.

Pre-treatment mean dissolved oxygen (DO) levels of seawater in BOD bottles prepared for hypoxic treatments were  $< 1 \text{ mg}\cdot\text{L}^{-1}$ . Post-treatment mean DO of seawater in the same bottles was  $< 2 \text{ mg}\cdot\text{L}^{-1}$  at  $10^{\circ}\text{C}$  and  $< 1 \text{ mg}\cdot\text{L}^{-1}$  at  $30^{\circ}\text{C}$  and was not measured at  $20^{\circ}\text{C}$ . Pre-treatment mean DO levels of seawater in BOD bottles for normoxic treatments ranged between  $7.24 - 7.56 \text{ mg}\cdot\text{L}^{-1}$  while post-treatment levels varied with temperature (Table 2).

After samples of water were taken for oxygen determinations, the leaf-containing BOD bottles were emptied and refilled with fresh filtered seawater and the bottles were placed in a temperature-controlled water bath under cool fluorescent light ( $120\text{-}160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) at the treatment temperature to allow the leaves to recover. Recovery times in the light were based on preliminary experiments and Hammer (1972). After allowing the plants to recover for the pre-determined time, each leaf was removed from the bottle and a 1.5 cm section of leaf from the middle of the leaf (Fig. 2) was cut in thirds and placed in a 20 ml glass scintillation vial with 1 ml of filtered seawater. The leaf was cut so it would fit in the vial. The rest of the leaf was placed in the dark in a resealable plastic bag and frozen for later chlorophyll analysis.

The radioisotope procedures were conducted in the radioisotope laboratory at Shannon Point Marine Center in Anacortes, Washington. The method that was used is outlined in Strickland and Parsons (1972):

radiocarbon-measured photosynthesis ( $\text{mg C}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) =

$$\frac{(R_s - R_b) \times W \times 1.05}{R \times N}$$

Table 2. Dissolved oxygen content of seawater ( $\text{mg}\cdot\text{L}^{-1}$ ) in BOD bottles pre-treatment and post-treatment. All treatments took place in the dark. Pretreatment means,  $n=5$ ,  $n=4^*$ . Post treatment means,  $n=9$ . Normoxia ( $> 2 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen). Hypoxia ( $> 0 \text{ mg}\cdot\text{L}^{-1}$  dissolved oxygen but  $\leq 2 \text{ mg}\cdot\text{L}^{-1}$ ). ND = no data. Saturation data from Strickland and Parsons (1972),  $30.5 \text{ }^{\circ}/\text{oo}$  salinity:  $10^{\circ}\text{C}$ :  $9.28 \text{ mg}\cdot\text{L}^{-1}$ ;  $20^{\circ}\text{C}$ :  $7.64 \text{ mg}\cdot\text{L}^{-1}$ ;  $30^{\circ}\text{C}$ :  $6.35 \text{ mg}\cdot\text{L}^{-1}$ .

Treatment	Dissolved oxygen (pre) mean	range	Dissolved oxygen (post) mean	range
<b>10°C</b>				
Normoxia 48 h	7.56	7.4-7.6	4.31	1.92-6.57
Normoxia 71.5 h	7.42	7.1-7.6	4.20	1.07-6.19
Normoxia 97 h	7.40	7.1-7.5	3.05	1.23-6.42
Hypoxia 48 h	0.49	0.25-0.9	1.29	0.61-1.96
Hypoxia 71.5 h	0.57	0.4-0.8	0.97	0.63-1.92
Hypoxia 97 h	0.66	0.5-0.8	0.68	0.55-1.30
<b>20°C</b>				
Normoxia 24 h	7.36*	7.2-7.5	ND	
Normoxia 36 h	7.24	7.1-7.4	ND	
Normoxia 48 h	7.54	7.2-7.7	ND	
Hypoxia 24 h	0.48*	0.3-0.8	ND	
Hypoxia 36 h	0.74	0.5-1.0	ND	
Hypoxia 48 h	0.50	0.4-0.6	ND	
<b>30°C</b>				
Normoxia 3.5 h	7.25	7.0-7.4	6.32	5.41-6.85
Normoxia 8 h	7.25	6.9-7.6	5.25	4.22-5.85
Normoxia 12 h	7.35	7.3-7.5	3.94	2.35-5.20
Hypoxia 3.5 h	0.54	0.5-0.6	0.74	0.53-1.09
Hypoxia 8 h	0.51	0.25-0.7	0.83	0.42-1.60
Hypoxia 12 h	0.48	0.3-0.8	0.75	0.26-0.61

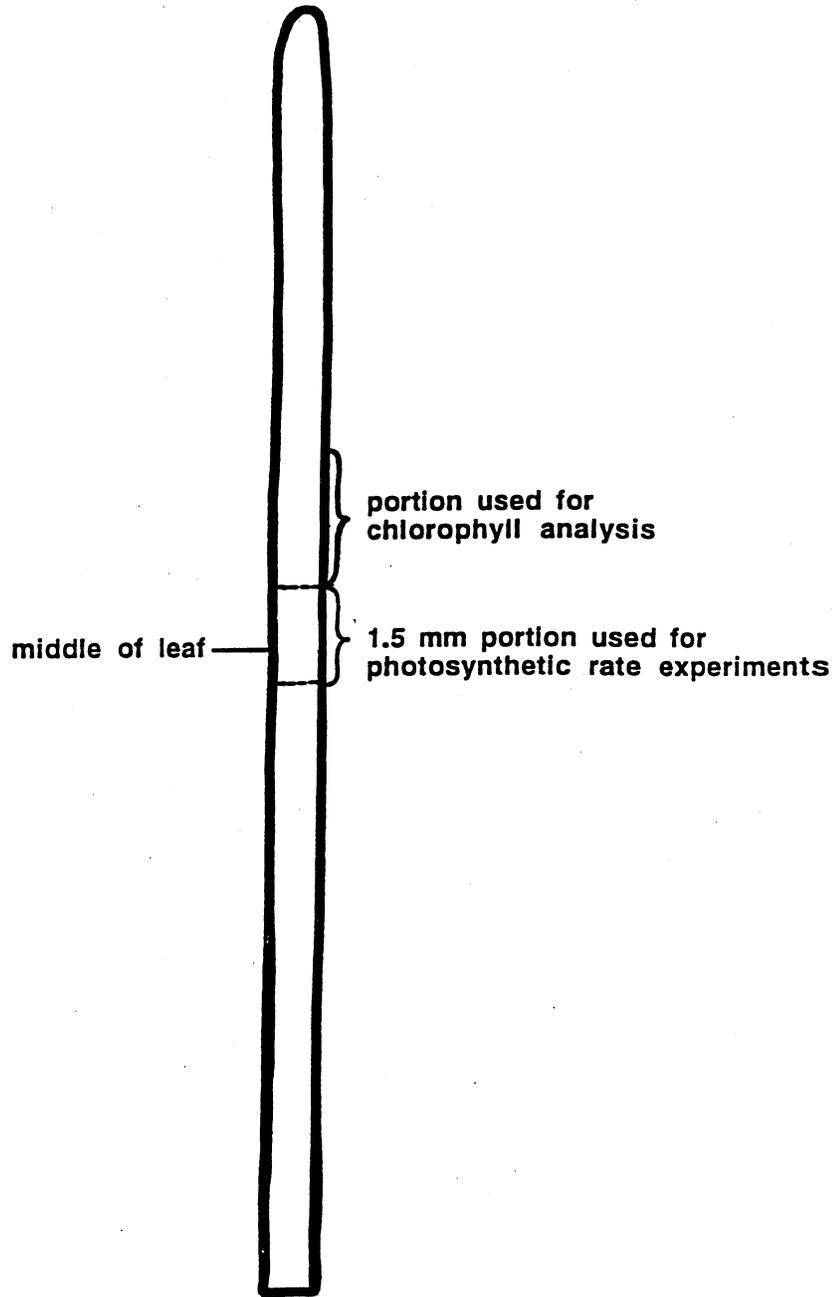


Figure 2. Portions of *Zostera marina* leaf used for photosynthetic rate experiments and for chlorophyll analysis.

where:

R = the total activity (dpm) of bicarbonate added to vials

R<sub>s</sub> = radioactivity of the sample (light bottle dpm)

R<sub>b</sub> = radioactivity of the blank (dark bottle dpm)

W = total carbonate content of sample (weight of total CO<sub>2</sub> present)

N = number of hours sample was exposed to light

1.05 = correction factor because <sup>14</sup>C is taken up more slowly than <sup>12</sup>C

Carbonate alkalinity was calculated as per Strickland and Parsons (1972) where F<sub>T</sub> is a factor for conversion of carbonate alkalinity to total carbon dioxide content (Table IX. in Strickland and Parsons, 1972):

Carbonate alkalinity = Total alkalinity - Borate alkalinity

Total CO<sub>2</sub> = Carbonate alkalinity x F<sub>T</sub> (mmoles·L<sup>-1</sup>)

mg C·L<sup>-1</sup> = (Total CO<sub>2</sub>(mmoles·L<sup>-1</sup>)) x (44 mg CO<sub>2</sub>/mmole CO<sub>2</sub>) x (12 mg C/44 mg CO<sub>2</sub>)

A sterile, aqueous radioisotope was used in this study, NaH<sup>14</sup>CO<sub>3</sub><sup>-</sup> (ICN Biomedical, Inc.). The distribution of carbon dioxide, carbonate, and bicarbonate (CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>2-</sup>) in seawater is determined by pH which, for seawater, is in the range of 7.8 to 8.2. The predominant ion in this range is HCO<sub>3</sub><sup>-</sup> (Larkum et al., 1989). Most seagrasses can use HCO<sub>3</sub><sup>-</sup> (Phillips and Menez, 1988; Larkum et al., 1989; Perez-Llorens and Niell, 1993).

One-tenth ml (0.05 microcuries) NaH<sup>14</sup>CO<sub>3</sub> in distilled water was added to each vial. Total activity was sampled by transferring 100 μl from one vial per series into small plastic scintillation vials, adding 5 ml scintillation fluid (Ecolume, ICN Biomedical, Inc.), and counting (disintegrations per minute [dpm], 10 minutes each vial) as soon as possible (< 3 minutes) in a Packard Model 1900TR Tri-Carb Liquid Scintillation Analyzer. Vials containing leaves exposed to hypoxic or normoxic water

were placed directly on the light table in the environmental chamber to achieve the highest light intensities possible. A dark bottle was run for each treatment to provide a correction for bacterial uptake of  $^{14}\text{C}$  and residual inorganic  $^{14}\text{C}$  in vials. The vials were incubated on the light table between 30-40 minutes. As time is factored into the equation for radiocarbon-measured photosynthesis (above), this small time discrepancy is not problematic nor does it affect the final results. The vials were then transferred to the radioisotope laboratory fume hood and  $^{14}\text{C}$  uptake was terminated with the addition of 0.3 ml 6 N HCl to drive off all  $\text{CO}_2$  not fixed by photosynthesis. The uncapped vials were held under a heat lamp for a short period of time to aid in degassing the samples before 0.3 ml 6 N NaOH and 0.5 ml ScintiGest tissue solubilizer (Fisher Scientific) were added. The vials were then capped and placed on a shaker table in the fume hood to speed the digestion of plant tissue. After six days, 20 ml of Ecolume scintillation fluid was added to each vial and the vials were counted (dpm) in the scintillation counter.

For chlorophyll analysis, *Zostera marina* leaves were removed from a freezer at the Shannon Point Marine Center and transported in a cooler to the laboratory at Padilla Bay N.E.R.R. for processing. A  $1\text{ cm}^2$  portion of leaf near the upper middle of the leaf (Fig. 2) was used for chlorophyll analysis. The leaves were macerated completely in 90% acetone in low light using a hand-held glass grinder placed in ice to reduce degradation of chlorophyll. When the tissue was completely macerated, the grinder was rinsed 3 times with 90% acetone and the extract was poured into a 15-ml Nalgene plastic centrifuge tube. The volume of acetone was recorded and the samples extracted overnight in a refrigerator. The samples were transported in a cooler from the laboratory at Padilla Bay N.E.R.R. to the Shannon Point Marine Center. The samples were centrifuged (Damon IEC Model HN-S) at 3/4 speed for 5 minutes. The

pellet and acetone supernatant volumes were recorded. The spectrophotometer (Hewlett Packard Model 8452A diode array spectrophotometer) was zeroed with a 90% acetone blank in a glass cuvette. The sample supernatants were then poured into quartz cuvettes and absorbances were read at wavelengths of 630, 646, 648, 664, and 750 nm with an integration time of one second. The 750 nm reading was used to correct for turbidity (i.e. that reading was subtracted from the readings at the other wavelengths). Chlorophylls *a* and *b* were determined using the following equations (Parsons et al., 1984):

$$\text{chlorophyll } a: 11.85A_{664} - 1.54A_{647} - 0.08A_{630}$$

$$\text{chlorophyll } b: 21.03A_{647} - 5.43A_{664} - 2.66A_{630}$$

### **Statistical analysis**

*Zostera marina* plants were collected randomly within a 17,662 m<sup>2</sup> circular plot and were randomly assigned to treatments. The data were analyzed using two-way ANOVA ( $\alpha = 0.05$ ) in Microsoft Excel. If an interaction was significant, then Tukey's HSD (a multiple range comparison test) was applied to test for differences between the treatment means ( $\alpha = 0.05$ ) (Zar, 1984).

One-way ANOVA ( $\alpha = 0.05$ ) was used to compare photosynthetic rate means of *Z. marina* leaves exposed to normoxia for one hour in the light (at the highest irradiance on the P-I curve) to photosynthetic rate means of leaves exposed to dark, environmental normoxia or hypoxia (at irradiances similar to the highest irradiance on P-I curve). If the analysis was significant, then Dunnett's test was used to compare the photosynthetic rate means (Zar, 1984).

### P-I curves

P-I curves have five characteristics: 1) the rate of photosynthesis at light saturation ( $P_{\max}$ ), 2) dark respiration, 3) initial slope, 4)  $I_k$  or the light intensity at photosynthetic saturation, and 5)  $I_c$  or the light compensation point. Three of these characteristics were determined for *Zostera marina* in this study: initial slope,  $P_{\max}$ , and  $I_k$  (Fig. 3). Most P-I curves that are generated for seagrasses use oxygen evolution as a measure of photosynthesis (Drew, 1979; Bulthuis, 1983; Marsh et al., 1986; Perez and Romero, 1992). With this method, plants evolve oxygen when they are exposed to light and are photosynthesizing and use oxygen in the dark when respiring. Therefore,  $P_{\text{net}}$  is measured and  $P_{\text{gross}}$  must be calculated ( $P_{\text{gross}} = P_{\text{net}} + \text{Respiration}$ ). This study used  $^{14}\text{C}$  fixed  $\cdot \text{cm}^{-2} \cdot \text{h}^{-1}$  as a measure of photosynthetic rate because  $^{14}\text{C}$  is taken up by actively photosynthesizing plants and can be measured. However, this method does not account for the respiratory loss of  $^{14}\text{C}$ -labeled photosynthate and therefore approximates gross photosynthesis, not net photosynthesis in short-term experiments (Harley and Findlay, 1994). Other researchers have used  $^{14}\text{C}$  uptake to measure photosynthesis in seagrasses such as *Thalassodendron ciliatum* (Parnik et al., 1992), *Cymodocea nodosa*, and *Posidonia oceanica* (Drew, 1978).

Crain (unpublished data, Padilla Bay N.E.R.R.) used an oxygen evolution method to measure photosynthesis in *Z. marina* from the same general location and season as was used in this study. Her P-I curves indicated  $P_{\max}$  would be achieved with photon flux densities (PFD) of  $220 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  or greater. A light table was designed and built to achieve PFD levels at least that great at all the experimental

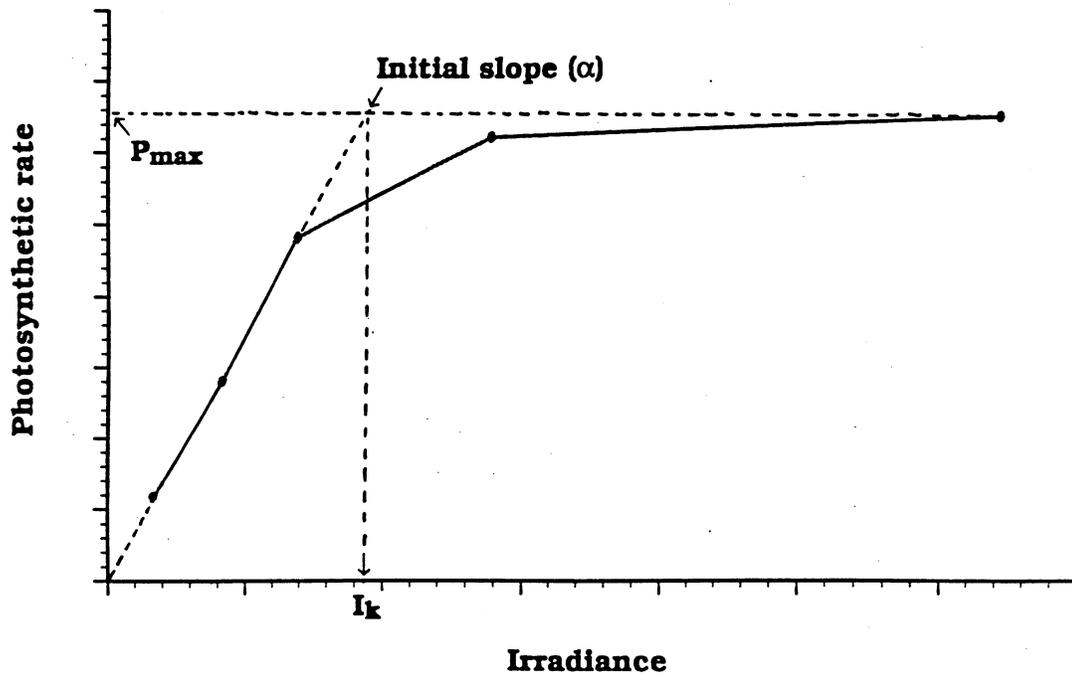


Figure 3. The P-I curve characteristics measured for this study were initial slope ( $\alpha$ ),  $P_{max}$  and  $I_k$ . The y-intercept is zero, or the light compensation point ( $I_c$ ), where respiration equals gross photosynthesis and net photosynthesis is zero.

temperatures (i.e.  $\sim 225 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$  at  $10^{\circ}\text{C}$ ,  $\sim 320 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at  $20^{\circ}\text{C}$ , and  $\sim 350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at  $30^{\circ}\text{C}$ ).

The P-I curves generated from data collected in this study were used for determining whether  $P_{\text{max}}$  was achieved at  $10^{\circ}$ ,  $20^{\circ}$ , and  $30^{\circ}\text{C}$ . The mean photosynthetic rates and chlorophyll concentrations determined for leaves exposed to normoxia or hypoxia in the dark were compared to the mean photosynthetic rates and chlorophyll concentrations achieved at the highest light intensities on the curve (hereafter referred to as "leaves exposed to normoxia for one hour in the light") and were used as a basis for comparison. This comparison assumed that leaves exposed to normoxia for one hour in the light were photosynthesizing at 100% capacity and that chlorophyll concentrations in these leaves were unaltered from natural field conditions.

#### ***Zostera marina* collection and laboratory procedures for P-I curves**

*Zostera marina* plants were collected on September 8, 12, and 18, 1993 (Table 1), in the same manner as described for "Photosynthetic rates and chlorophyll concentrations" above (p. 6). *Zostera marina* leaves were held at  $10^{\circ}$ ,  $20^{\circ}$ , or  $30^{\circ}\text{C}$  in light for one hour in the environmental chamber before processing leaves for experimental  $^{14}\text{C}$  uptake and chlorophyll analysis at each temperature. Radioisotope was added as described above in "Photosynthetic rates and chlorophyll concentrations" (p. 14). The vials containing *Z. marina* leaves were exposed to different light intensities (5 light intensities,  $n = 8$  at each light intensity) on the light table in the environmental chamber to provide data for P-I curves. Different light intensities for the P-I curves were achieved on the light table by the use of light-reduction screens around individual vials. Highest intensity light was achieved by

using no screen and subsequent light reductions by using one to four layers of screen around the scintillation vials. A dark bottle was run for each replicate curve to allow a correction for bacterial uptake of  $^{14}\text{C}$  and to zero the P-I curve at the y-intercept. Vials for P-I curves were held on the light table between 30-40 minutes. The samples were further processed as described under "Photosynthetic rates and chlorophyll concentrations, Laboratory procedures and calculations" (p. 15).

#### **Data analysis for P-I curves**

The initial slopes at  $10^{\circ}$ ,  $20^{\circ}$ , and  $30^{\circ}\text{C}$  were determined by least squares regression (using the origin as the first point and photosynthetic rates at the first two irradiances on the P-I curves as the second and third points). These slopes were compared statistically between temperatures using analysis of covariance (Zar, 1984).

$P_{\text{max}}$  is the photosynthetic rate at saturating irradiance. P-I curves provided the basis for determining whether  $P_{\text{max}}$  was attained at  $10^{\circ}$ ,  $20^{\circ}$ , and  $30^{\circ}\text{C}$  in this study. Graphically, if it appeared that  $P_{\text{max}}$  had been reached (i.e. the curve flattened, Fig. 3); then the mean photosynthetic rate value achieved at the highest light intensity was considered  $P_{\text{max}}$  for that temperature. The light intensity at photosynthetic saturation ( $I_k$ ) was calculated as per Fourqurean and Zieman (1991) using mean  $P_{\text{max}}$  and mean initial slope ( $\alpha$ ) ( $I_k = P_{\text{max}}/\alpha$ ). ANOVA was used to compare the effect of temperature on  $P_{\text{max}}$  and  $I_k$ .

## RESULTS

### 10°C experiment

#### Photosynthetic rates

Photosynthetic rates of leaves exposed to normoxic water were significantly greater than for leaves exposed to hypoxic water (Fig. 4, Table 3). The length of time leaves were held in normoxia or hypoxia (or "duration of treatment" on graphs) is hereafter referred to as "time". There were no significant differences in mean photosynthetic rates for time (Fig. 4, Table 3) and the interaction for oxygen and time was not significant.

Mean photosynthetic rates measured after leaves were exposed to normoxia or hypoxia in the dark were compared to the photosynthetic rate mean for *Z. marina* leaves exposed to normoxic water for one hour under light at 10°C (Fig. 5). One-way ANOVA of the photosynthetic rate means indicated significant treatment effects. Dunnett's test further showed mean photosynthetic rates measured after leaves were exposed to normoxic or hypoxic water in the dark at 10°C were significantly less than for *Z. marina* leaves exposed to normoxic water for one hour under light (Fig. 5).

In another comparison, photosynthetic rate means measured in leaves after they were exposed to normoxic or hypoxic conditions were expressed as a percentage of the photosynthetic rate mean for *Z. marina* leaves exposed to normoxic water for one hour under light, assuming it represented 100% photosynthetic capacity for intertidal *Z. marina* leaves at 10°C (Table 4). Percent reduction in mean photosynthetic rates increased from 27.9 to 48.3% with increased length of exposure to dark normoxia and from 56.8 to 64.7% with increased exposure to dark hypoxia.

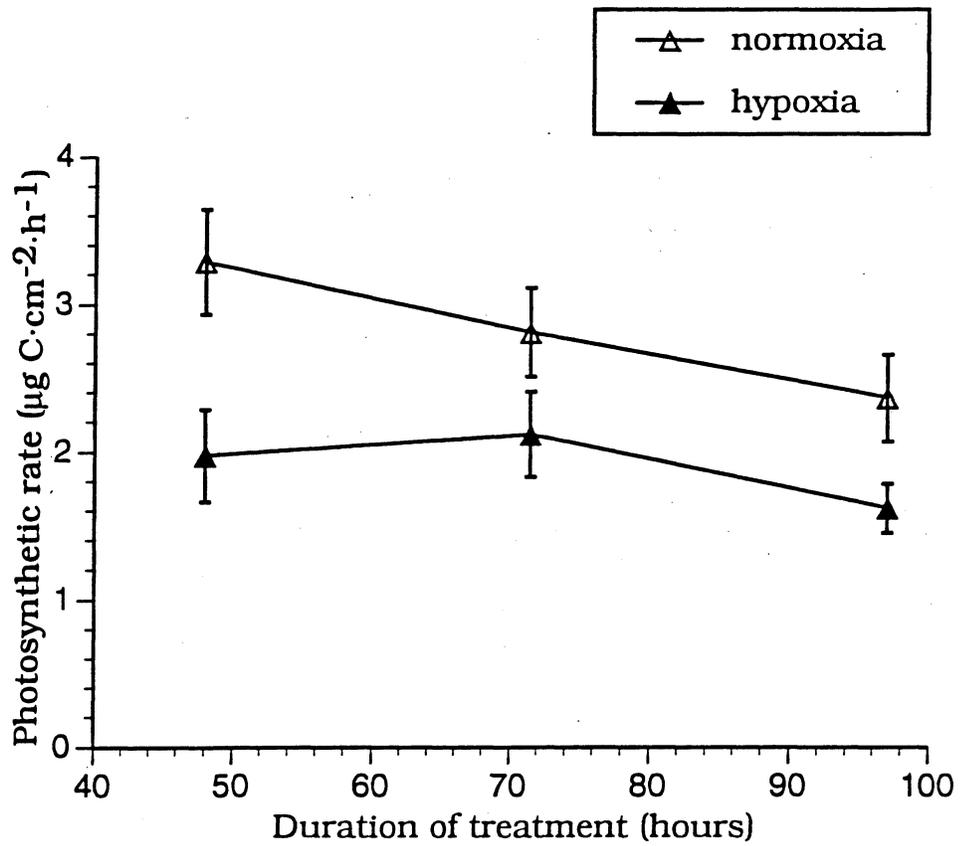


Figure 4. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 10°C on mean photosynthetic rates in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=8,  $\pm$  s.e.).

Table 3. Analysis of variance for photosynthetic rates in *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 10°C for 48, 71.5 and 97 hours ( $\alpha = 0.05$ ).

Source	df	F	p
Oxygen	1	14.73	<0.001
Time	2	2.61	0.086
Oxygen x Time	2	0.70	0.504
Error	42		
Total	47		

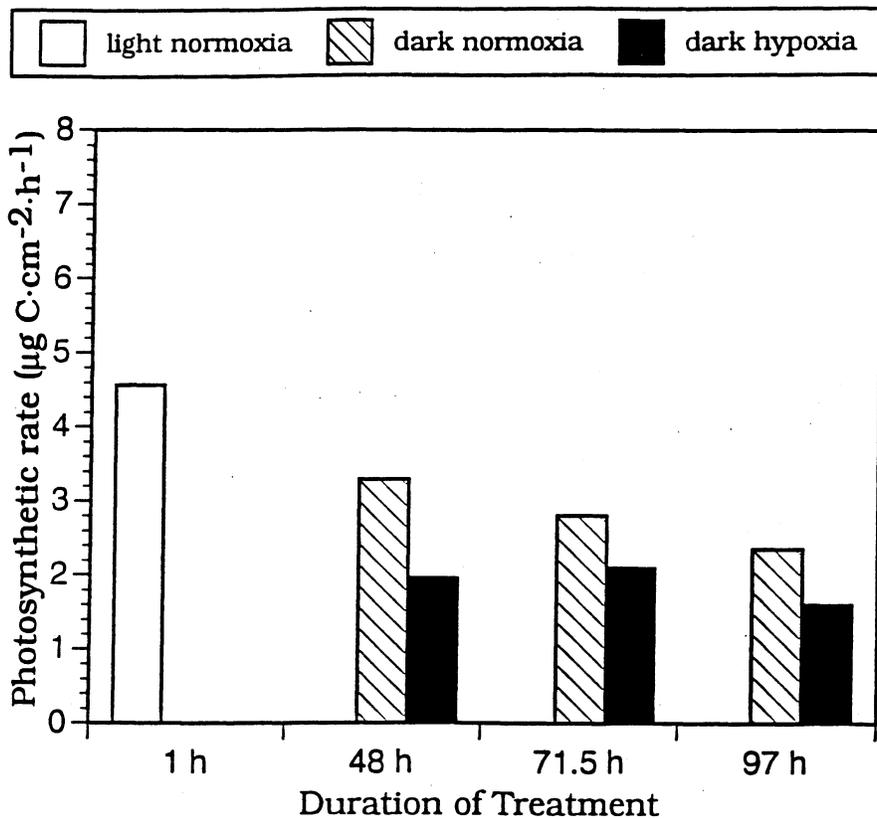


Figure 5. Comparison of photosynthetic rates for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 10°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours. Photosynthetic rates for leaves exposed to normoxic or hypoxic water in the dark were significantly less than for leaves exposed to normoxic water in the light.

Table 4. Mean photosynthetic rates of intertidal *Zostera marina* leaves at 10°C (n = 8, ± s.e.). Normoxic (> 2 mg·L<sup>-1</sup> dissolved oxygen) treatments were in the dark. Hypoxic (> 0 mg·L<sup>-1</sup> dissolved oxygen but ≤ 2 mg·L<sup>-1</sup>) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light.

Treatment	Photosynthetic Rate Means ( $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ )	Percent of Unexposed Mean	Percent Reduction of Photosynthesis
Not exposed	4.56 (± 0.74)	100.0	0
Normoxia 48 h	3.29 (± 0.36)	72.1	27.9
Normoxia 71.5 h	2.81 (± 0.30)	61.6	38.4
Normoxia 97 h	2.36 (± 0.30)	51.7	48.3
Hypoxia 48 h	1.97 (± 0.31)	43.2	56.8
Hypoxia 71.5 h	2.11 (± 0.29)	46.3	53.7
Hypoxia 97 h	1.61 (± 0.17)	35.3	64.7

### **Chlorophyll concentrations**

There were no significant differences in chlorophyll *a* or *b* concentrations in *Z. marina* leaves for the main factors oxygen level and time (Figs. 6 and 7) and no significant interaction between oxygen and time. An ANOVA comparing the chlorophyll *a* concentration in leaves exposed to normoxia in the light for one hour and in leaves exposed to normoxia and hypoxia in the dark was significant ( $p < 0.05$ ), but Dunnett's test indicated there was no significant difference between treatments. Dunnett's test was not sensitive enough to determine where the differences occurred (Fig. 8). Chlorophyll *b* concentrations were also compared using ANOVA and were significantly different ( $p < 0.05$ ). Dunnett's test showed that the chlorophyll *b* concentration in leaves exposed to normoxia for one hour in the light was significantly different from the chlorophyll *b* concentration in leaves exposed to 71.5 h of hypoxia in the dark but was not significantly different from chlorophyll *b* concentrations in all other treatments at 10°C (Fig. 9).

The chlorophyll *a:b* ratio was significantly higher in *Z. marina* leaves exposed to environmental normoxia than environmental hypoxia and differed significantly over time (Fig. 10, Table 5). A Tukey's multiple range comparison test of the factor time (which combines normoxic and hypoxic means) showed the chlorophyll *a:b* ratio was significantly greater in leaves exposed for 48 hours than for 71.5 or 97 hours (Table 6).

## **20°C experiment**

### **Photosynthetic rates**

There was a significant interaction between oxygen level and time on the photosynthetic rates at 20°C (Fig. 11, Table 7). The main effects of the factors oxygen

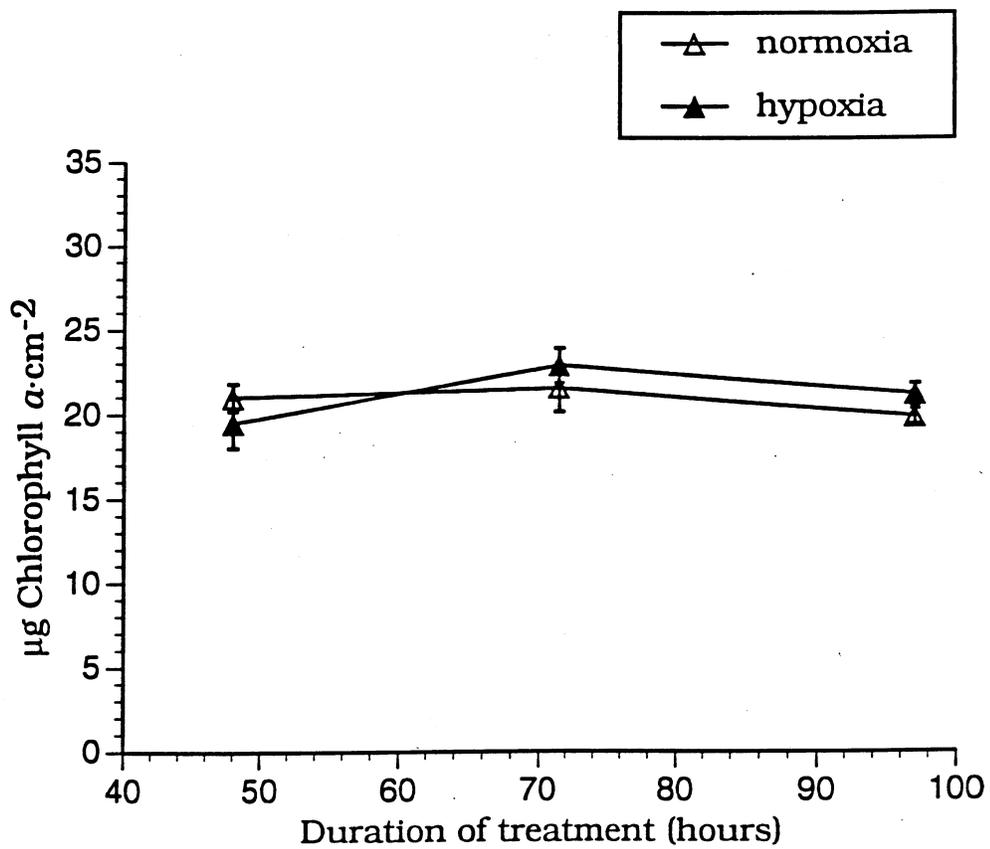


Figure 6. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 10°C on mean chlorophyll *a* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9,  $\pm$  s.e.).

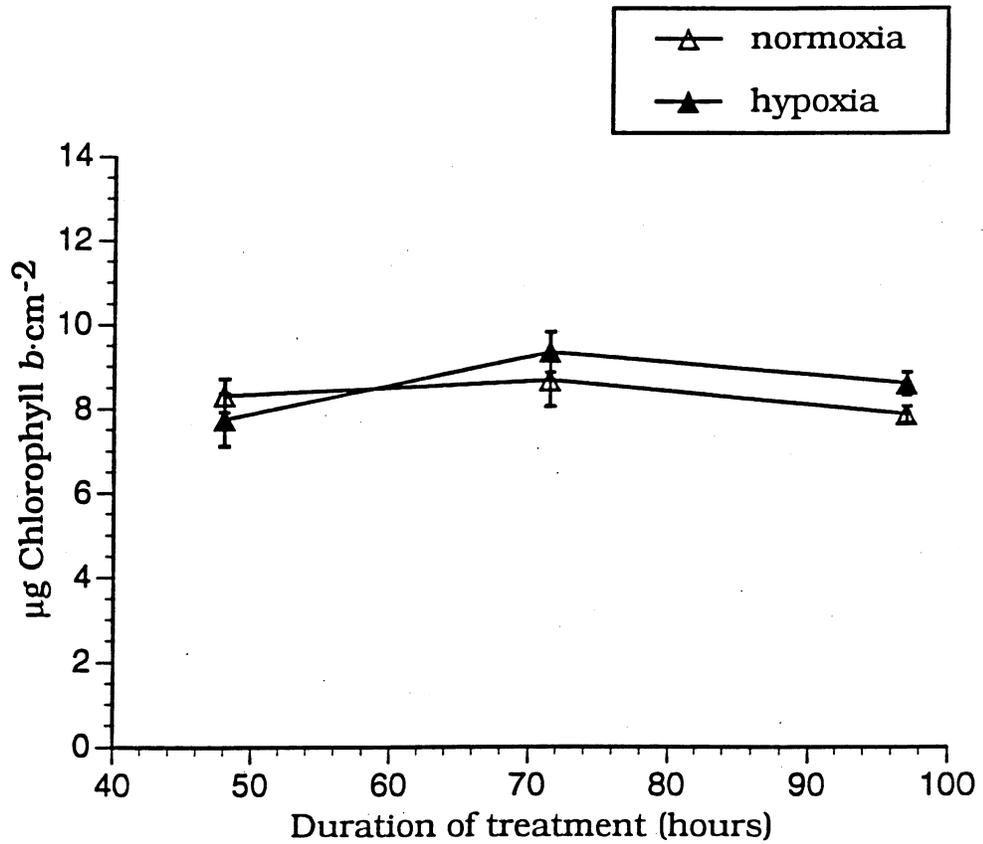


Figure 7. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 10°C on mean chlorophyll *b* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.).

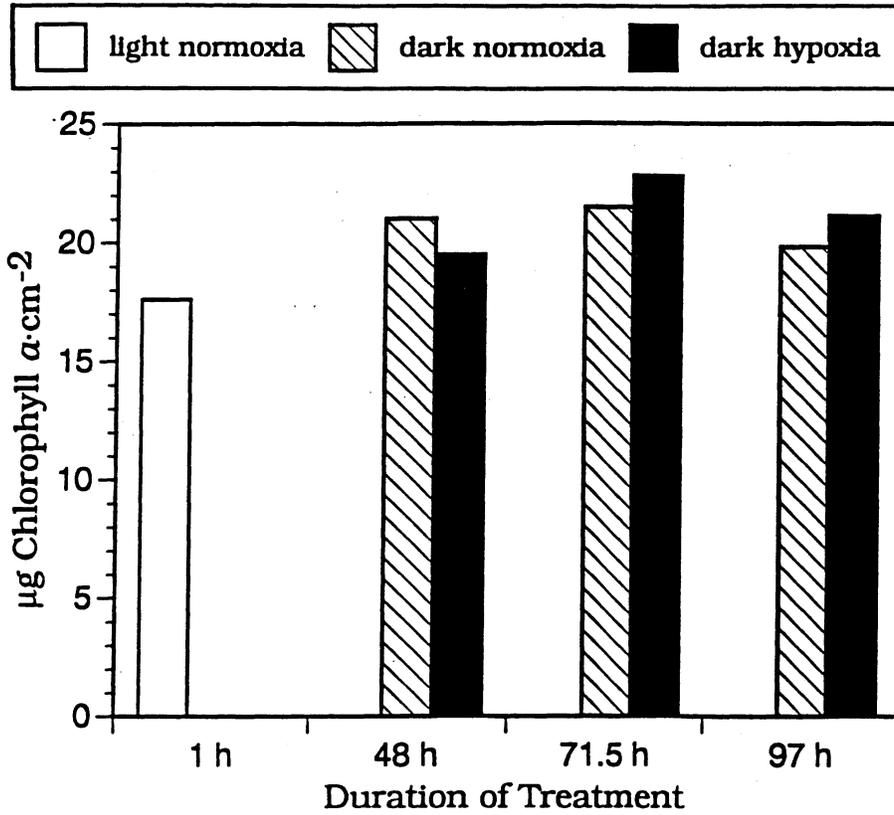


Figure 8. Comparison of chlorophyll *a* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 10°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours.

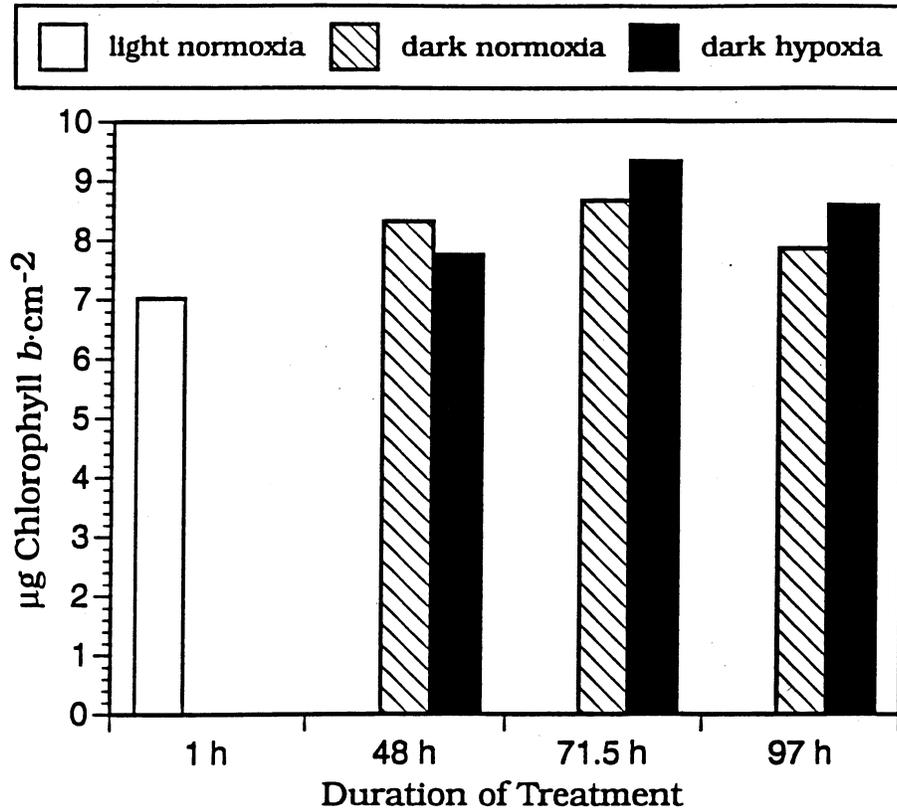


Figure 9. Comparison of chlorophyll *b* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 10°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 48, 71.5, and 97 hours. Chlorophyll *b* concentrations in leaves exposed to normoxic water in the light were significantly less than chlorophyll *b* concentrations in leaves exposed to hypoxic water for 71.5 hours but were not significantly different from all other treatments.

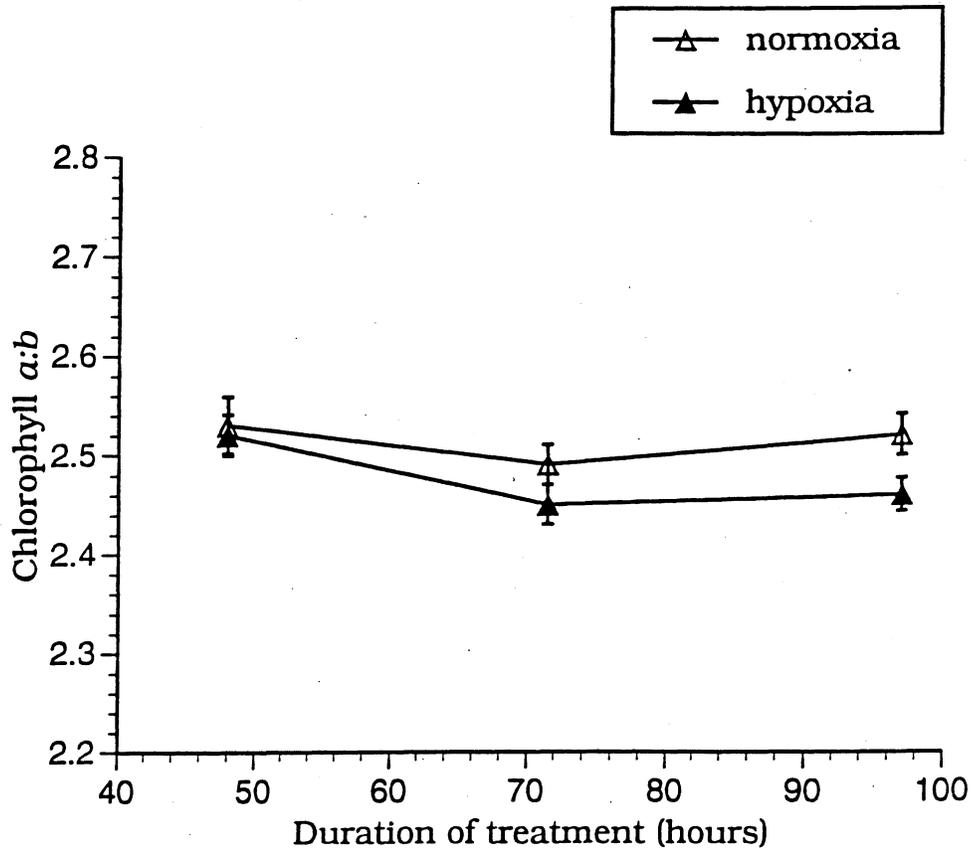


Figure 10. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 10°C on mean chlorophyll *a:b* ratios in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9,  $\pm$  s.e.).

Table 5. Analysis of variance for chlorophyll *a:b* ratios in *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 10°C for 48, 71.5 and 97 hours ( $\alpha = 0.05$ ). There were no significant differences among treatment means for chlorophylls *a* or *b*.

Source	df	F	<i>p</i>
Oxygen	1	4.45	0.035
Time	2	4.13	0.027
Oxygen x Time	2	0.69	0.554
Error	48		
Total	53		

Table 6. Results of Tukey's multiple range comparison test for chlorophyll *a:b* ratio over time at 10°C for intertidal *Z. marina* leaves collected from Padilla Bay, Washington. Treatments connected by an underline are not significantly different ( $\alpha = 0.05$ ). Treatment duration was 48, 71.5, and 97 hours.

**Means:**

**2.47**

**2.49**

**2.53**

**Treatments:**

**71.5 h**

**97 h**

**48 h**

---

---

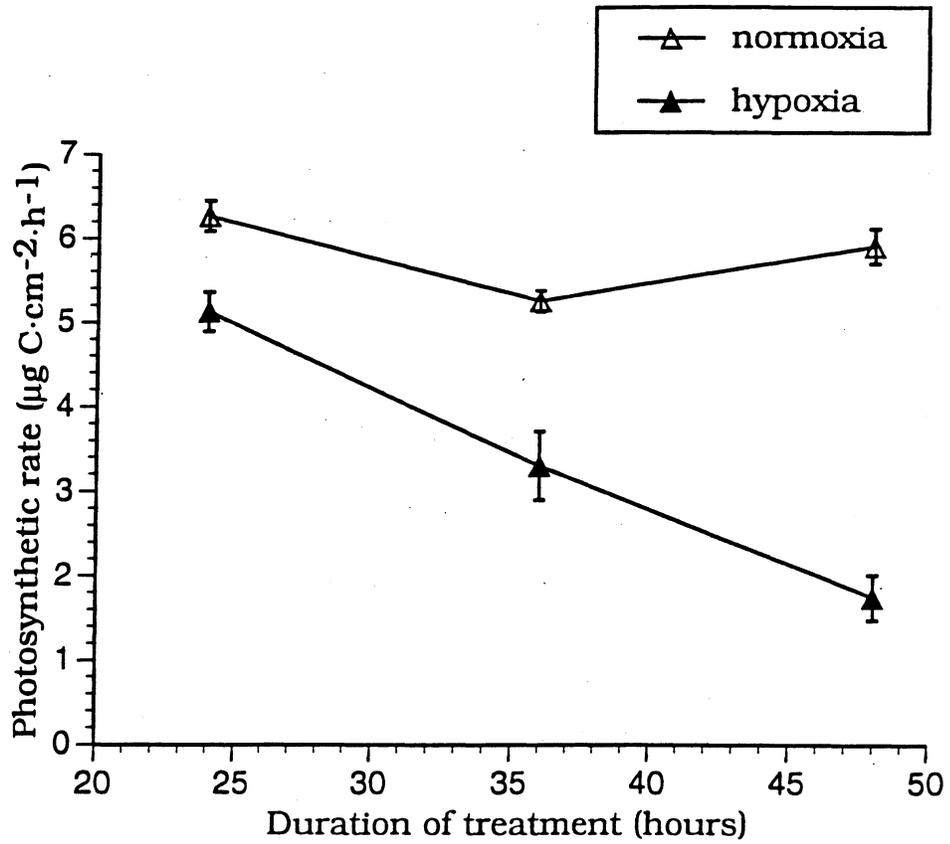


Figure 11. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 20°C on mean photosynthetic rates in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=8,  $\pm$  s.e.).

Table 7. Analysis of variance for photosynthetic rate in *Zostera marina* leaves exposed to normoxia or hypoxia at 20°C for 24, 36, and 48 hours ( $\alpha = 0.05$ ).

Source	df	F	p
Oxygen	1	136.70	<0.001
Time	2	29.66	<0.001
Oxygen x Time	2	19.06	<0.001
Error	42		
Total	47		

and time on photosynthetic rates were also significant. A Tukey's multiple range test of all the photosynthetic rate means showed time was a significant factor for hypoxic treatments but did not significantly affect normoxic treatments (Table 8). The photosynthetic rate means for leaves exposed to hypoxic conditions were significantly lower than for those exposed to normoxic conditions for each time (Table 8).

Photosynthetic rate means measured in leaves after exposure to normoxic or hypoxic water under dark conditions were compared to the photosynthetic rate mean for *Z. marina* leaves exposed to normoxic water for one hour under light (Fig. 12). One-way ANOVA of these treatment means indicated there were significant treatment effects. Dunnett's test indicated the photosynthetic rates measured for leaves after exposure to normoxia in the dark for 24 and 48 hours were not significantly different from leaves exposed to normoxia in the light (Fig. 12). However, mean photosynthetic rates for all other treatments were significantly less than means of leaves exposed to normoxic water for one hour in the light.

Photosynthetic rate means measured in leaves after they were exposed to normoxia and hypoxia in the dark were also expressed as a percentage of the photosynthetic rate mean for *Z. marina* leaves exposed to 20°C for one hour in the light, assuming it represented 100% photosynthetic capacity for intertidal *Z. marina* leaves at 20°C. Percent reduction of photosynthetic rate for *Z. marina* leaves exposed to normoxic water in the dark ranged from 3.7 - 19.1%, with the highest reduction following 36 hours of exposure. Leaves exposed to hypoxic water in the dark experienced 21.2 - 73.2% reductions in photosynthetic rates, with the greatest reduction occurring after 48 hours of exposure (Table 9).

Table 8. Results of Tukey's multiple range comparison test for photosynthetic rates ( $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) at 20°C for *Z. marina* leaves collected from Padilla Bay, Washington. Treatments connected by an underline are not significantly different ( $\alpha = 0.05$ ). Treatment duration was 24, 36, and 48 hours.

**Means:**

1.7                    3.3                    5.1                    5.3                    5.9                    6.3

**Treatments:**

Hypoxic 48	Hypoxic 36	Hypoxic 24	Normoxic 36	Normoxic 48	Normoxic 24
			<hr/>		
<hr/>	<hr/>	<hr/>			

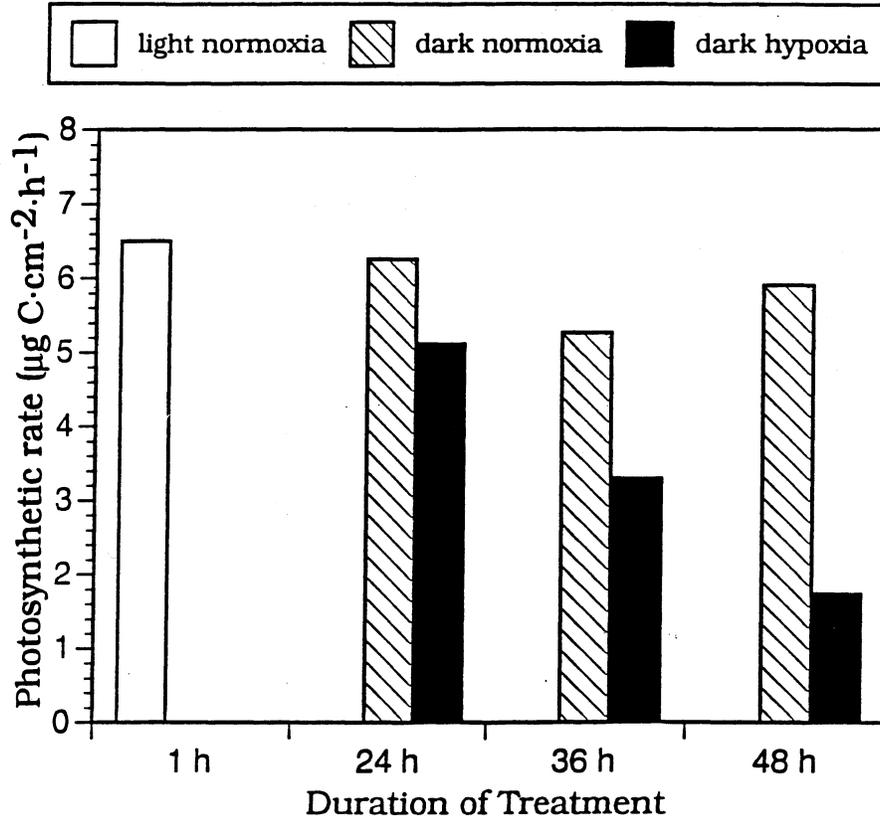


Figure 12. Comparison of photosynthetic rates for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to dark normoxic or hypoxic water for 24, 36, and 48 hours. Photosynthetic rates for leaves exposed to dark normoxic water for 24 and 48 hours were not significantly different from leaves exposed to normoxic water in the light. All other treatment means were significantly less than those of the leaves exposed to normoxia in the light.

Table 9. Mean photosynthetic rates of intertidal *Zostera marina* leaves at 20°C (n= 8, ± s.e.). Normoxic (> 2 mg·L<sup>-1</sup> dissolved oxygen) treatments were in the dark. Hypoxic (> 0 mg·L<sup>-1</sup> dissolved oxygen but ≤ 2 mg·L<sup>-1</sup>) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light.

Treatment	Photosynthetic Rate Means (μg C·cm <sup>-2</sup> ·h <sup>-1</sup> )	Percent of Unexposed Mean	Percent Reduction of Photosynthesis
Unexposed	6.50 (± 0.79)	100.0	0
Normoxia 24 h	6.26 (± 0.18)	96.3	3.7
Normoxia 36 h	5.26 (± 0.12)	80.9	19.1
Normoxia 48 h	5.90 (± 0.21)	90.8	9.2
Hypoxia 24 h	5.12 (± 0.23)	78.8	21.2
Hypoxia 36 h	3.30 (± 0.41)	50.8	49.2
Hypoxia 48 h	1.74 (± 0.27)	26.8	73.2

### **Chlorophyll concentrations**

There were no significant differences in chlorophyll *a* or *b* concentrations in leaves for the factors oxygen level or time (Figs. 13 and 14) and no significant interactions. ANOVA comparing the means of chlorophyll *a* and *b* concentrations in leaves exposed to normoxia for one hour in the light and leaves exposed to normoxia or hypoxia in the dark was not significant ( $p > 0.05$ , Figs. 15 and 16).

Chlorophyll *a:b* ratios were significantly lower for *Z. marina* leaves exposed to normoxic water vs. hypoxic water (Fig. 17, Table 10). However, chlorophyll *a:b* ratios in leaves were not significant for time or interaction.

### **30°C experiment**

#### **Photosynthetic rates**

Mean photosynthetic rates of leaves exposed to normoxic conditions were significantly greater than those of leaves exposed to hypoxic water (Fig. 18, Table 11). Time was also shown to significantly affect photosynthetic rate (Table 11). However, the interaction between oxygen and time was not significant. A Tukey's test compared mean photosynthetic rates (normoxic + hypoxic) for *Z. marina* leaves over time. The mean photosynthetic rates of leaves were not significantly different for 3.5 and 8 hours of exposure to normoxic and hypoxic water but were significantly higher than photosynthetic rates of leaves exposed for 12 hours to normoxic and hypoxic conditions (Table 12).

Mean photosynthetic rates measured after leaves were exposed to normoxic and hypoxic water in the dark were compared to the photosynthetic rate mean for *Z. marina* leaves exposed to 30°C for one hour in the light. A one-way ANOVA of these photosynthetic rate means indicated there were significant treatment effects.

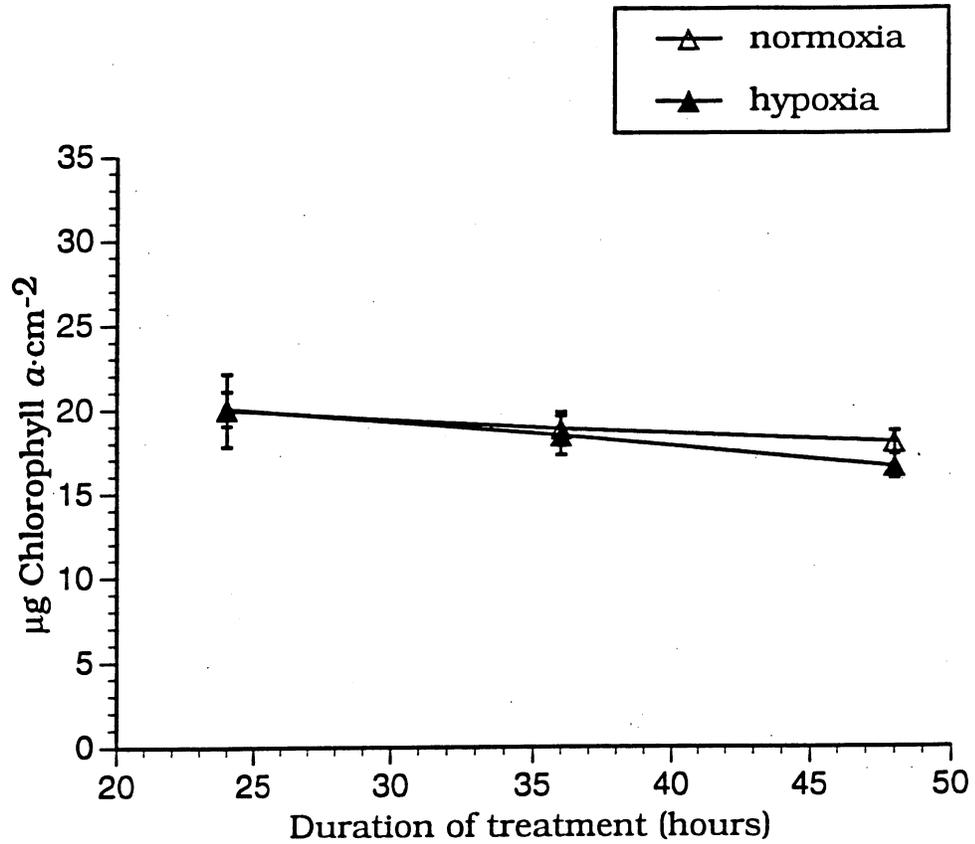


Figure 13. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 20°C on mean chlorophyll *a* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9,  $\pm$  s.e.).

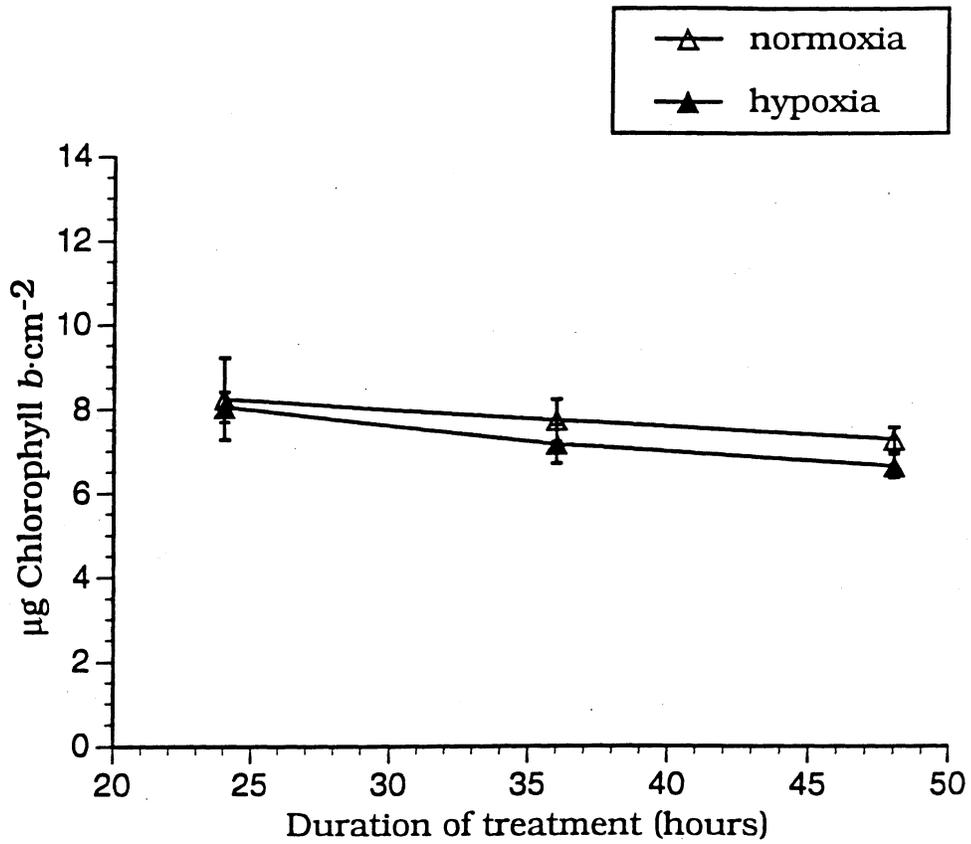


Figure 14. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 20°C on mean chlorophyll *b* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.).

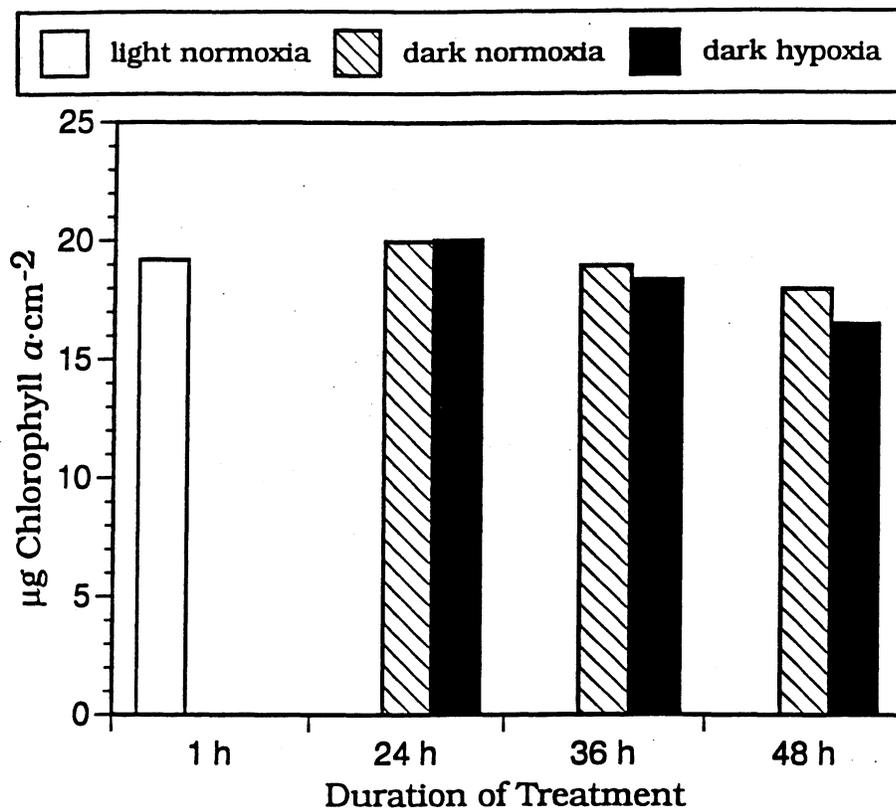


Figure 15. Comparison of chlorophyll *a* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 24, 36, and 48 hours. Mean chlorophyll *a* concentration in leaves exposed to normoxia for one hour in the light were not significantly different from all other treatments.

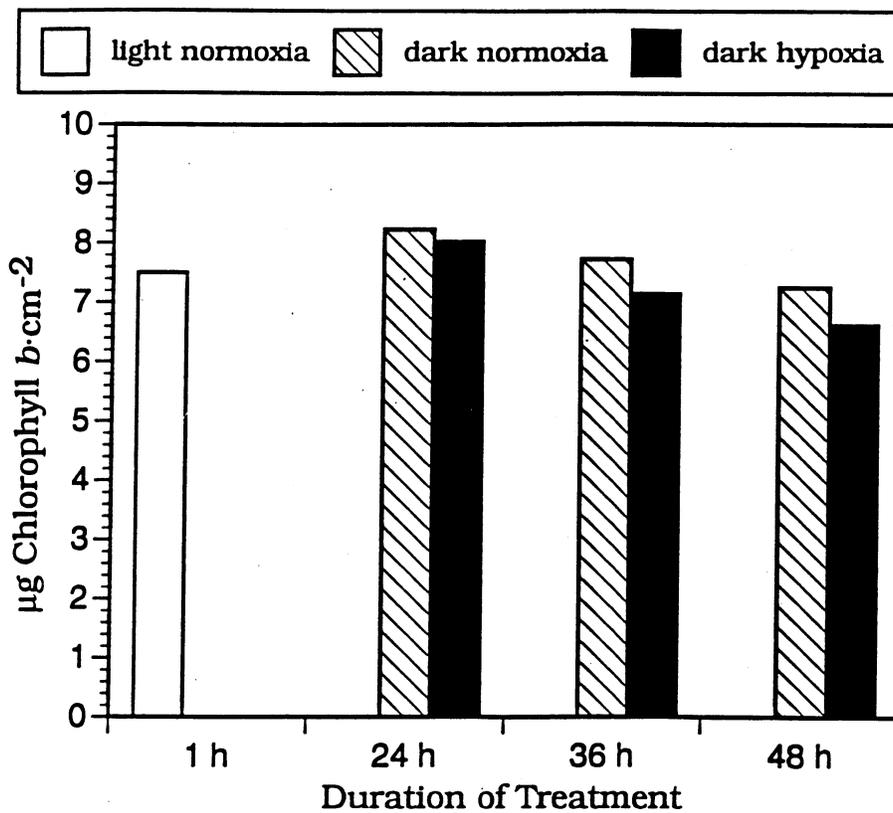


Figure 16. Comparison of chlorophyll *b* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 20°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 24, 36, and 48 hours. Chlorophyll *b* concentrations in leaves exposed to normoxic water in the light were not significantly different from all other treatment means.

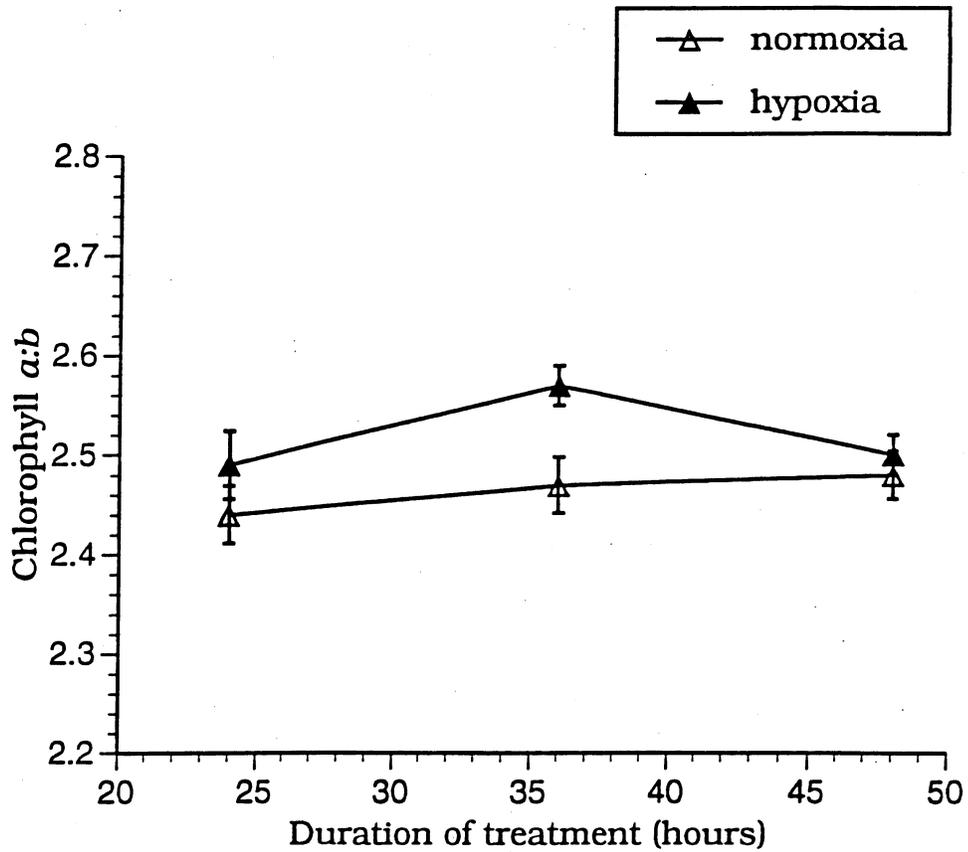


Figure 17. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 20°C on mean chlorophyll *a:b* ratios in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 90 minute recovery period in the light (n=9, ± s.e.).

Table 10. Analysis of variance for chlorophyll *a:b* ratio in *Zostera marina* leaves exposed to normoxia or hypoxia at 20°C for 24, 36, and 48 hours ( $\alpha = 0.05$ ). There were no significant differences among treatment means for chlorophylls *a* or *b*.

Source	df	F	<i>p</i>
Oxygen	1	6.80	0.012
Time	2	2.31	0.110
Oxygen x Time	2	1.55	0.222
Error	48		
Total	53		

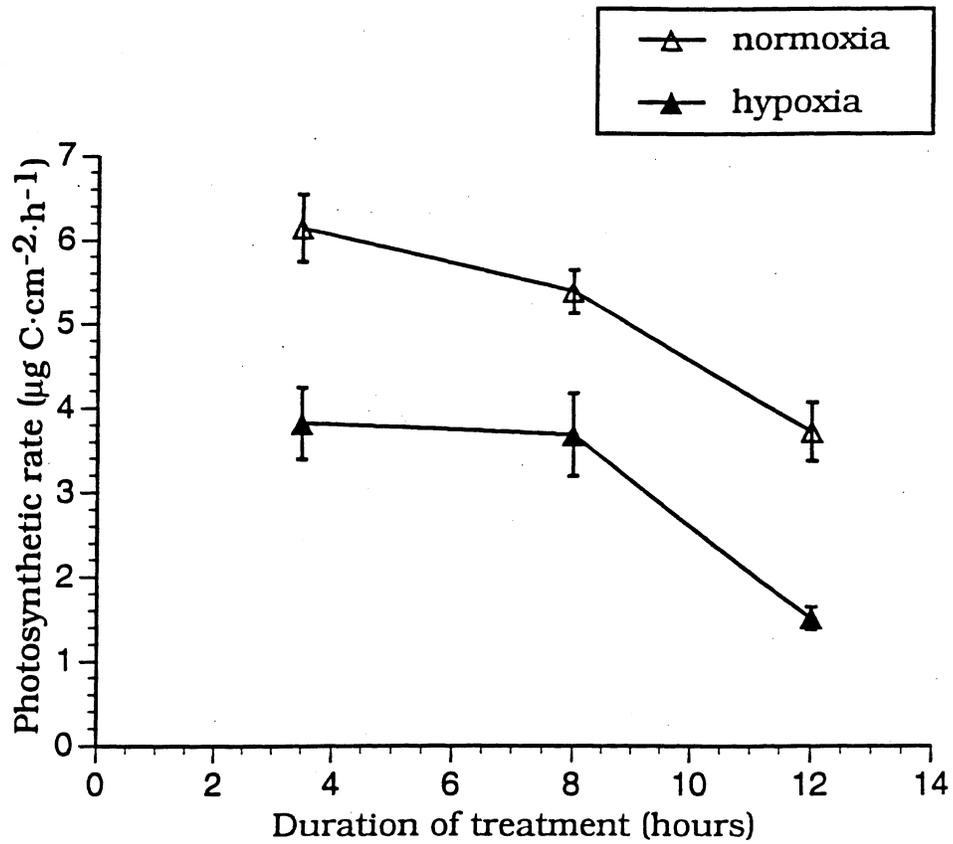


Figure 18. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 30°C on mean photosynthetic rates in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=8,  $\pm$  s.e.).

Table 11. Analysis of variance for photosynthetic rates for *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 30°C for 3.5, 8, and 12 hours ( $\alpha = 0.05$ ).

Source	df	F	p
Oxygen	1	49.78	<0.001
Time	2	24.38	<0.001
Oxygen x Time	2	0.41	0.665
Error	42		
Total	47		

Table 12. Results of Tukey's multiple range comparison test for photosynthetic rates ( $\mu\text{gC}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) over time at 30°C for intertidal *Z. marina* leaves collected from Padilla Bay, Washington. Treatments connected by an underline are not significantly different ( $\alpha = 0.05$ ). Treatment duration was 3.5, 8, and 12 hours.

**Means:**

**2.6**

**4.5**

**5.0**

**Treatments:**

**12 h**

**8 h**

**3.5 h**

---

---

Dunnett's test showed mean photosynthetic rates of leaves exposed to normoxic or hypoxic water at 30°C were significantly less than the mean for *Z. marina* leaves exposed to normoxic water for one hour under light (Fig. 19).

Mean photosynthetic rates of leaves measured after leaves were exposed to environmental normoxia or hypoxia in the dark were also expressed as a percentage of the photosynthetic rate mean for *Z. marina* leaves exposed to 30°C for one hour under light, assuming it represented 100% photosynthetic capacity for intertidal *Z. marina* leaves at 30°C (Table 13). The reduction in mean photosynthetic rates ranged from 16.8 - 49.7% for *Z. marina* leaves exposed to dark normoxic conditions as compared to 48.2 - 79.5% for leaves exposed to dark hypoxic conditions (Table 13). Leaves exposed to hypoxia in the dark for 12 hours experienced the greatest percent reduction in photosynthetic rate (79.5%).

### **Chlorophyll concentrations**

Mean chlorophyll *a* concentration was significantly higher in *Z. marina* leaves exposed to normoxic vs. hypoxic water (Fig. 20, Table 14). The main factor time was not significant for mean chlorophyll *a* concentration and there was no significant interaction. There were no significant differences in mean chlorophyll *b* concentration for oxygen level or time (Fig. 21) and there was no significant interaction. ANOVA comparing the mean chlorophyll *a* and *b* concentrations in leaves exposed to normoxia for one hour in the light and leaves exposed to normoxia or hypoxia in the dark were not significant ( $p > 0.05$ , Figs. 22 and 23). The factors oxygen and time were not significant for the chlorophyll *a*: *b* ratio at 30°C (Fig. 24), nor was the interaction.

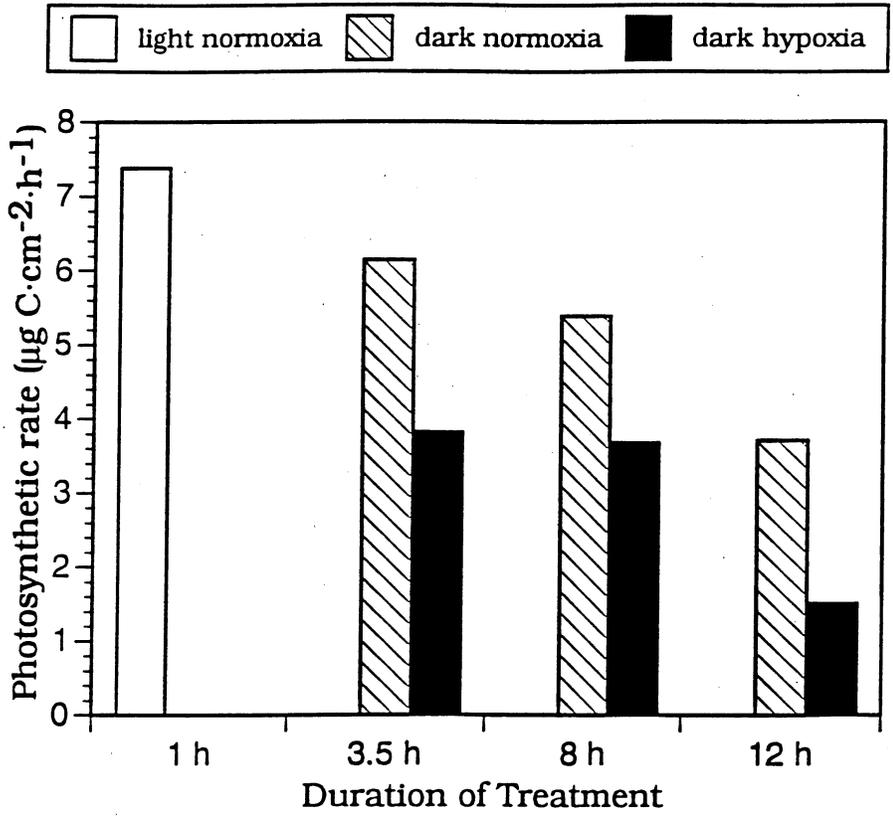


Figure 19. Comparison of photosynthetic rates for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 30°C and *Z. marina* leaves exposed to dark normoxic or hypoxic water for 3.5, 8, and 12 hours. Photosynthetic rates for leaves exposed to dark normoxic or hypoxic water were significantly less than for leaves exposed to normoxic water in the light.

Table 13. Mean photosynthetic rates of intertidal *Zostera marina* leaves at 30°C (n = 8, ± s.e.). Normoxic (> 2 mg·L<sup>-1</sup> dissolved oxygen) treatments were in the dark. Hypoxic (> 0 mg·L<sup>-1</sup> dissolved oxygen but ≤ 2 mg·L<sup>-1</sup>) treatments were in the dark. Leaves not exposed to dark normoxia or hypoxia were held for one hour at the experimental temperature under light.

Treatment	Photosynthetic Rate Means (μg C·cm <sup>-2</sup> ·h <sup>-1</sup> )	Percent of Unexposed Mean	Percent Reduction of Photosynthesis
Unexposed	7.38 (± 0.55)	100.0	0
Normoxia 3.5 h	6.14 (± 0.40)	83.2	16.8
Normoxia 8 h	5.39 (± 0.26)	73.0	27.0
Normoxia 12 h	3.71 (± 0.34)	50.3	49.7
Hypoxia 3.5 h	3.82 (± 0.42)	51.8	48.2
Hypoxia 8 h	3.68 (± 0.49)	49.9	50.1
Hypoxia 12 h	1.51 (± 0.14)	20.5	79.5

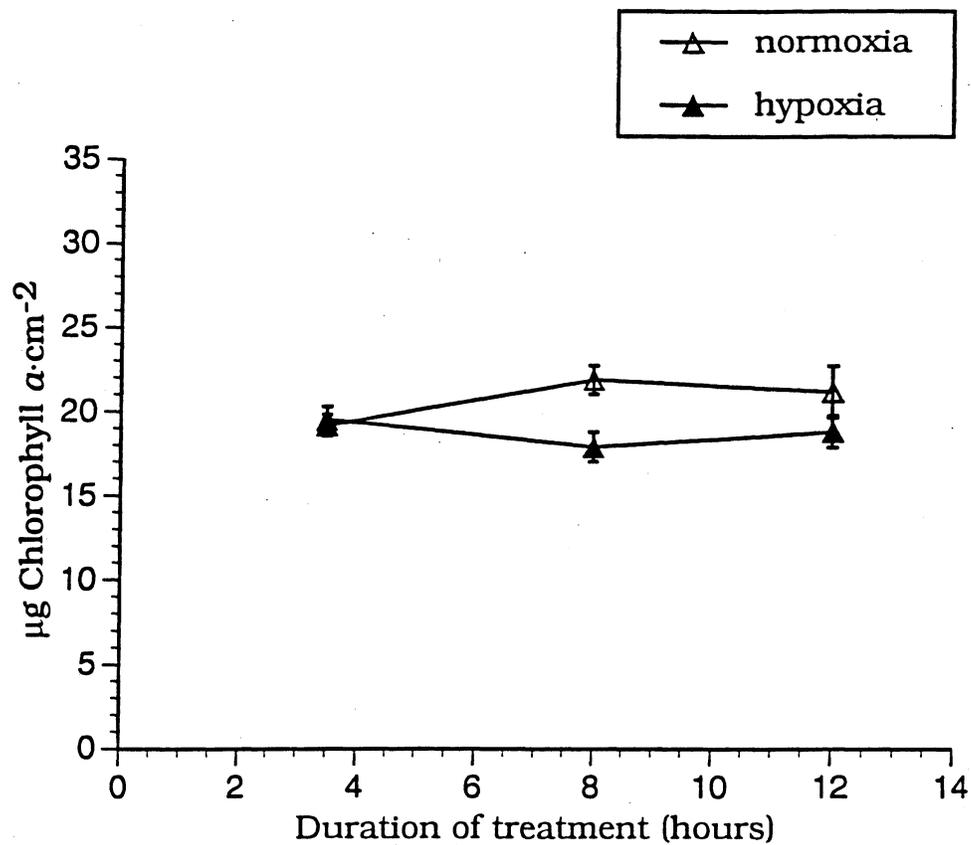


Figure 20. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 30°C on mean chlorophyll *a* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9, ± s.e.).

Table 14. Analysis of variance for chlorophyll *a* for *Zostera marina* leaves exposed to normoxia or hypoxia in the dark at 30°C for 3.5, 8, and 12 hours ( $\alpha = 0.05$ ). There were no significant differences among treatment means for chlorophyll *b* or chlorophyll *a:b* ratios.

Source	df	F	<i>p</i>
Oxygen	1	5.34	0.015
Time	2	0.37	0.786
Oxygen x Time	2	2.91	0.095
Error	48		
Total	53		

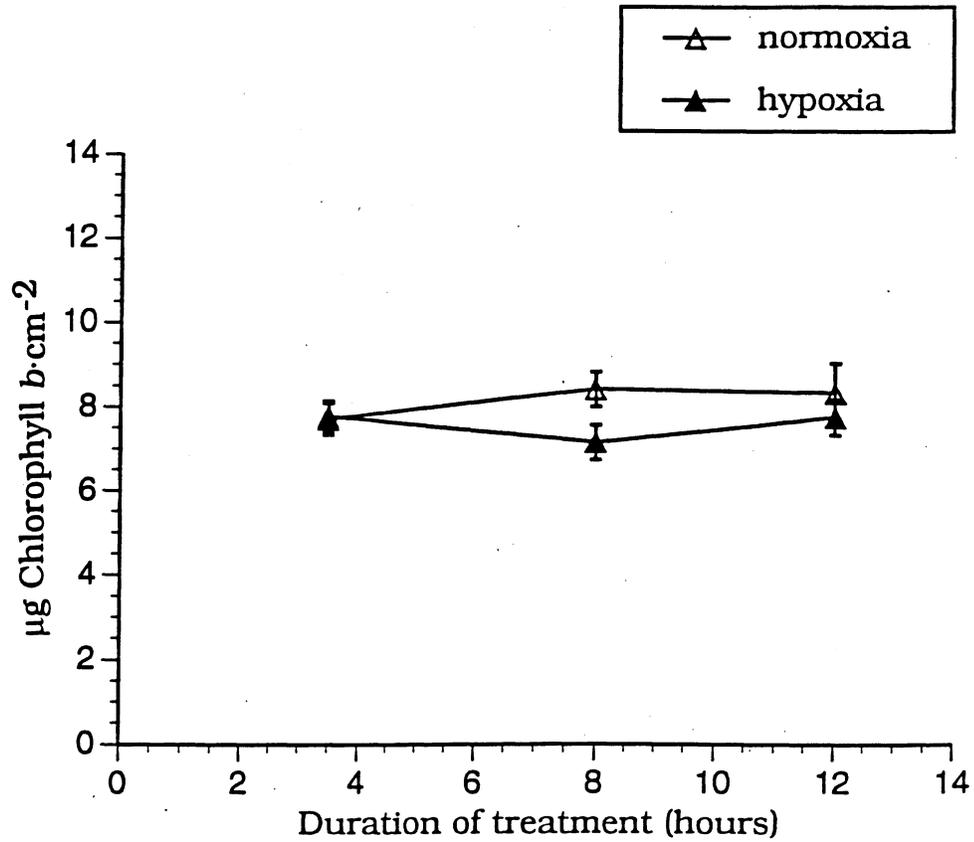


Figure 21. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 30°C on mean chlorophyll *b* concentrations in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9, ± s.e.).

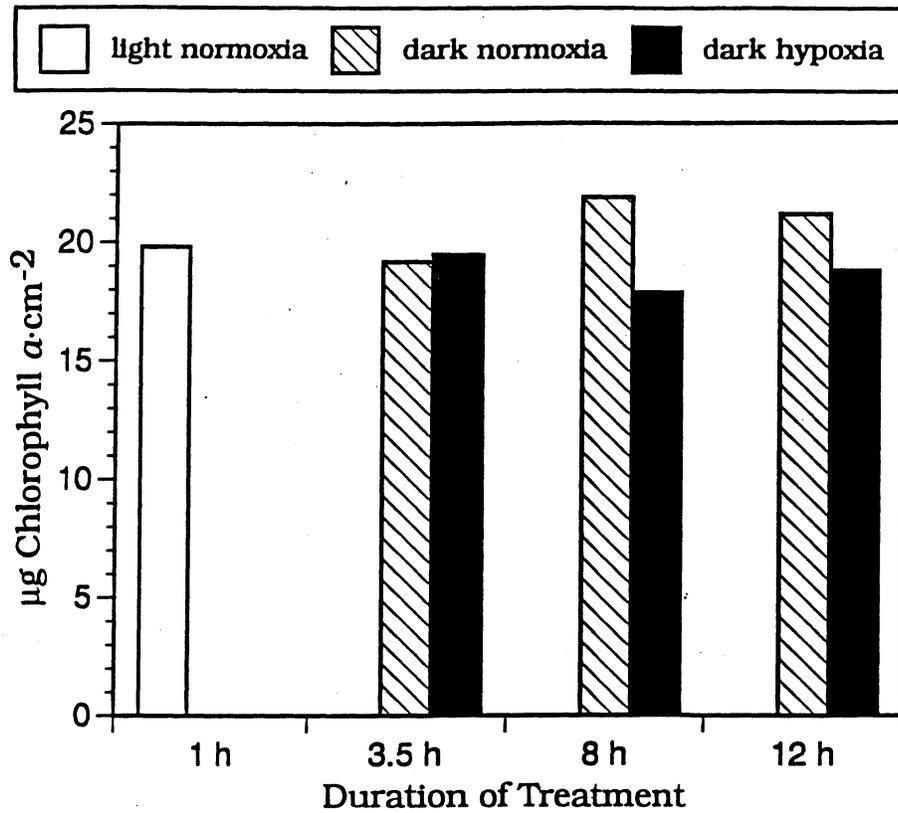


Figure 22. Comparison of chlorophyll *a* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 30°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 3.5, 8, and 12 hours. Mean chlorophyll *a* concentration in leaves exposed to normoxia for one hour in the light were not significantly different from all other treatments.

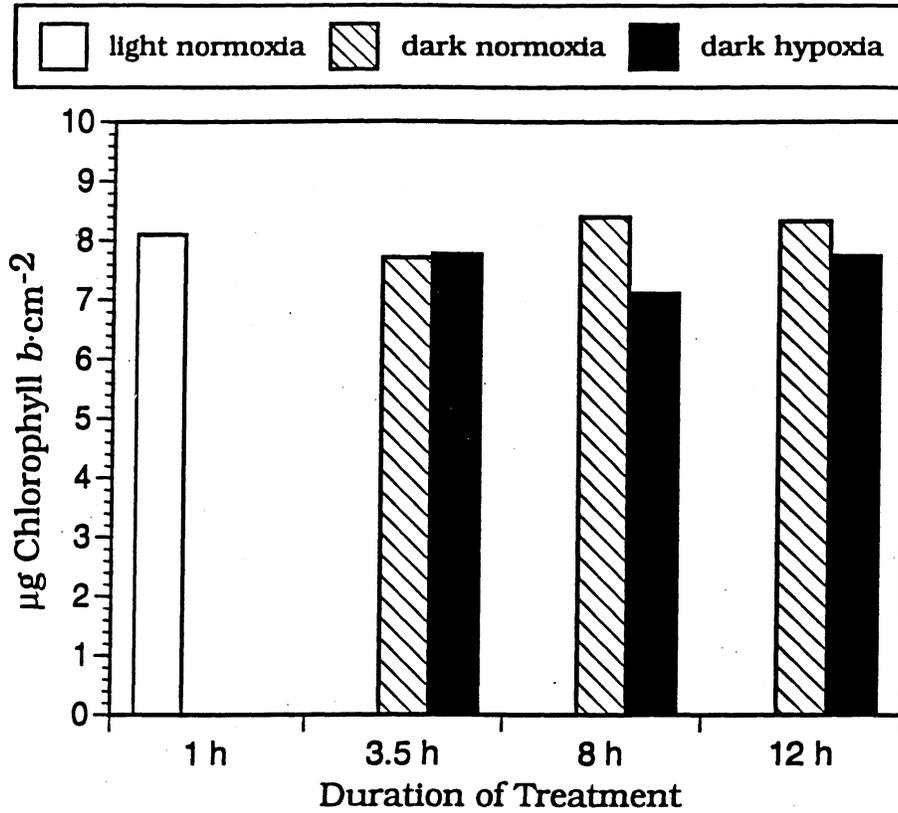


Figure 23. Comparison of chlorophyll *b* concentrations for intertidal *Zostera marina* leaves exposed to normoxic water in the light for one hour at 30°C and *Z. marina* leaves exposed to normoxic or hypoxic water in the dark for 3.5, 8, and 12 hours. Chlorophyll *b* concentrations in leaves exposed to normoxic water in the light were not significantly different from all other treatment means.

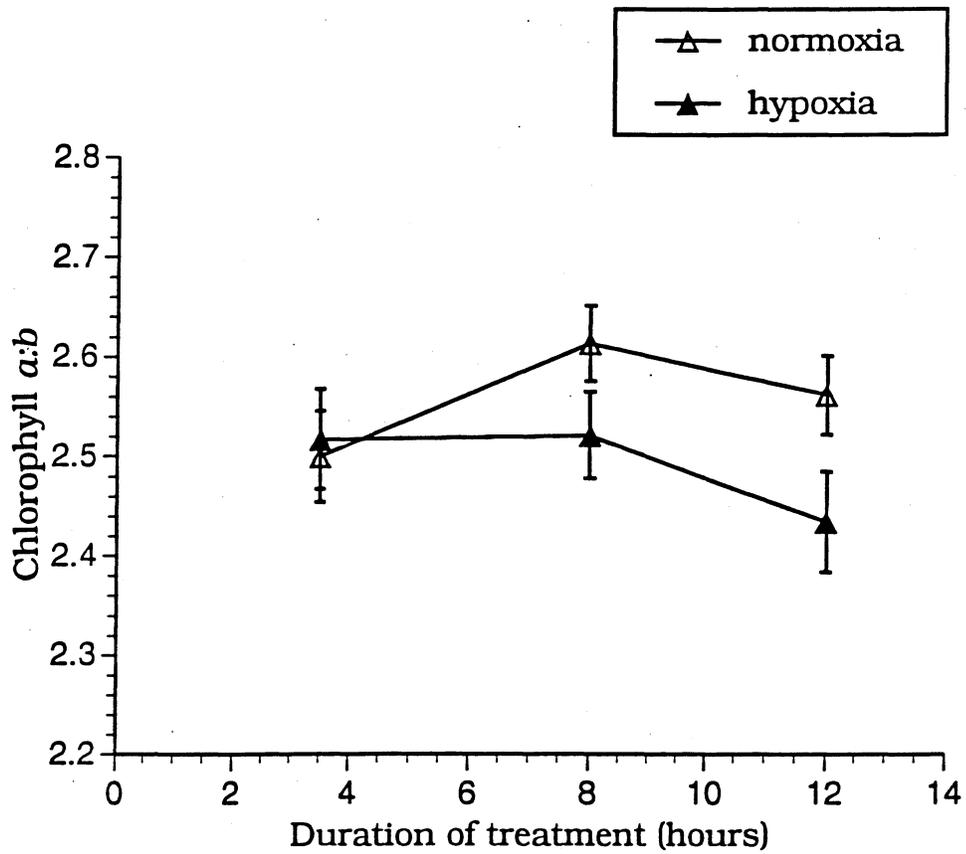


Figure 24. Profile of the main effects of normoxic and hypoxic treatments over time in the dark at 30°C on mean chlorophyll *a:b* ratios in intertidal *Zostera marina* leaves from Padilla Bay, Washington, following a 150 minute recovery period in the light (n=9,  $\pm$  s.e.).

### **Photosynthesis vs. irradiance curves**

Photosynthesis vs. irradiance (P-I) curves were plotted for *Z. marina* leaves exposed to normoxic water for one hour under light at each treatment temperature (Figs. 25, 26, and 27). Mean initial slopes, mean  $P_{\max}$ , and  $I_k$  values are reported in Table 15. An ANOVA showed no significant differences in initial slopes between temperatures.  $P_{\max}$  and  $I_k$  did not differ significantly between 10° and 20°C.  $P_{\max}$  and  $I_k$  could not be tested statistically between 10°C and 30°C or between 20°C and 30°C because light-saturated photosynthesis was not reached at 30°C at the irradiances (photon flux densities) used in this study (Fig. 27).

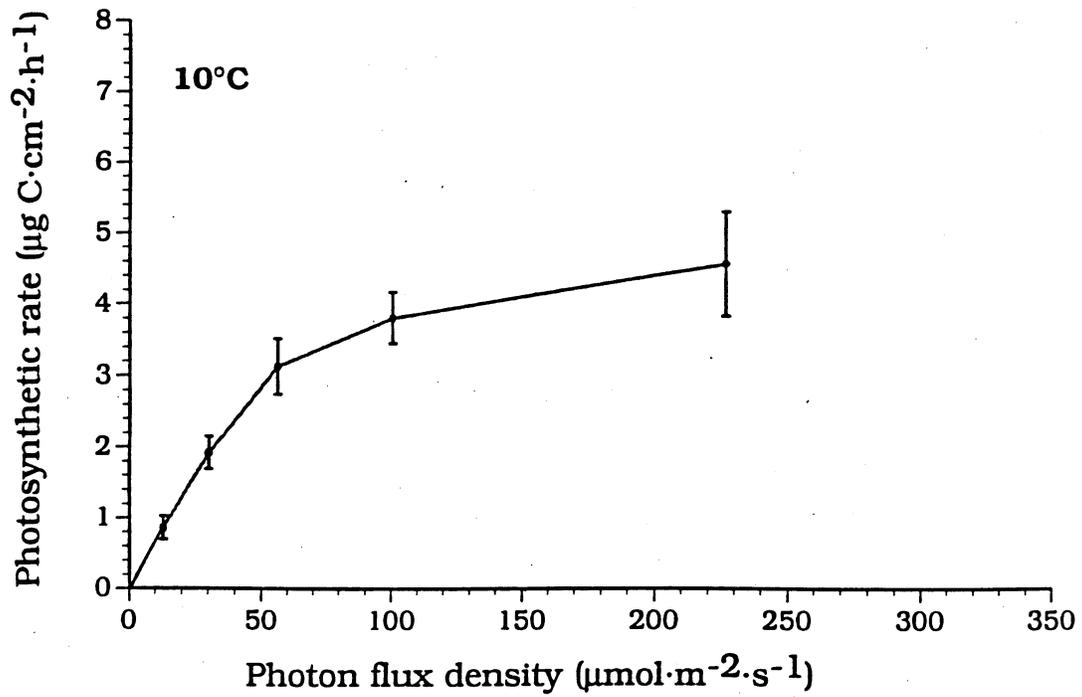


Figure 25. P-I curve for intertidal *Zostera marina* leaves exposed to normoxia in the light for one hour at 10°C. Each point represents mean photosynthetic rate ( $n=8, \pm$  s.e.).

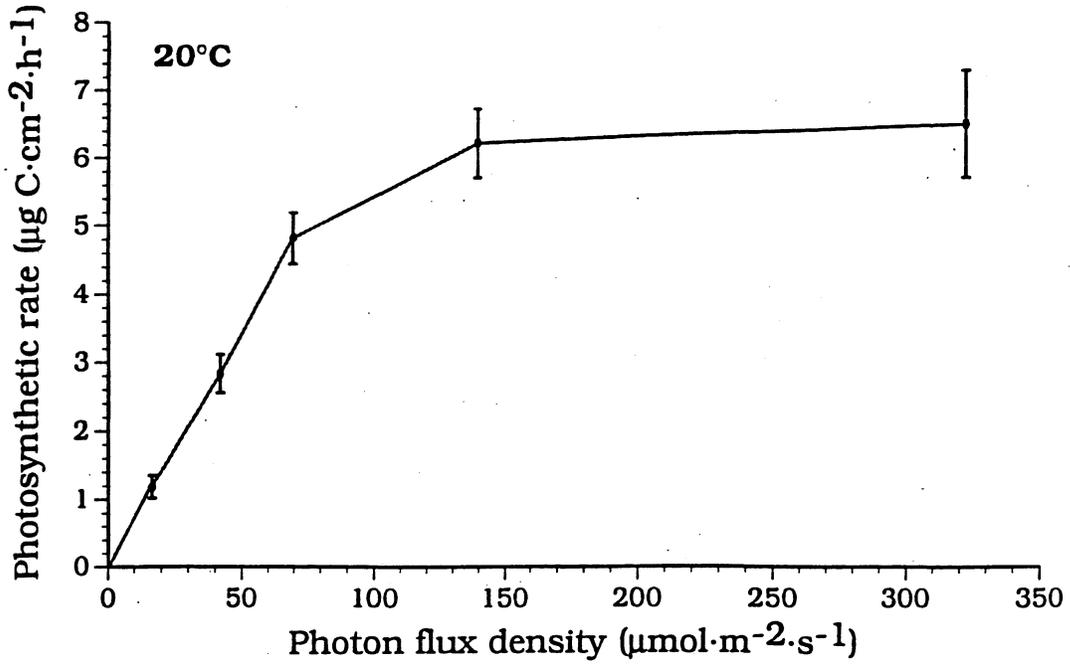


Figure 26. P-I curve for intertidal *Zostera marina* leaves exposed to normoxia in the light for one hour at 20°C. Each point represents mean photosynthetic rate (n=8,  $\pm$  s.e.).

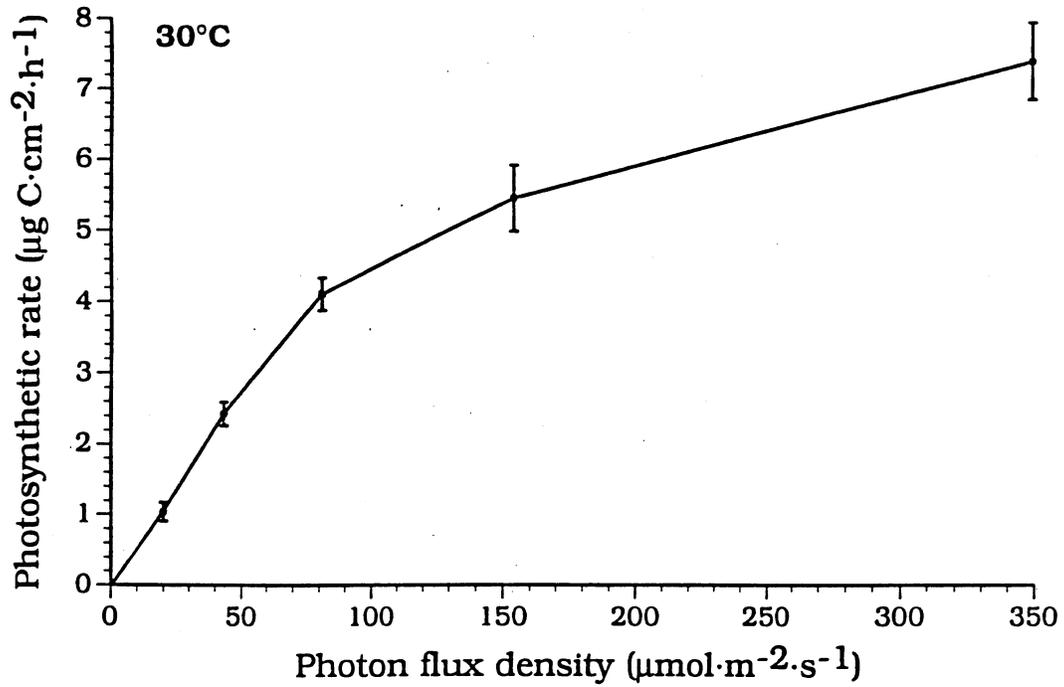


Figure 27. P-I curve for intertidal *Zostera marina* leaves exposed to normoxia in the light for one hour at 30°C. Each point represents mean photosynthetic rate ( $n=8$ ,  $\pm$  s.e.).

Table 15. P-I curve characteristics for intertidal *Zostera marina* leaves exposed to normoxia for one hour in the light. Least squares regression was performed on mean (n=8) initial slopes.  $P_{\max}$  values are means (n=8).  $P_{\max}$  was not reached at 30°C.  $I_k = P_{\max}/\alpha$ , where  $\alpha$  = initial slope.

Temperature (°C)	Initial slope	$P_{\max}$ $\mu\text{g C}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$	$I_k$ $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
10	0.065	4.56	71.2
20	0.065	6.50	94.2
30	0.055	ND	ND

## DISCUSSION

### Photosynthetic rates and chlorophylls

Significant reductions in photosynthetic rate in intertidal *Z. marina* leaves exposed to environmental hypoxia in darkness compared to *Z. marina* leaves exposed to environmental normoxia for one hour in the light occurred at 10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C and for all lengths of exposure tested (Figs. 5, 12, and 19). The photosynthetic rates of intertidal *Z. marina* leaves exposed to environmental hypoxia in the dark were significantly lower than photosynthetic rates of leaves exposed to environmental normoxia in the dark at 10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C (Figs. 4, 11, and 18) and the reductions in photosynthetic rate were greater for the hypoxic than the normoxic treatments. The lengths of exposure to hypoxia in the dark in this study were longer than those encountered in Padilla Bay except for the 3.5 and 8 hour exposures (30<sup>o</sup>C experiment). Spring and summer minus low tides in Padilla Bay expose the mudflat, ponds, and associated *Z. marina* meadows and macroalgal mats for up to nine hours. A strong potential exists for high temperatures and low oxygen concentrations to develop in ponded areas in estuaries during minus daytime tides in spring or summer. The photosynthetic rate results indicate that photosynthesis and growth of *Z. marina* in estuaries that are prone to ponding and/or eutrophication can be impacted if the right set of conditions exists to encourage hypoxic or anoxic conditions, especially where *Zostera marina* is growing under macroalgal mats.

Physiological normoxia for *Z. marina* has not been defined in the literature. Environmental normoxia (> 2 mg·L<sup>-1</sup> dissolved oxygen) in these experiments cannot be considered normoxia as defined in a physiological sense (Dr. David Schneider, Western Washington University, personal communication). Significant reductions in photosynthetic rates would not occur in leaves experiencing physiological normoxia,

but would occur in leaves experiencing physiological hypoxia. With the exception of the 3.5 and 8 hour normoxic exposures in the dark at 30°C (Table 2, dissolved oxygen post-treatment), environmental normoxia in this study probably represented physiological hypoxia for the leaves. The reductions in photosynthetic rate in leaves exposed to environmental hypoxia in the dark were likely a result of physiological hypoxia. Physiological anoxia and hypoxia are known to cause changes in intracellular pH, enzyme levels, ATP, and metabolic rates of plants. *Zostera marina* leaves held in the dark are not photosynthesizing (i.e. are not producing oxygen) but are respiring and using oxygen. When hypoxic or anoxic conditions are reached inside the leaf, anaerobic respiration occurs. Anaerobic respiration uses more of the plant's stored energy reserves and generates less ATP than aerobic respiration (Crawford, 1978; Berry and Bjorkman, 1980) so the leaves in this study may have experienced an energy deficit during darkness.

One effect of detaching a seagrass leaf from the whole plant is disruption of the lacunal system. In seagrass leaves, this system is continuous from leaf tip to rhizome (Larkum et al., 1989). It has been suggested that oxygen in the lacunae of the seagrass *Thalassia* might provide some protection from anoxia (Hammer, 1972). An alternative view is that the lacunal system may instead help transport harmful volatile products, such as result from anaerobic respiration, from the plant (Crawford et al., 1989). It is likely that anaerobic respiration occurred in the current study due to the lengthy exposure of *Z. marina* leaves to hypoxia in the dark and the fact that leaves may have had limited oxygen or energy reserves due to being detached from the whole plant.

The photosynthetic rates of intertidal *Z. marina* leaves exposed to environmental normoxia in the dark were significantly lower than photosynthetic

rates of leaves exposed to environmental normoxia for one hour in the light at all temperatures and for all lengths of exposure (Figs. 5 and 19) except for 24 and 48 hours at 20°C (Fig. 12). Mean dissolved oxygen levels in the BOD bottles for normoxic treatments in the dark were, except for two treatments, below saturation (Table 2). Therefore, leaves in these bottles likely experienced varying degrees of physiological hypoxia and responded with the same physiological changes as discussed above. The leaves were not photosynthesizing in the dark and dark exposures at 10° and 20°C were longer than would occur in nature. Lengthy exposures to dark conditions combined with the possible physiological hypoxia probably caused the photosynthetic rate reductions observed in this comparison.

Reductions in photosynthetic rate are indicative of some compromise to the photosynthetic system or other factor involved in the photosynthetic process. The leaf may or may not be able to repair the damage (Berry and Bjorkman, 1980), depending on the level of damage and which systems (e.g. enzymes, photosynthetic apparatus) have been compromised. An attempt was made to delineate maximum times at each temperature after which leaves exposed to treatments would not recover and fail to photosynthesize. These exposure times were based on preliminary experiments but it appears longer times were needed at all temperatures to see complete photosynthetic failure. The leaves were able to partially recover and photosynthesize for all the exposure times tested. However, the greatest percent reduction in photosynthetic rate (79.5%, Table 13) was recorded at 30°C for the longest dark hypoxic exposure (12 hours). Complete photosynthetic failure does not have to occur in order for the failure to be ecologically relevant. If the plant is compromised in any way by incomplete photosynthetic recovery after exposure to normoxia or hypoxia in darkness, potential ecological impacts exist (e.g. reduced

growth or productivity, increased susceptibility to disease). The present study was not designed to determine how long full recovery of photosynthesis takes in *Z. marina* leaves exposed to normoxia or hypoxia in the dark, or if full recovery is even possible. Further studies would help clarify these issues.

Studies are also needed to determine the causes of reduced photosynthetic rates observed in the present study. Elements which were not considered in this study but which can contribute to changes in photosynthetic rates include temperature effects (Sutcliffe, 1977), gas exchange (Larkum et al., 1989), and metabolic adaptations (Crawford, 1978). Enzymes are particularly sensitive to changes in temperature and may deactivate at high temperatures as their proteins denature (Weier et al., 1974). Photosynthesis, which is controlled by enzymes, increases with increasing temperature to about 30°C in *Z. marina* (Drew, 1979; Evans et al., 1986; Perez and Romero, 1992). Carbon dioxide is necessary for photosynthesis in vascular plants, including seagrasses. Bicarbonate is the predominant ion at seawater pH, but mechanisms for the transport of bicarbonate across seagrass leaf surfaces are only hypothesized at present (Larkum et al., 1989). Plants tolerant of anoxia produce metabolites when subjected to low-oxygen stress. Synthesis of these metabolites results in more oxidation than reduction reactions and an excess of protons. Tissues must dispose of these excess protons and one way is by forming acids (Crawford, 1978). These acids can change intracellular pH. Studies of agricultural crops show anoxia causes changes in intracellular pH in rice and wheat (Menegus et al., 1989; Menegus et al., 1991) and hypoxia in rice leads to decreased synthesis of mitochondrial proteins (Couee et al., 1992). Similar changes could be occurring in *Z. marina* exposed to hypoxia.

I hypothesized that if reductions in photosynthetic rates were observed following exposure of *Z. marina* leaves to normoxia or hypoxia in the dark, then reduced chlorophyll concentrations might be one factor causing the reduction in photosynthetic rates. However, the only significant decrease in chlorophyll concentrations occurred for chlorophyll *a* in *Z. marina* leaves exposed to hypoxia in the dark at 30°C. It is possible that enzymatic processes associated with the synthesis of chlorophyll *a* were negatively affected at this temperature. Because chlorophyll concentrations were not significantly affected by exposure to dark environmental normoxia or hypoxia at 10° or 20°C, I conclude that some mechanism other than chlorophyll concentration was responsible for the reductions in photosynthetic rates at those temperatures.

Chlorophyll ratios are used to compare changing chlorophyll concentrations in plants exposed to different light cycles or shading. In the present study, the chlorophyll *a:b* ratios for *Z. marina* leaves exposed to normoxia or hypoxia in dark conditions ranged from 2.3 ( $\pm$  0.20 s.e.) to 2.6 ( $\pm$  0.04 s.e.) which are more in the range of values for marine green algae (2.4 to 3.2) than vascular plants (3.9 to 6.0) (Dring, 1982). Other investigators have reported chlorophyll *a:b* ratios for seagrasses which are similar to values in this study or lower. Dennison and Alberte (1986) reported chlorophyll *a:b* ratios of 1.8 for intertidal *Z. marina* plants and 2.1 for subtidal *Z. marina* plants. Chlorophyll *a:b* ratios for other seagrasses growing intertidally ranged from 2.9 to 3.3 for *Heterozostera tasmanica* in Australia (Bulthuis, 1983), 2.3 to 4.0 for *Cymodocea nodosa* in the Mediterranean (Drew, 1978), and 1.6 to 3.2 for *Posidonia oceanica*, also a Mediterranean species (Drew, 1978).

Photosynthesis in intertidal *Zostera marina* is significantly reduced by exposure to dark low-oxygen conditions like those occurring under macroalgal mats

and this reduction is seen over a range of temperatures. Since chlorophyll concentration generally was not significantly affected by hypoxic exposure in the dark, the physiological causes for the photosynthetic reductions seen here need further study.

### P-I curves

Photosynthetic rate is directly proportional to irradiance in the initial portion of the curve known as initial slope and depends on the physical light-capture ability of the photosynthetic apparatus (Geider and Osborne, 1992). Marsh et al. (1986) found relatively constant initial slopes for *Z. marina* between 5<sup>o</sup>-30<sup>o</sup>C and initial slopes for *Z. marina* in this study were not significantly different between 10<sup>o</sup>, 20<sup>o</sup>, or 30<sup>o</sup>C.

The maximum photosynthetic rate of the P-I curve ( $P_{\max}$ ), is related to the rate of the light-independent reaction which is temperature dependent (Bulthuis, 1987).  $P_{\max}$  increases with increasing temperatures (10<sup>o</sup>, 20<sup>o</sup>, and 30<sup>o</sup>C) in the seagrass *Heterozostera tasmanica* (Bulthuis, 1983). In the current study,  $P_{\max}$  did not significantly increase for *Z. marina* leaves as temperature increased from 10<sup>o</sup> to 20<sup>o</sup>C (Table 15). Comparison of values reported in the literature is difficult due to differing units and measurements of photosynthesis (e.g.  $P_{\max}$ :  $\mu\text{mol O}_2 \cdot \text{dm}^{-2} \cdot \text{min}^{-1}$ ,  $\text{nmol O}_2 \cdot (\text{g Chl})^{-1} \cdot \text{h}^{-1}$ ,  $\mu\text{g C} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ ,  $\mu\text{g C} \cdot \text{h}^{-1} \cdot \text{shoot}$ ).

It is not known if treatment leaves would have reached the  $P_{\max}$  values of *Zostera marina* leaves exposed to normoxia in the light if they had been allowed more recovery time. The only reference regarding the effect of anoxia on photosynthesis in seagrass leaves is Hammer (1972), who indicates that even after a relatively short

exposure (24 hours) to anaerobiosis and 300 minutes of recovery, the seagrass *Halophila decipiens* is unable to photosynthesize at pre-treatment levels. In contrast, after 32 hours of exposure to anoxia and ~240 minutes of recovery, the seagrass *Thalassia testudinum* is able to photosynthesize at about 95% of the pre-treatment level (Hammer, 1972). A plant's ability to recover from damage caused by exposure to hypoxia or anoxia may depend on a variety of factors, including differences between individuals, species, and length of stress exposure.

$I_k$  values for seagrasses usually increase with increasing temperature (Drew, 1979; Bulthuis, 1983; Marsh et al., 1986; Pollard and Greenway, 1993) but did not change significantly with an increase in temperature from 10<sup>o</sup> to 20<sup>o</sup>C which was the only possible comparison for this data (Table 15).

## LITERATURE CITED

- Berry, J., and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Ann. Rev. Plant Physiol.* 31:491-543.
- Biebl, R., and C.P. McRoy. 1971. Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. *Mar. Biol.* 8:48-56.
- Brändle, R.A. 1991. Flooding resistance of rhizomatous amphibious plants, p. 35-46. *In* M.B. Jackson, D.D. Davies, and H. Lambers, eds. *Plant life under oxygen deprivation*. SPB Academic Publishing, The Hague, The Netherlands.
- Broekhuysen, G.J. Jr. 1935. The extremes in percentages of dissolved oxygen to which the fauna of a *Zostera* field in the tide zone at Nieuwediep can be exposed. *Arch. Neerlandaises de Zoologie* 1:339-346.
- Bulthuis, D.A. 1983. Effects of temperature on the photosynthesis-irradiance curve of the Australian seagrass, *Heterozostera tasmanica*. *Mar. Biol. Letters* 4:47-57.
- Bulthuis, D.A. 1987. Effects of temperature on photosynthesis and growth of seagrasses. *Aquat. Bot.* 27:27-40.
- Burkholder, J.M., K.M. Mason, and H.B. Glasgow, Jr. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. *Mar. Ecol. Prog. Ser.* 81:163-178.
- Cassidy, P.M. and G. McKeen. 1986. Padilla Bay baseline water quality record. Shannon Point Marine Center, Western Washington University, Anacortes, Washington. Washington Department of Ecology, Padilla Bay National Estuarine Research Reserve Reprint Series No. 2.
- Churchill, A. C. 1992. Growth characteristics of *Zostera marina* seedlings under anaerobic conditions. *Aquat. Bot.* 43:379-392.
- Cooper, S.R., and G.S. Brush. 1993. A 2,500-year history of anoxia and eutrophication in Chesapeake Bay. *Estuaries* 16:617-626.
- Couee, I., S. Defontaine, J-P. Carde, and A. Pradet. 1992. Effects of anoxia on mitochondrial biogenesis in rice shoots. *Plant Physiol.* 98:411-421.
- Crawford, R.M.M. 1978. Metabolic adaptations to anoxia. *In* D.D. Hook and R.M.M. Crawford, eds. *Metabolic adaptations to anoxia*. Ann Arbor Science, Ann Arbor.
- Crawford, R.M.M., C. Studer, and K. Studer. 1989. Deprivation indifference as a survival strategy in competition: advantages and disadvantages of anoxia tolerance in wetland vegetation. *Flora* 182:189-201.
- Day, J.W., Jr., C.A.S. Hall, W.M. Kemp, and A.Yanez-Arancibia. 1989. *Estuarine ecology*. John Wiley and Sons, Inc., New York.

- Den Hartog, C. 1970. The seagrasses of the world. North-Holland Publishing Company, Amsterdam.
- Den Hartog, C. 1994. Suffocation of a littoral *Zostera* bed by *Enteromorpha radiata*. *Aquat. Bot.* 47:21-28.
- Dennison, W.C., and R.S. Alberte. 1986. Photoadaptation and growth of *Zostera marina* L. (eelgrass) transplants along a depth gradient. *J. Exp. Mar. Biol. Ecol.* 98:265-282.
- Dortch, Q., N.N. Rabalais, R.E. Turner, and G.T. Rowe. 1994. Respiration rates and hypoxia on the Louisiana shelf. *Estuaries* 17:862-872.
- Drew, E.A. 1978. Factors affecting photosynthesis and its seasonal variation in the seagrasses *Cymodocea nodosa* (Ucria) Aschers., and *Posidonia oceanica* (L.) Delile in the Mediterranean. *J. Exp. Mar. Biol. Ecol.* 31:173-194.
- Drew, E.A. 1979. Physiological aspects of primary production in seagrasses. *Aquat. Bot.* 7:139-150.
- Dring, M.J. 1982. The biology of marine plants. Edward Arnold Publishers, London.
- Enriquez, S., S. Agustí, and C.M. Duarte. 1992. Light absorption by seagrass *Posidonia oceanica* leaves. *Mar. Ecol. Prog. Ser.* 86:201-204.
- Evans, A.S., K.L. Webb, and P.A. Penhale. 1986. Photosynthetic temperature acclimation in two coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. *Aquat. Bot.* 24:185-197.
- Fahy, E., R. Goodwillie, J. Rochford, and D. Kelly. 1975. Eutrophication of a partially enclosed estuarine mudflat. *Mar. Poll. Bull.* 6:29-31.
- Fourqurean, J.W., and J.C. Zieman. 1991. Photosynthesis, respiration and whole plant carbon budget of the seagrass *Thalassia testudinum*. *Mar. Ecol. Prog. Series* 69:161-170.
- Geider, R.J., and B.A. Osborne. 1992. Algal photosynthesis. Chapman and Hall, New York.
- Hammer, L. 1972. Anaerobiosis in marine algae and marine phanerogams, p. 414-419. In Proceedings of the 7th International Seaweed Symposium.
- Harley, M.T., and S. Findlay. 1994. Photosynthesis-irradiance relationships for three species of submersed macrophytes in the tidal freshwater Hudson River. *Estuaries* 17:200-205.
- Harlin, M.M., and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. *Mar. Biol.* 65:221-229.
- Kordan, H.A., and M. Ashraf. 1990. Environmental anoxia is unnecessary for inhibiting chloroplast photomorphogenesis in rice coleoptiles (*Oryza sativa* L.). *J. Exp. Bot.* 42:435-440.

- Larkum, A.W.D., G. Roberts, J. Kuo, and S. Strother. 1989. Gaseous movement in seagrasses, p. 686-722. In A.W.D. Larkum, A.J. McComb and S.A. Sheperd, eds. *Biology of seagrasses*. Elsevier, New York.
- Lüning, K., and W. Freshwater. 1988. Temperature tolerance of northeast Pacific marine algae. *J. Phycol.* 24:310-315.
- Marsh, J.A. Jr., W.C. Dennison, and R.S. Alberte. 1986. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *J. Exp. Mar. Biol. Ecol.* 101:257-267.
- McLusky, D.S. 1974. *Ecology of estuaries*. Heinemann Educational Books, London.
- McRoy, C.P. 1966. The standing stock and ecology of eelgrass (*Zostera marina* L.) in Izembek Lagoon, Alaska. MS Thesis. University of Washington, Seattle.
- Menegus, F., L. Cattaruzza, A. Chersi, and G. Fronza. 1989. Differences in the anaerobic lactate-succinate production and in the changes of cell sap pH for plants with high and low resistance to anoxia. *Plant Physiol.* 90:29-32.
- Menegus, F., L. Cattaruzza, M. Mattana, N. Beffagna, and E. Ragg. 1991. Response to anoxia in rice and wheat seedlings. *Plant Physiol.* 95:760-767.
- Mertens, E., Y. Larondelle, and H-G. Hers. 1990. Induction of pyrophosphate: fructose 6-phosphate 1-phosphotransferase by anoxia in rice seedlings. *Plant Physiol.* 93:584-587.
- Monk, L.S., R. Braendle, and R.M.M. Crawford. 1987. Catalase activity and post-anoxic injury in monocotyledonous species. *J. Exp. Bot.* 38(187):233-246.
- Moore, K.A., R.J. Orth, and J.F. Nowak. 1993. Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. *Aquat. Bot.* 45:79-91.
- Parnik, T., K. Bil, P. Kolmakov, and E. Titlyanov. 1992. Photosynthesis of the seagrass *Thalassodendron ciliatum* leaf anatomy and carbon metabolism. *Photosynthetica* 26:213-223.
- Parsons, T.R., Y. Maita, and C.M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, New York.
- Penhale, P.A. 1977. Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) community. *J. Exp. Mar. Biol. Ecol.* 26:211-224.
- Perez, M., and J. Romero. 1992. Photosynthetic response to light and temperature of the seagrass *Cymodocea nodosa* and the prediction of its seasonality. *Aquat. Bot.* 43:51-62.
- Perez-Llorens, J.L., and F. X. Niell. 1993. Temperature and emergence effects on the net photosynthesis of two *Zostera noltii* Hornem. morphotypes. *Hydrobiologia* 254:53-64.

- Phillips, R.C., and E.G. Menez. 1988. Seagrasses. Smithsonian Contributions to the Marine Sciences, Number 34.
- Pollard, P.C., and M. Greenway. 1993. Photosynthetic characteristics of seagrasses (*Cymodocea serrulata*, *Thalassia hemprichii* and *Zostera capricorni*) in a low-light environment, with a comparison of leaf-marking and lacunal-gas measurements of productivity. *Aust. J. Mar. Freshwater Res.* 44:127-139.
- Rabalais, N.N., W.J. Wiseman, Jr., and R.E. Turner. 1994. Comparison of continuous records of near-bottom dissolved oxygen from the hypoxia zone along the Louisiana coast. *Estuaries* 17:850-861.
- Raven, P.H., R.F. Evert, and H. Curtis. 1981. *Biology of plants*. Worth Publishers, Inc., New York.
- Smith, R.D., A.M. Pregnall, and R.S. Alberte. 1988. Effects of anaerobiosis on root metabolism of *Zostera marina* (eelgrass): implications for survival in reducing sediments. *Mar. Biol.* 98:131-141.
- Strickland, J.D.H., and T.R. Parsons. 1972. *A practical handbook of seawater analysis*. Bulletin 167 (2nd ed.). Fisheries Research Board of Canada, Ottawa.
- Sutcliffe, J. 1977. *Plants and temperature*. Edward Arnold Publishers, London.
- Waters, I., P.J.C. Kuiper, E. Watkin, and H. Greenway. 1991a. Effects of anoxia on wheat seedlings. I. Interaction between anoxia and other environmental factors. *J. Exp. Bot.* 42:1427-1435.
- Waters, I., S. Morrell, H. Greenway, and T.D. Colmer. 1991b. Effects of anoxia on wheat seedlings. II. Influence of O<sub>2</sub> supply prior to anoxia on tolerance to anoxia, alcoholic fermentation, and sugar levels. *J. Exp. Bot.* 42:1437-1447.
- Weier, T.E., C.R. Stocking, and M.G. Barbour. 1974. *Botany: an introduction to plant biology*. John Wiley and Sons, New York.
- Wetzel, R.L., and H.A. Neckles. 1986. A model of *Zostera marina* L. photosynthesis and growth: simulated effects of selected physical-chemical variables and biological interactions. *Aquat. Bot.* 26:307-323.
- Zar, J.H. 1984. *Biostatistical analysis* (2nd ed). Prentice-Hall, Englewood Cliffs, New Jersey.

