

CO₂-ENRICHMENT EFFECTS ON EELGRASS (*ZOSTERA MARINA* L.) AND BULL KELP (*NEREOCYSTIS LUETKEANA* (MERT.) P. & R.)

RONALD M. THOM

*Battelle/Pacific Northwest Laboratory, Marine Sciences Laboratory, 1529 W. Sequim Bay Road,
Sequim, WA 98382*

(Received 29 June, 1993; accepted 25 July, 1995)

Abstract. I investigated the effect of CO₂-enrichment on productivity of two aquatic plant species [*Zostera marina* L., *Nereocystis luetkeana* (Mert.) P. & R.] that form significant components of coastal ecosystems in the Pacific Northwest. Short-term (i.e., 2-hr) experiments showed that doubling CO₂ resulted in up to a 2.5-fold increase in *Zostera* net apparent productivity (NAP). *Nereocystis* NAP was increased 2.2 – 2.8 fold. In experiments involving seven enrichment treatments, NAP increased with increasing CO₂ between ambient (1.0x) and 2.5x CO₂ in both *Zostera* and *Nereocystis*. *Nereocystis* and *Zostera* NAP was lowest at highest (i.e., 5x) CO₂ concentrations. In growth experiments, mean growth rate of *Zostera* increased with increasing CO₂ during one of the two trials. I conclude that increasing CO₂ in the surface waters of the coastal ocean would predictably result in increased NAP of these two species. These results supplement limited published data showing that shallow estuarine and marine systems are vulnerable to increased carbon dioxide.

1. Introduction

It is estimated that human activities, such as burning fossil fuels, contribute significant amounts of CO₂ (i.e., 6 Pg yr⁻¹) to the atmosphere (e.g., Lugo 1992). Experimental evidence is growing in terms of the effects of increasing CO₂ on plants, and that sustained, enhanced photosynthesis occurs in some species, in particular those with the C₃ photosynthetic pathway (e.g., Bazzaz and Fajar 1992, Lugo 1992, Ziska *et al.*, 1990).

One of the key findings from the 'Natural Sinks of CO₂' conference held in 1992 was that 'There is a clear need to add the marine coastal system to the ... models of the carbon cycle because this sector ... has a high rate of carbon sequestration that has not been accounted for in terrestrial and oceanic carbon models' (Wisniewski and Lugo 1992). Coastal plains and shallow coastal seas comprise 8% of the world's surface and contribute roughly 25% of the global biological production (Holligan and deBoois 1993). These systems tend to have a high production to mean annual biomass (P:B) ratio which means that turnover and export (i.e., herbivory, burial, loss to adjacent systems) is relatively important on a global scale (Walsh *et al.*, 1978, Longhurst, 1991). Smith and Hollibaugh (1993) showed that coastal-system metabolism may be a significant component (30%) of the total oceanic carbon budget, and that coastal systems are highly vulnerable to human perturbations, which can significantly alter metabolic activity. Coastal

margin productivity provides the basis of energy that supports the majority of commercial fishery products world-wide (e.g., Mann 1982). An understanding of the effects of global warming and increased CO₂ on fundamental processes such as coastal primary production is highly relevant to the global carbon budget, and to the fishery resources supported by primary production in coastal systems.

The purpose of the present study was to test effect of CO₂-enrichment on productivity of two plant species [*Zostera marina* L., *Nereocystis luetkeana* (Mert.) P. & R.] that form significant components of coastal ecosystems in the Pacific Northwest. *Zostera marina* is a widespread seagrass species which dominates many subtropical to subarctic nearshore systems (Phillips and Meñez, 1988). The massive brown bull kelp *Nereocystis luetkeana* occurs from central California through the Aleutian Islands, and forms forests on rocky shores through much of the mid-portion of this range.

2. Methods and Materials

Three sets of experiments were conducted. Experiment 1 measured changes in net apparent photosynthesis (NAP; as O₂-flux) of *Z. marina* and *N. luetkeana* caused by enrichment treatments of 1.0x and 2.0x ambient CO₂. In Experiment 2, the effect on NAP of both species was tested under an enrichment series of 1.0x, 1.25x, 1.5x, 2.0x, 2.5x, 4.0x and 5.0x ambient CO₂. Experiment 3 tested the effect of 1.0x, 1.25x, 1.5x, 1.75x and 2.0x ambient CO₂-enrichments on the growth rate of *Z. marina*. Experiments 1 and 3 were conducted twice (trials 1 and 2). The measurements of NAP evaluated the effect of higher CO₂ on short-term (i.e., 2-hr) photosynthetic response, whereas the growth rate experiment tested longer-term (i.e., several days) photosynthetic response. Experiment 1 verified that there could be a response, and Experiment 2 illustrated the response curve of NAP vs CO₂. Experiment 3 evaluated a longer, and perhaps more ecologically-meaningful, response.

Experiments were conducted using plants collected near Battelle Marine Sciences Laboratory (MSL), Sequim, Washington (48°05'N, 123°03'W). In Experiment 1, two 10cm-long sections of healthy portions of *Z. marina* leaves were placed in a 1-L glass jar that was filled with either ambient (20 μm-filtered) seawater or seawater enriched to a level of 2.0x ambient CO₂. Similarly, a 5 × 5 cm section of a healthy *N. luetkeana* blade was placed into a jar. Ten replicate jars were prepared for each treatment along with ten replicate jars with water only. DO changes for all treatments were compared to controls which received enrichments but contained no plant material. Following the methods of Littler and Arnold (1985), the initial dissolved oxygen (DO) was recorded with an oxygen meter (Yellow Springs Instruments), the jars were then carefully sealed and placed in a water bath at ambient sea temperature and incubated for 2 hrs under ambient mid-day sunlight. The end DO was recorded after the incubation period. Experiment 1 was conducted twice

during July 1992. Experiment 2 methods were the same as experiment 1 except that seven levels of enrichment were tested instead of two. Experiment 2 was conducted in July 1993.

In experiment 3, eelgrass shoot growth was measured using the leaf-marking method (Kentula and McIntire, 1986). Twenty-eight to 30 shoots, planted in plastic flowers pots containing sediment from the meadow, were placed in each of five 130L tanks containing ambient seawater. Epiphytes were carefully removed by gentle scraping from the eelgrass leaves. Small (ca. 0.5 mm diameter) holes were then punched using a hypodermic needle through the leaves at a point immediately above the sheath. Water in the tanks was then enriched with CO₂ (see below). CO₂ levels were maintained in the standing water by re-enrichment two or three times during the day. Ambient seawater was flushed through the tanks at night. Growth was determined by harvesting the shoots and recording the net leaf material produced in g dry wt. relative to a reference hole punched in the oldest leaf (assumed to not grow). The experiment was conducted during July-August 1993. The plants were grown for 10 and 7 days in trials 1 and 2, respectively.

Enrichment of the water was accomplished by bubbling CO₂ into ambient seawater from the mouth of Sequim Bay. Differences between treatments in dissolved inorganic carbon (DIC = CO₂, HCO₃⁻) concentration was estimated using a pH meter with reference to the relationship between DIC and pH in Stumm and Morgan (1983). Ambient water was from a narrow inlet where water is well-mixed by vigorous tidal action. Repeated measurements pH of the water during the tests showed standard deviations of <0.1 pH units, which indicated that relative differences in concentration among treatments within a trial were maintained. All experiments were run under conditions where photosynthesis was light-saturated (i.e., photosynthetically active radiation >350 μM⁻² s⁻¹; LI-COR Radiation Sensor) and at water temperatures ranging from 13–16 °C.

3. Results

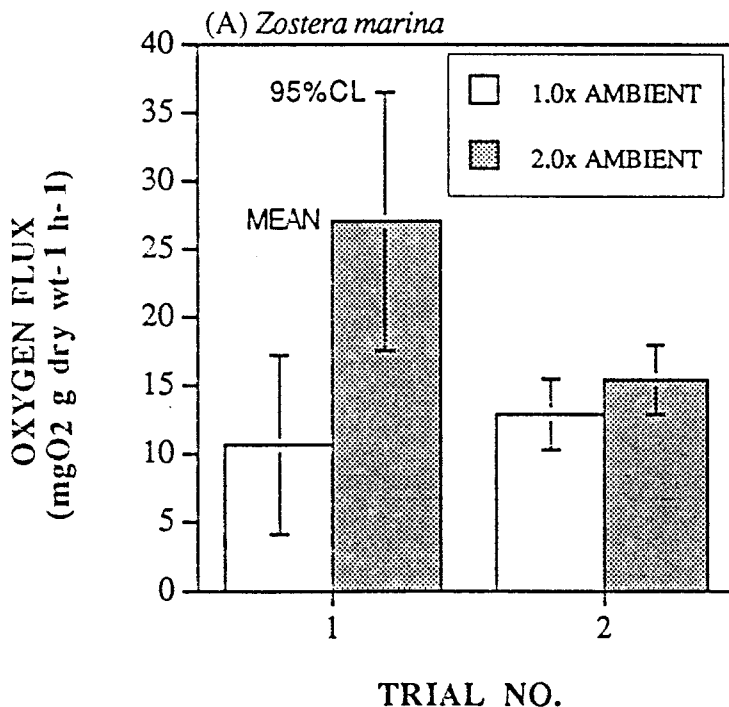
3.1. SHORT-TERM RESPONSE OF NAP TO CO₂ ENRICHMENT

Doubling CO₂ resulted in a 2.5-fold increase in *Zostera* NAP in trial 1 and no appreciable response in trial 2 (Figure 1A). *Nereocystis* NAP was increased 2.2 and 2.8-fold in trials 1 and 2, respectively (Figure 1B).

There was a general pattern of increased NAP with increasing CO₂ between ambient (1.0x and 2.5x CO₂ in both *Zostera* and *Nereocystis* (Figure 2A, B). *Nereocystis* NAP showed lowest NAP at 5.0x CO₂, whereas *Zostera* NAP showed low but extremely variable results at this concentration. The polynomial regressions between NAP and CO₂ (x) and correlation coefficients (R) shown in Figure 2 were:

$$\text{Zostera NAP} = -0.59x^2 + 3.74x + 2.16 \quad (R = 0.46)$$

$$\text{Nereocystis NAP} = -39.57x^2 + 223.63x - 6.017 \quad (R = 0.75)$$



Figs. 1(a)–(b). Effect of 2x CO₂ enrichment on the net productivity of (A) *Zostera marina*, and (B) *Nereocystis luetkana*.

3.2. GROWTH-RATE RESPONSE OF *Zostera* TO CO₂ ENRICHMENT

Mean growth rate of *Zostera* increased with increasing CO₂ during trial 1 but not trial 2 (Figure 3). Growth rates in controls (1.0x) were the same in each trial, which indicated that the growth potential of plants did not differ between trials. Growth rates in trial 2 at were lowest at intermediate concentrations, and were essentially the same at the highest and lowest CO₂ concentrations.

4. Discussion

I conclude that increasing CO₂ in the surface waters of the coastal ocean would predictably result in increased NAP of these two species. Whether actual growth rate would increase in *Zostera* is speculative due to inconclusive results during the growth rate experiment. Under the common scenario of a doubling of CO₂ (Bazzaz and Fajer, 1992), both species could approximately double their productivity. Results for bull kelp were consistent between experiments, whereas eelgrass showed highly variable responses to the treatments. Previous physiological studies indicate that this variability is due to a number of potential factors including availability of carbon in source water, initial use of carbon, the storage and translocation

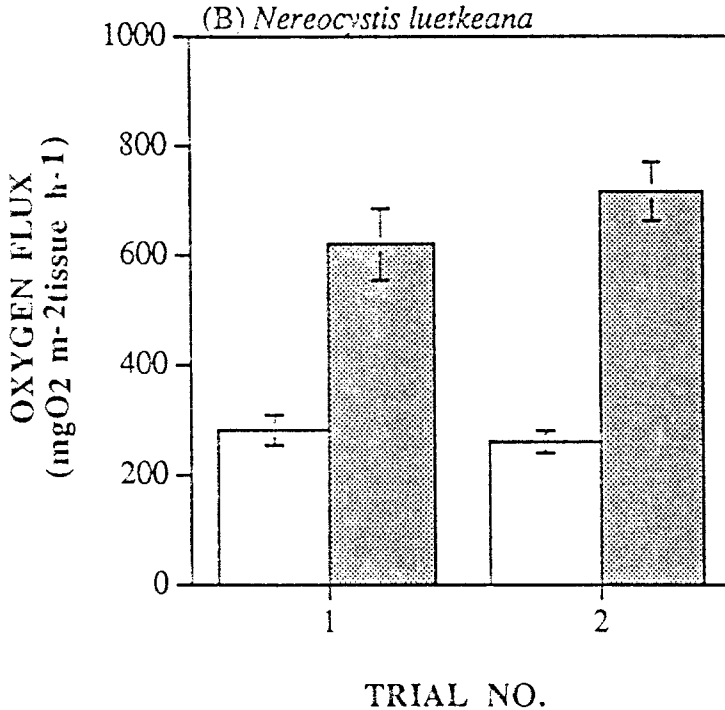
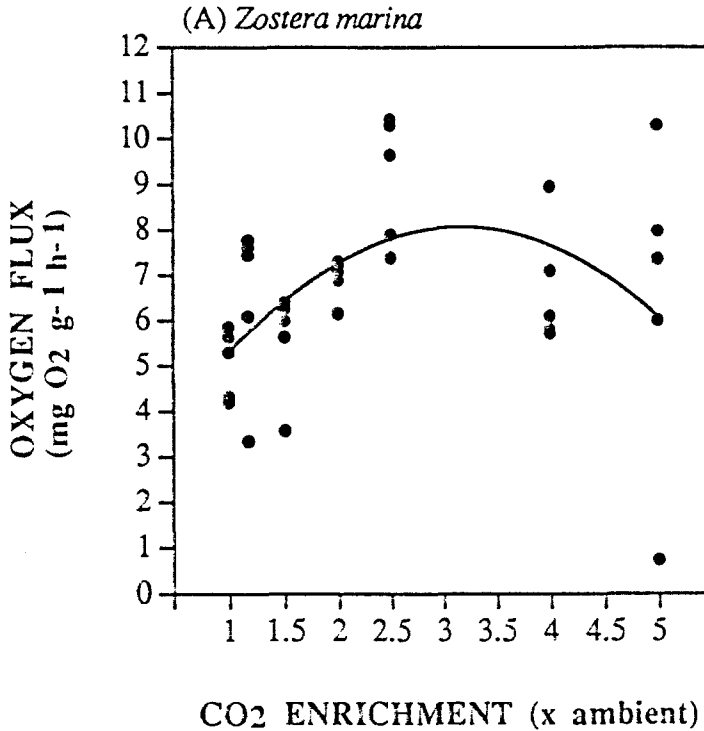


Fig. 1b.

of gases, products of photosynthesis and anatomical structure (e.g., Sodergaard and Wetzel, 1980; Kraemer and Alberte, 1993). At present, I have no evidence to support any conclusions regarding the relative effect of these factors. Factors such as temperature and nutrient limitation, which I also did not evaluate in the present study, could interact to alter the production response (Zimmerman *et al.*, 1987; Williams and Ruckelshaus, 1993).

There are very limited analogous data on the effects of enriched CO₂ or dissolved inorganic carbon on the photosynthetic rates of coastal plants. However, Kirk (1994) in a literature review of factors affecting photosynthesis of aquatic plants, makes a convincing case that inorganic carbon-limitation is a major factor controlling nearshore production. In addition, a general indication of C-limitation can be derived from a summary of C:N:P ratios from marine macrophytes (Atkinson and Smith, 1983). The mean C:N was 22, and approximately 13% of the plant species presented in the review by Atkinson and Smith had a C:N ratio of between 5 and 10, and the lowest C:N ratios were found under conditions of high nitrogen concentrations. Duarte (1992) found that macroalgae tissues had low % C, seagrasses had higher values and freshwater angiosperms had the highest values (related the higher amounts of cellulose and lignin). Either very low C:N ratios or high levels of carbon that are unavailable for use by the plant, may indicate C-limitation. Most of the seagrass species that have been evaluated utilize the



Figs. 2(a)–(b). Effect of serial CO₂ enrichment on the net productivity of (A) *Zostera marina*, and (B) *Nereocystis luetkana*.

C₃ photosynthetic pathway, which indicates potential carbon limitation (Beer and Wetzel, 1982). A growing body of experimental work has proven that dissolved inorganic carbon is frequently at low enough concentrations in ambient seawater to limit seaweed photosynthesis (e.g., Levassasseur *et al.*, 1991). However, analysis of carbon limitation is complicated in several seaweed species by a CO₂ concentrating mechanism which is active under emersed conditions (Surif and Raven, 1990). Kelp beds can have very high standing crops, and it is probable that these dense beds have a major requirement for carbon during periods of active growth (reviewed in Foster and Shiel, 1985). Taken as a whole, there is good evidence from a variety of different investigations indicating carbon limitation in coastal waters dominated by benthic macrophytes.

What is the relevance of these experiments to coastal systems as well as the global carbon budget? Rooted angiosperms and kelp occur in shallow depths over major areas of freshwater systems and coastlines globally. These systems are both highly productive and harbor large number of fisheries resources. Annual rates of net primary productivity (NPP) for tidal freshwater marshes, salt marshes, mangroves and seagrasses range from about 300–1000 gC m⁻² (Mann, 1982). Algae, including seaweeds and kelps have NPP rates on the order of 400–1900 gC m⁻² y⁻¹ (Mann, 1982). As such, these systems are key components of that part

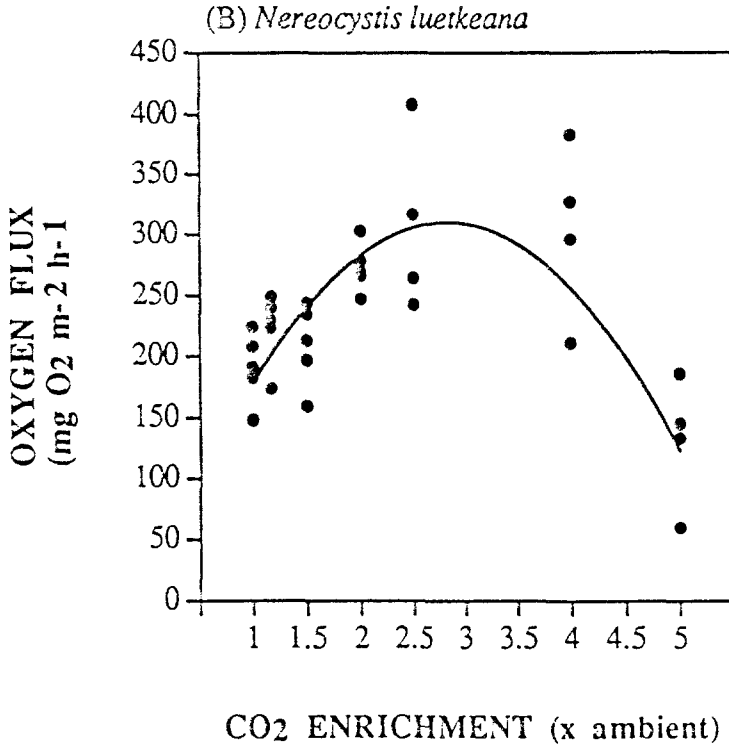


Fig. 2b.

of the biosphere (i.e., coastal plains and shallow seas) which accounts for 25% of the global biological production (Holligan and de Boois, 1993). Limited data show that these shallow systems are vulnerable to increased carbon dioxide. A doubling of CO₂ would increase productivity in the coastal zone significantly, and may then account for a larger percentage of global biological production in a CO₂-enriched biosphere.

Finally, as pointed out by Smith and Hollibaugh (1993) and Houghton and Woodwell (1983), variability in the flux of carbon exists among coastal systems. Preliminary evidence summarized by the IGBP does indicate that coastal systems may be a major sink of carbon derived from terrestrial and coastal primary producers (Holligan and de Boois, 1993). Hence, determining loss due to export and other potential large sinks is in need of study to resolve how these systems might act in terms of a global carbon sink. There is an obvious need to focus global climate change research on the coastal ecosystem.

Acknowledgements

The excellent technical assistance of B. Claiborne and V. Gaedecki is gratefully acknowledged. V. Gaedecki was funded by the Teacher Research Associate program

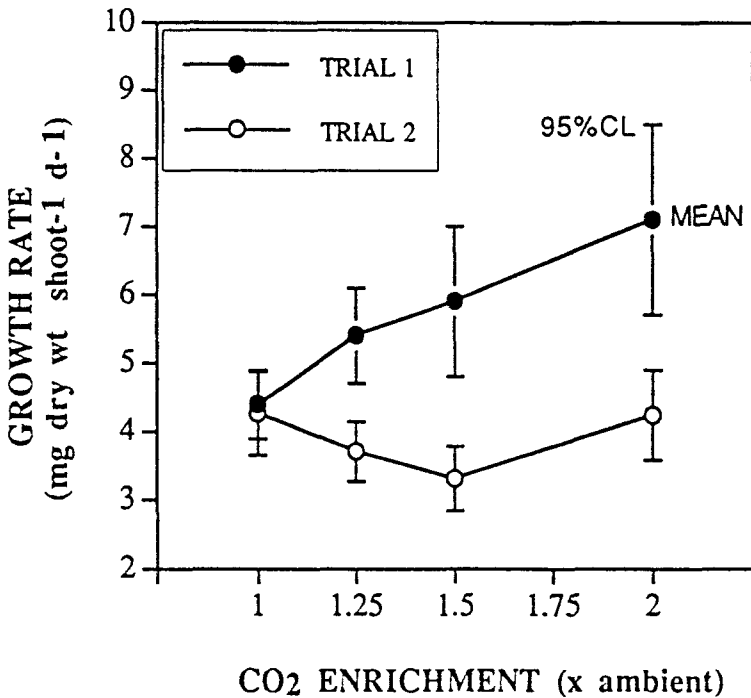


Fig. 3. Effect of serial CO₂ enrichment on growth of *Zostera marina*.

of the U.S. Department of Energy. W. Asher discussed aspects of the measurement of carbon dioxide in seawater. J. Downing and two anonymous reviewers provided excellent comments on the manuscript. Partial support for preparation of this manuscript was provided by the U.S. Department of Energy under contract DE-AC06-76LO-1830 to Battelle Memorial Institute.

References

- Atkinson, M. J. and Smith, S. V.: 1983. C:N:P Ratios Benthic Marine Plants. *Limnology and Oceanography* **28**, 568-574.
- Bazzaz, F. A. and Fajer, E. D.: 1992, 'Plant Life in a CO₂-rich World', *Scientific American* **266**, 68-74.
- Beer, S. and Wetzel, R. G.: 1982, 'Photosynthetic Carbon Fixation Pathways in *Zostera marina* and Three Florida Seagrasses', *Aquatic Botany* **13**, 141-146.
- Duarte, C. M.: 1992, 'Nutrient Concentration of Aquatic Plants: Patterns Across Species', *Limnology and Oceanography* **37**, 882-889.
- Foster, M. S. and Schiel, D. R.: 1985, 'The Ecology of Giant Kelp Forests in California: a Community Profile'. *U.S. Fish Wildl. Serv. Biol. Rep.* **86**(7.2). 152 pp.
- Holligan, P. M. and de Boois, H. (eds.): 1993, 'Land-ocean Interactions in the Coastal Zone (LOICZ) Science Plan'. International Geosphere-Biosphere Program: A Study of Global Change (IGBP) of the International Council of Scientific Unions (ICSU), Stockholm.

- Houghton, R. A. and Woodwell, G. M.: 1983, 'Effect of Increased, C, N, P, and S on the Global Storage of C'. Pages 327–342 in B. Bolin and R. B. Cook, (eds.), *The Major Biogeochemical Cycles and Their Interactions*. SCOPE.
- Kentula, M. E. and McIntire, C. D.: 1986, 'The Autecology and Production Dynamics of Eelgrass (*Zostera marina* L.) in Netarts Bay, Oregon', *Estuaries* **9**, 188–199.
- Kirk, J. T. O.: 1994, 'Light and Photosynthesis in Aquatic Ecosystems', Cambridge Univ. Press. Second Edition. 509 p.
- Kraemer, G. P. and Alberte, R. S.: 1993, 'Age-Related Patterns of Metabolism and Biomass in Subterranean Tissues of *Zostera marina* (Eelgrass)', *Mar. Ecol. Prog. Ser.* **95**, 193–203.
- Levassasseur, G., Edwards, G. E., Osmund C. B. and Ramus, J.: 1991, 'Inorganic Carbon Limitation of Photosynthesis in *Ulva rotundata* (Chlorophyta)', *J. Phycology* **27**, 667–672.
- Littler, M. M. and Arnold, K. E.: 1985, '17: Electrodes and Chemicals'. Pages 349–375 in Mark M. Littler and Diana S. Littler, (eds.), *Ecological Field Methods: Macroalgae*. Handbook of Physiological Methods. Cambridge Univ. Press, Cambridge, New York.
- Longhurst, A. R.: 1991, 'Role of the Marine Biosphere in the Global Carbon Cycle', *Limnology Oceanography* **36**, 1507–1526.
- Lugo, A. E.: 1992, 'The Search for Carbon Sinks in the Tropics', *Water, Air, and Soil Pollution* **64**, 3–9.
- Mann, K. H.: 1982, 'Ecology of Coastal Waters'. University of California Press, 322 pp.
- Phillips, R. C. and Meñez E. G.: 1988, 'Seagrasses. Smithsonian Contributions to the Marine Sciences No. 34'. Smithsonian Institution Press, Washington DC.
- Sodergaard, M. and Wetzel, R. G.: 1980, 'Photorespiration and Internal Recycling of CO₂ in the Submersed Angiosperm *Scirpus subterminalis*'. *Torr. Can. J. Bot.* **58**, 591–598.
- Smith, S. V. and Hollibaugh, J. T.: 1993, 'Coastal Metabolism and the Oceanic Organic Carbon Balance', *Reviews of Geophysics* **31**, 75–89.
- Stumm, W. and Morgan, J. J.: 1983, 'Aquatic Chemistry'. Wiley and Sons.
- Surif, M. B. and Raven, J. A.: 1990, 'Photosynthetic Gas Exchange under Emerged Conditions in Eulittoral and Normally Submersed Members of the Fucales and the Laminariales: Interpretation in Relation to C Isotope and N and Water Use Efficiency', *Oecologia* **82**, 68–80.
- Walsh, J. J., Rowe, G. T., Iverson, R. L. and McRoy, C. P.: 1978, 'Biological Export of Shelf Carbon is a Sink of the Global CO₂ Cycle', *Nature* **291**, 196–201.
- Williams, S. and Ruckleshaus, M.: 1993, 'Effects of Nitrogen Availability and Herbivory on Eelgrass (*Zostera marina*) and Epiphytes', *Ecology* **74**, 904–918.
- Wisniewski, J. and Lugo, A. E.: 1992, 'Natural Sinks of CO₂'. Kluwer Academic Publishers.
- Zimmerman, R. C., Smith, R. C., and Alberte, R. S.: 1987, 'Is Growth of Eelgrass Nitrogen Limited? A Numerical Simulation of the Effects of Light and Nitrogen on the Growth Dynamics of *Zostera marina*'. *Mar. Ecol. Prog. Ser.* **41**, 167–176.
- Ziska, L. H., Drake, B. G. and Chamberlain, S.: 1990, 'Long-term Photosynthetic Response in Single Leaves of a C₃ and C₄ Salt Marsh Species Grown at Elevated Atmospheric CO₂ *in situ*'. *Oecologia* **83**: 469–472.