PHOTORESPIRATION AND RELEASE OF ORGANIC CARBON IN SUBMERSED AQUATIC VASCULAR PLANTS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
RICHARD ANTON HOUGH
1973

This is to certify that the

thesis entitled

PHOTORESPIRATION AND RELEASE OF ORGANIC CARBON
IN SUBMERSED AQUATIC VASCULAR PLANTS

presented by

Richard Anton Hough

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany and Plant
Pathology

Major professor

Date June 22, 1973

O-7639

ABSTRACT

PHOTORESPIRATION AND RELEASE OF ORGANIC CARBON IN SUBMERSED AQUATIC VASCULAR PLANTS

By

Richard Anton Hough

A ^{14}C-assay for photorespiration (the light induced uptake of oxygen and release of CO_2 resulting from glycolate metabolism) was developed for use in submersed aquatic plants both in the laboratory and *in situ*. Laboratory studies with axenic cultures of *Najas flexilis* (Willd.) Rostk. and Schmidt indicated that respired carbon dioxide is refixed extensively in the light, similar to the activity of plants with the C_4 photosynthetic pathway, although analyses of leaf cross sections and of first ^{14}C fixation products showed that *N. flexilis* is not a C_4 plant. Hence, to the extent that CO_2 is refixed in the light, the ^{14}C photorespiration assay is a measure of net, rather than gross, photorespiration. Respiration in the light in *N. flexilis* increased with increasing dissolved oxygen concentration, indicating presence and enhancement of photorespiration, and indicating that net photosynthesis would decrease with increasing oxygen concentration consistent with the Warburg effect.

In situ experiments indicated that photorespiration varies within a day's photosynthetic period in *Najas flexilis*, in which afternoon decrease in net photosynthesis was correlated with afternoon increase in photorespiration, and a causal relationship was suggested. Photorespiration and dark respiration were 10-fold higher in fall than in summer in *N. flexilis*, which reflects senescence characteristic of annual plants. Net photorespiration in the perennial *Scirpus subterminalis* Torr. in the fall was similar to that in *N. flexilis* in the summer, but was increased somewhat under the ice in late winter.

The ^{14}C-assay evaluates total release of dissolved organic carbon from submersed plants simultaneously with respiration. Kinetics of release of organic carbon with respect to light vs. dark and to oxygen concentration suggest that glycolate was not excreted extensively by *Najas flexilis*, consistent with the evidence for oxidation of glycolate in photorespiration. Release of organic carbon *in situ* was relatively low in *N. flexilis* in summer, but increased 10-fold in the fall during senescence, which suggests an increased source of substrate for microbial metabolism and subsequent enhancement of fall phytoplankton bloom phenomena. Release of organic carbon was relatively low in *Scirpus subterminalis* in the fall but increased somewhat under ice in the winter.

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A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1973

This work is dedicated to my father, Jack L. Hough,
and to the memory of my mother, Alice C. Hough.

ACKNOWLEDGMENTS

Initially I must acknowledge the profound influence of four individuals, without which this work would never have been undertaken. Foremost is that of my father, Dr. Jack L. Hough, who engendered my aptitude for natural science and provided the opportunity for early shipboard experience in aquatic science. My grandfather, the late Dr. Anton J. Carlson, kindled my early interest in biology and physiology. Dr. Lester Ingle provided outstanding instruction and undergraduate laboratory experience in biology. Dr. John C. Ayers joined my father in exposing me to an intangible philosophy of aquatic science and to numerous practical field techniques.

Early graduate work under Dr. George W. Saunders resulted in my strong interest in the role of carbon metabolism in the aquatic ecosystem.

For the direction of the present work I extend most sincere thanks to Dr. Robert G. Wetzel, who constantly gave unlimited time and effort in assistance with the work and provided crucial encouragement of my ideas and approaches to the problem. I thank the other members of my guidance committee, Drs. Brian Moss, Clifford J. Pollard, and N. Edward Tolbert, for helpful advice during the study. In

particular, I gratefully acknowledge the help of Dr. Tolbert, from whose work and ideas my interest in photorespiration originally developed.

I thank Madelaine J. Hewitt, Karen E. Hogg, Jane Holt, Evelyn M. Hough, and Jayashree Sonnad for able technical assistance, and Mary Hughes and Evelyn Hough for help in preparation of the manuscript. Numerous discussions with Dr. Michael J. Klug, Dr. Bruce A. Manny, Kelton R. McKinley, and W. Sedgefield White were most helpful to me. Assistance of Dr. Kenneth W. Cummins and Robert C. Petersen in scintillation counting techniques is gratefully acknowledged. Dr. Steven Stephenson generously provided advice in preparation of leaf sections with the Hooker microtome and in interpretation of material.

The research was supported financially by subventions from AEC Grant AT-(11-1)-1599, COO-1599-71, and NSF Grants GB-15665 and GB-31018X. I wish to thank Drs. George H. Lauff and R. G. Wetzel for ensuring that generous support was consistently available.

I have warmest appreciation for my wife, Lynn, who shared and endured with me the ordeal, and for my sons Jack Brian and Robert Anton, who flourished in spite of it.

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INTRODUCTION

Productivity of Aquatic Macrophytes

In the long history of investigations of the ecology and productivity of aquatic macrophytes (macroalgae and aquatic vascular plants), the majority of the work has involved descriptions of plant associations and their distribution, with estimates of primary productivity based on measurements of standing crop biomass, as reviewed by Penfound (1956), Westlake (1963, 1965), Wetzel (1964, 1965) and Sculthorpe (1967). Early extensive work in physiological aspects of macrophyte growth was performed and reviewed by Arens (1934), Blackman (1895), Blackman and Smith (1911a, 1911b), Gessner (1937, 1955, 1959), Gorski (1929, 1935), Ruttner (1926) and others. Ecological studies of these plants generally have not included sophisticated physiological techniques, especially in coupled laboratory and field studies with manipulative experimental approaches, and controlling mechanisms in macrophyte productivity remain poorly known (Wetzel and Hough, 1973). In particular, very little is known of photosynthesis, respiration, and environmental factors affecting this metabolism in these plants.

The present work was directed toward aspects of these questions in the light of increasing developments in the area of plant photorespiration and its relationship to photosynthetic efficiency and plant productivity.

Net photosynthetic carbon fixation in aquatic macrophytes is reduced to varying degrees by losses of respiratory CO_2 and secretion of soluble organic compounds, and photosynthetic efficiency is influenced directly by the rates of these processes. In calculations of primary productivity, the respiration rate is usually assumed to be the same in light as in the dark. Physiological evidence increasingly suggests that such an assumption is erroneous. The normal process of mitochondrial or dark respiration may be inhibited in the light in some plants, perhaps by suppression of glycolysis (Jackson and Volk, 1970). Furthermore, refixation of respired CO_2 in the light may be extensive in submersed aquatic plants (Wetzel and Hough, 1973). These processes would restrict loss of respiratory CO_2.

Photorespiration

Alternately, the phenomenon of photorespiration, well known in terrestrial plants (reviewed in Fock and Egle, 1966; Gibbs, 1970; Goldsworthy, 1970; Hatch, et al., 1971; Jackson

and Volk, 1970; Tolbert, 1963; Zelitch, 1964, 1971), enhances loss of CO_2 in the light and may be a significant factor in reduction of photosynthetic efficiency of aquatic macrophytes. In this process O_2 is consumed and CO_2 is generated in the light as a result of synthesis and oxidation of glycolic acid, a process directly associated with the C_3 Calvin cycle of photosynthesis.

The precise pathways of oxygen and carbon dioxide in photorespiration have been investigated intensively, but remain in some dispute. Evidence from the laboratories of Tolbert (Andrews, et al., 1971, 1973; Lorimer, et al., 1973; Tolbert, 1971b) and Ogren (Bowes, et al., 1971; Bowes and Ogren, 1972; Chollet and Ogren, 1972a, 1972b) strongly suggests that glycolate is synthesized in chloroplasts from phosphoglycolate resulting from oxygenation of ribulose diphosphate (RuDP; Figure 1). In this reaction oxygen competes directly with CO_2 for reaction with RuDP. Alternative hypotheses for glycolate synthesis were proposed by Gibbs (1970), involving oxidation of a transketolase addition complex arising from a hexose phosphate in (or produced by) the Calvin cycle, and by Zelitch (1971), involving reductive condensation of CO_2. The pathway depicted in Figure 1 appears to be consistent with the widest range of physiological characteristics of photorespiration (Andrews, et al., 1973).

Glycolate leaves the chloroplasts and, in higher plants, enters the glycolate-glyoxylate shuttle in peroxisomes

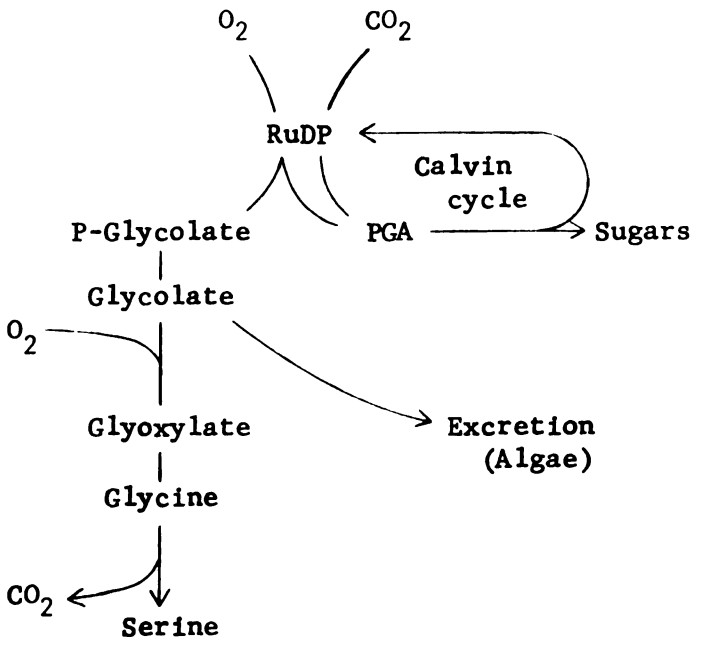

Figure 1. The paths of oxygen and carbon in plant photorespiration.

(Tolbert, 1971a), in which glycolate is oxidized by glycolate oxidase, with uptake of O_2, to glyoxylate and hydrogen peroxide. Tolbert (1971b) proposes that glyoxylate is transaminated to glycine, which leaves the peroxisomes and subsequently is condensed (perhaps in mitochondria) to serine with loss of CO_2, presumably the CO_2 of photorespiration; Zelitch (1972) proposes direct decarboxylation of glyoxylate. Serine then may enter a series of interconversions leading to, among several possibilities, phosphoglyceric acid (PGA).

The rate of glycolate metabolism (i.e. photorespiration) is highly influenced by, and is proportional to, oxygen concentration, light intensity, and temperature. Glycolate metabolism is also enhanced when low CO_2 limits photosynthesis. The rate at which photorespired CO_2 is actually lost from the plant depends on the efficiency of CO_2 refixation. Regardless of whether the CO_2 is lost from the plant or recycled, considerable photosynthetic assimilatory reducing power is consumed in photorespiration.

Terrestrial C_3 plants, in which all green cells photosynthesize by the C_3 Calvin cycle, can lose up to 50% of fixed carbon immediately through the glycolate pathway (Tolbert, 1963). In C_4 plants, CO_2 is fixed by the C_4 carboxylation pathway (Hatch and Slack, 1970; Kortschak, et al., 1965) in the mesophyll cells and transported via malic and/or aspartic acids to highly developed bundle sheath cells, where the CO_2 is transferred by decarboxylation to the

C_3 Calvin cycle (Johnson, et al., 1971). The mesophyll cells lack photorespiration; they also efficiently refix photorespiratory CO_2 lost from the bundle sheath cells, and little or no CO_2 is lost from these plants in the light.

C_3 and C_4 plants can be distinguished by the following combination of characteristics, any one of which is reasonable evidence for the distinction (adapted from Black, 1971, and from review articles cited above): C_4 plants have highly developed bundle sheath cells, best observed in leaf cross sections, with unusually high concentrations of chloroplasts, other organelles, and starch (elucidated by use of iodine-potassium iodide stain); C_3 leaves lack this "Krantz type" anatomy. The first CO_2 fixation products are primarily malic and/or aspartic acids in C_4 plants, and PGA and sugar phosphates in C_3 plants. Photosynthesis is difficult to light-saturate in C_4 plants, while in C_3 plants saturation illuminance is in the range of 10,000 to 45,000 lux. Photosynthetic temperature optima are 30° - 40° C in C_4 plants, 10° - 25° C in C_3 plants. Photosynthetic CO_2 compensation points are low for C_4 plants (0-10 ppm CO_2), and high for C_3 plants (30-70 ppm CO_2). Response of net photosynthesis to O_2 concentration is not detectable in C_4 plants, whereas C_3 plants exhibit the Warburg effect (Warburg, 1920), the progressive inhibition of net photosynthesis with increasing O_2. Photorespiration is not detectable in C_4 plants at any O_2 concentration, but is detectable and is enhanced with increasing O_2 in C_3 plants

(the probable cause of the Warburg effect). Glycolate synthesis and glycolate oxidase activity are low in C_4 plants and high in C_3 plants; glycolate metabolism can be inhibited by hydroxymethanesulfonates, with corresponding reduction in photorespiration in C_3 plants. An extensive and growing list of C_4 species exists (Black, et al., 1969; Downton, 1971), with obvious implications as to presence or absence of photorespiration. However, the list consists entirely of terrestrial plants, and little is known of the role of photorespiration in higher aquatic plants.

Emergent hydrophytes are exposed to terrestrial environmental conditions, and photorespiration undoubtedly can be extensive. C_3 metabolism and photorespiration have been demonstrated in the cattails Typha anqustifolia and T. latifolia (Forrester, et al., 1966b; McNaughton, 1966, 1969; McNaughton and Fullem, 1969). The C_4 photosynthetic system thus likely would be of adaptive value in many emergent hydrophytes, particularly in regions of high temperature and high light intensity, and also in situations where salt content of the environment adversely affects internal CO_2 and water balance (Bjorkman, 1971; Slatyer, 1971). In the latter context, Spartina, the dominant plant of the salt marshes of the east coast of the United States, appears to be a C_4 plant (Krenzer and Moss, 1969). Also evidently of C_4 type is a highly productive floating species of Alternanthera (C. C. Black, personal commun.) of the family Amaranthaceae, which includes numerous C_4 plants.

Submersed hydrophytes in a given locality are exposed to lower maximum O_2, light, and temperature than are terrestrial plants, and photorespiration correspondingly may be generally of somewhat lesser magnitude in the submersed plants. Also, the greater resistance of water to diffusion of CO_2 relative to air (Raven, 1970) and presence of massive internal gas lucanae may retard loss of CO_2 from submersed hydrophytes and facilitate refixation of CO_2 regardless of presence or absence of the C_4 photosynthetic system. For these reasons the C_4 system may not be of major adaptive value, and although few studies have been made, there are no known C_4 submersed hydrophyte species. On the other hand, the glycolate pathway operates in all C_3 plants, and it is probable that photorespiration affects photosynthetic efficiency in submersed plants in some circumstances, especially in view of the complexity and variability of environmental conditions in the littoral zones of lakes. If photorespiration does exist significantly in some aquatic plants, its magnitude may vary in different species in relation to the C_3 and C_4 photosynthetic pathways and to environmental conditions, with evolutionary and ecological implications particularly in terms of competitive capabilities under conditions of CO_2-, O_2-, and temperature stress, both in natural and culturally altered water bodies (Hough and Wetzel, 1972). Such stress often reaches greatest extremes in the latter situation.

Background of Methods

At the time that oxygen inhibition of net photosynthesis was first observed by Warburg (1920), an associated acceleration of respiration in light was suspected by several workers (Meyer and Deleano, 1913; Spoer and McGee, 1923; reviewed in James, 1928). Strong evidence that CO_2 evolution in the light can exceed that in the dark in green plants was reported initially by Decker (1955, 1957, 1959a, 1959b), who observed a "post-illumination burst" of CO_2 in suddenly darkened plants, followed by greatly reduced CO_2 evolution in continued darkness. Similar effects were reported by Krotkov's group (Krotkov, et al., 1958; Forrester, et al., 1966a, 1966b; Tregunna, et al., 1966), along with other evidence that light and high O_2 concentrations stimulate CO_2 production. Holmgren and Jarvis (1967) and Moss (1966) devised measurements of steady state light and dark CO_2 release into CO_2-free air using a gas flow-through system and infrared CO_2 analysis. This system was modified for use with ^{14}C-labeled plants by Goldsworthy (1966), and further so by Zelitch (1968), in which $^{14}CO_2$ evolving into CO_2-free air flowing past prelabeled leaf discs is followed in the light and dark; this method is based on the assumption that photorespiratory CO_2 is derived from the most recently synthesized carbohydrates.

The use of ^{14}C in measurements of photosynthesis has been particularly valuable in studies of aquatic plants, as first developed for phytoplankton by Steeman Nielsen (1952a).

The ^{14}C technique in aquatic macrophytes was treated in detail by Wetzel (1964), and was viewed as a superior approach for studies of carbon metabolism in these plants. Accordingly, the ^{14}C-assay of Goldsworthy and Zelitch appeared to be well suited for investigations of photorespiration in submersed plants, with appropriate modification to an aqueous flow-through system (Hough and Wetzel, 1972). Flow-through systems have been used previously for analyses of photosynthesis and respiration in submersed macrophytes, initially by Blackman and Smith (1910), and later by Meyer (1939), Westlake (1967), and McDonnell (1971; McDonnell and Weeter, 1971). These studies involved measuring changes in O_2 or CO_2 content of water flowing past plants; flow-through techniques do not appear to have been used in conjunction with ^{14}C in aquatic macrophytes prior to the preliminary study of Hough and Wetzel (1972). Investigations of ^{14}C loss from prelabeled macrophytes or algae by Carr (1969), Steeman Nielsen (1955), and Ryther (1956)(cf. Discussion) were not performed with flow-through systems. Recycling of respired $^{14}CO_2$ in the light, both internally and from the medium, is a serious problem in the method (cf. Discussion) and a flow-through system at least minimizes recycling of $^{14}CO_2$ leaving the plants. Other problems associated with closed-bottle techniques (Sculthorpe, 1967), especially the rapid changes in O_2 and CO_2 concentrations, also are avoided with a flow-through system.

Scope of the Study

In the initial stages of the present study, techniques of the ^{14}C photorespiration assay were developed as an aqueous system. Najas flexilis (Willd.) Rostk. and Schmidt was selected for the major portion of the work because reliable techniques were available for obtaining axenic (algal-and bacteria-free) cultures, to ensure that all data would apply to the plants alone.

Major emphasis was placed on the effects of variation of dissolved oxygen concentration on rates of respiration in the light and dark. Influence of carbon dioxide concentration was also tested, partly as a prelude to in situ studies in the field. Effects of presence of epiphytic algae and other microorganisms also were examined, inasmuch as these organisms are always present on naturally growing macrophytes and are highly active metabolically.

To aid the assessment of photorespiration, the status of the plants used in the study as C_3 or C_4 was examined, both in terms of first ^{14}C fixation products and leaf cross section anatomy.

In situ ^{14}C photorespiration assays were initiated on natural plants in Lawrence Lake, southwestern Michigan. Lawrence Lake is a small (4.9 ha) marl lake in the outwash apron of a glacial moraine system and receives water of very high calcium carbonate content. As in most hard-water marl lakes, rates of phytoplanktonic production are moderate to low, largely as a result of several nutrient interactions

associated with calcium carbonate precipitation (Wetzel, 1965, 1966a, 1966b, 1968, 1972, 1973). The littoral zone is well developed, and while diversity of submersed macrophytes is low, the species present dominate the total primary production of the lake (Rich, et al., 1971). Najas flexilis, a common annual rooted angiosperm in marl lakes, is fairly widely distributed in the littoral zone of this lake, largely at depths less than 1 meter. The perennial rooted angiosperm Scirpus subterminalis Torr. completely dominates the macrophytic growth between 1 and 7 meters depth, below which no macrophytes are present. In situ studies were initiated with natural N. flexilis as an extension of the work in axenic cultures, and with S. subterminalis because of its major role in the productivity of the lake. Photorespiration assays of two marine angiosperms performed at this writing are summarized in the Discussion.

Of particular interest in the in situ work was the initial testing of a hypothesis (Hough and Wetzel, 1972) concerning possible variations in photorespiration during a day's photosynthetic period as an explanation for the "afternoon depressions" in net photosynthesis in freshwater macrophytes and phytoplankton, a phenomenon observed by several workers (cf. Discussion). Inasmuch as net productivity often is maximal in the mornings, and decreases thereafter regardless of constant or increasing light intensity, photorespiration may increase through the day

with increasing light intensity, oxygen tension, and temperature, and possible decreasing CO_2 availability. Conditions are known to develop in this manner particularly in littoral zones with dense macrophytic growth (Sculthorpe, 1967), especially in calm weather with little turbulence, and these are precisely the conditions conducive to accelerated photorespiration. Accordingly, multiple *in situ* photorespiration assays were performed in a single day with concurrent measurements of net photosynthesis and environmental conditions.

The *in situ* studies were performed in summer, fall and late winter, which allowed consideration of seasonal aspects of respiration.

The ^{14}C photorespiration assay in an aqueous system automatically provides data on total released dissolved organic carbon as well as CO_2. The release of organic carbon may be significant in terms of glycolate metabolism. A large body of literature exists concerning glycolate metabolism in algae (e.g. Hess and Tolbert, 1967; Bruin, *et al.*, 1970; and many others) which is useful in planning and interpreting experiments on photorespiration in higher aquatic plants. However, important differences between glycolate metabolism in algae and that in vascular plants have been demonstrated. In particular, some algae oxidize glycolate via a dehydrogenase enzyme in a reaction which does not use O_2 as the terminal electron acceptor, as opposed to the oxidase universally present in terrestrial vascular plants (Bruin,

et al., 1970; Nelson and Tolbert, 1969). Activity of glycolate dehydrogenase in these algae is low in relation to the oxidase in higher plants, and much of the glycolate is excreted rather than oxidized. Glycolate excretion by algae, first reported by Tolbert and Zill (1956), has received much attention by workers in phytoplankton ecology (reviewed in Fogg, 1971). While this aspect of the glycolate pathway has not been documented in higher aquatic plants, the loss of various amounts of recently synthesized unidentified organic carbon as secreted dissolved organic compounds has been demonstrated for a few submersed and floating angiosperms (Khailov, 1970, 1971; Khailov and Burlakova, 1969; Wetzel, 1969; Wetzel and Manny, 1972; Hough and Wetzel, 1972). This loss of organic carbon represents a significant reduction in net photosynthetic efficiency similar to the effects of photorespiration, and may involve glycolate excretion to varying degrees, depending on plant biochemistry and environmental conditions. Tolbert (personal commun.) recently has found glycolate dehydrogenase in several marine macrophytes.

The release of dissolved organic matter by submersed hydrophytes enhances the development of a productive epiphytic microflora (Allen, 1971; Wetzel and Allen, 1972), with resulting symbiotic nutrient interactions of much greater magnitude than exist in the more diluted planktonic regime. Physiologically the release of organic matter may represent an incomplete adaptation to the aquatic environ-

ment, but the resulting community interaction may be viewed in an evolutionary context as a community adaptation to environmental conditions imposing physiological stress on the macrophytes (Wetzel and Hough, 1973). Hence, attention was given in this study to release of organic matter, including its relation to respiration and photorespiration, environmental variables, and epiphytic microbial interactions.

MATERIALS AND METHODS

^{14}C Assay for Photorespiration

Photorespiration was evaluated in terms of CO_2 loss in the light in a ^{14}C-assay adapted from the Zelitch (1968) technique of following $^{14}CO_2$ evolution from prelabeled leaf discs. The assay was modified from the flowing gas system employed by Zelitch to a flowing aqueous system (Hough and Wetzel, 1972). In each case plant material was incubated in the light in aqueous medium containing $NaH^{14}CO_3$ to develop a ^{14}C-labeled cellular organic carbon pool. Plants then were rinsed thoroughly and placed in specially constructed tubular glass flow-through chambers (Figure 2) situated between fluorescent light tubes. The chambers were equipped with removable opaque jackets and with Tygon tubing at the intake and outlet ports. Aqueous media were pumped unidirectionally through the chambers at constant rates, during which the effluent medium was collected in five-minute fractions for 30, 50, or 55 minutes and radioassayed for $^{14}CO_2$ and acid stable organic compounds released by the plants. Carbon dioxide was separated from organic carbon by acidification (pH 2.5) and N_2 purging.

Laboratory experiments were performed with axenic cultures of *Najas flexilis* (Willd.) Rostk. and Schmidt, a submersed, rooted, freshwater angiosperm of the family Najadaceae. Methods of axenic culture were as described in

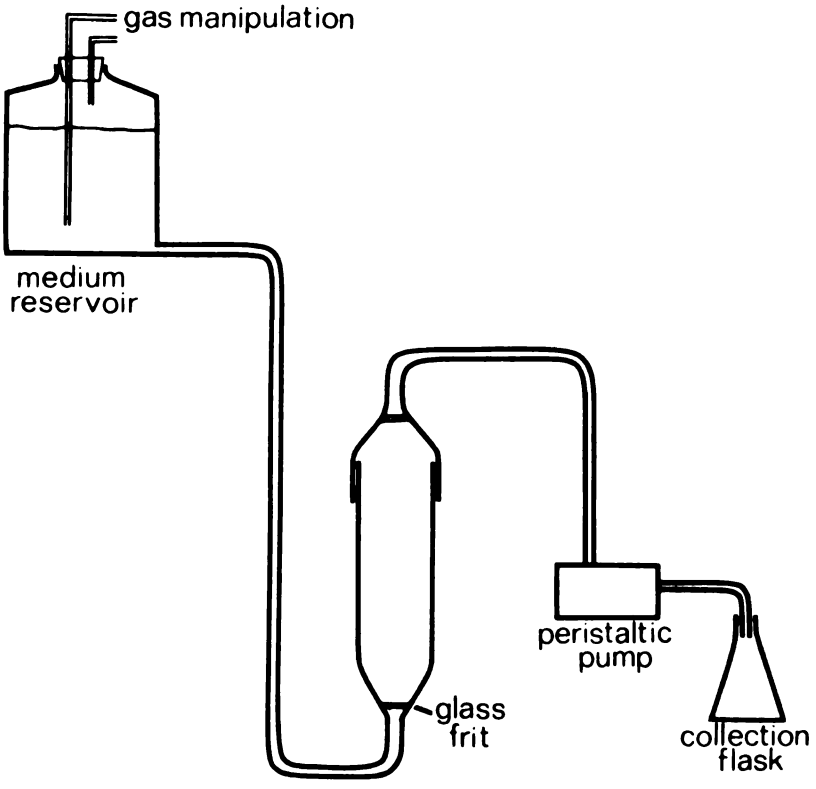

Figure 2. Apparatus for obtaining carbon dioxide and organic carbon released from ^{14}C labeled submersed aquatic plants. Flow-through chamber: length 15 cm, diameter 3 cm, volume 110 ml.

Wetzel and McGregor (1968), in which seeds are collected from natural populations, surface-sterilized, and germinated (after cold treatment) in sterile growth medium. Cultures of ca. 60 to 100 seedlings were maintained in erlenmeyer flasks containing 200 ml defined synthetic lake water growth medium at 25°C and 5500 lux (16 hr day) in controlled environmental chambers. Experience enabled the visual recognition of contaminated cultures, because the relatively large amount of organic buffer in the medium provided sufficient substrate for rapid bacterial growth, resulting in cloudiness of the medium. Sterility tests with Difco nutrient broth, as well as microscopic examinations, were performed periodically to support the visual examinations.

Prelabeling for the ^{14}C photorespiration assays was accomplished by injecting an appropriate volume (usually 1 ml) of known high specific activity (88.6 or 97.2 $\mu Ci\ ml^{-1}$) aqueous carrier-free $NaH^{14}CO_3$ into the culture flasks and incubating for 30, 60, or 190 minutes at 25°C and 5500 lux. A final isotope concentration of about 0.5 to 1 $\mu Ci\ ml^{-1}$ and an incubation time of 60 minutes resulted in optimum plant radioactivity.

A CO_2-free environment is recommended in the ^{14}C photorespiration assay to avoid dilution of the labeled cellular organic carbon pool by ^{12}C fixed in the light during the analysis (Zelitch, 1968). Experimental media were prepared by omitting additions of carbonate, autoclaving

to remove dissolved gases, and then allowing contact only with CO_2-free N_2 or O_2. Infrared analyses (Beckman 215A) of CO_2 purged from acidified medium samples collected from the flow-through system under N_2 showed presence of 0.19 ± 0.02 mg C (inorganic) l^{-1}, or ca. 0.7 mg CO_2 l^{-1}. This value was higher than expected, and exceeded the theoretical concentration of CO_2 in water at equilibrium with air (ca. 0.5 mg l^{-1} at 22C; Raven, 1970); the system of CO_2 analysis was operating near its lower limit of sensitivity, and the figure may be somewhat high. Because of the high solubility of CO_2 in water, it is difficult to render and maintain water absolutely free of CO_2. Media treated for CO_2-removal thus are designated "low-CO_2" in this study.

In initial experiments the medium consisted of phosphate buffer (0.025 M monobasic and dibasic potassium phosphate); in later laboratory experiments a modified synthetic lake water growth medium was used containing (in mg l^{-1}): $CaCl_2$ (54), NH_4Cl (3.82), $MgSO_4 \cdot 7H_2O$ (100), KCl (30), K_2HPO_4 (0.56), NTA[1] (20), and tris buffer[2] (500). All media were prepared with deionized, glass distilled water. Adjustments of pH (8.1 - 8.2) were made with 1N NaOH or HCl by syringe through the stopper in the medium reservoir, or by pipet, with N_2 in the space over the medium. Dissolved

---

1 nitriolotriacetic acid
2 tris (hydroxymethyl) aminomethane

oxygen concentrations in the media were determined by the unmodified Winkler titrimetric method (Welch, 1948).

At the beginning of an experiment, the chambers and tubing were flushed with N_2, and aqueous medium was allowed to flow by gravity into the lower portion of the chamber (Figure 2); the flow was then stopped and the prelabeled plants placed in the chamber. The upper portion of the chamber was seated on the lower portion (silicone-greased ground-glass fitting), and the flow of medium was resumed. Rates of flow were controlled at ca. 15 ml min^{-1} with a multi-channel peristaltic pump. The rates were similar to those found by James (1928) to be optimal for measurements of photosynthesis and respiration.

In initial experiments, the five-minute effluent fractions were acidified and purged with N_2 in a closed system in which the gas was passed through 10 ml hyamine-OH to absorb evolved CO_2. After each fraction was purged, a 1 ml aliquot of hyamine was collected and radioassayed in 14 ml PPO[1]/toluene (5 g/l) scintillation mixture with a Beckman LS-150 scintillation counter. Purged fractions were radioassayed for non-volatile ^{14}C organic carbon by evaporating 3 ml aliquots on planchets and counting with a Nuclear-Chicago G-M counter (D-47) of known efficiency.

In later experiments, radioassay of the fractions was accomplished in a simpler procedure, in which two-ml

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1 2,5-Diphenyloxazole

aliquots of each fraction, and of each purged fraction, were radioassayed directly by liquid scintillation in a mixture of 7 ml Triton X-100 and 6 ml butyl PBD[1]/PBBO[2]/ toluene (8 g l^{-1}, 0.5 g l^{-1}). Counting efficiency was calculated, using external standard ratios, with an efficiency regression for chemical quenching obtained by radioassay of aqueous ^{14}C standards in the scintillation mixture. Carbon dioxide radioactivity was calculated by subtracting organic ^{14}C counts min^{-1}(cpm) in purged fractions from total cpm in non-purged fractions.

To relate isotope released in each experiment to total activity in the prelabeled plant material (allowing comparison of performance of plants in separate assays), radioactivity of the plants was determined at the end of the experiment by combustion of lyophilized plants with a Packard tritium-carbon oxidizer and radioassay of CO_2 evolved (into ethanolamine) in 15 ml PPO/bis-MSB[3]/toluene (15 g l^{-1}, 1 g l^{-1}) scintillation mixture. Total cpm released in the experiment were added to the final radioactivity of the plants to give initial plant radioactivity, which also allowed calculation of ^{14}C fixation rates during prelabeling. Carbon dioxide cpm present in each effluent fraction were added consecutively, as were organic carbon cpm, to give cumulative cpm released at each time interval. Cumulative

1 2-(4'-t-Butylphenyl)-5-(4"-biphenyl)-1,3,4,-oxidazole
2 2-(4'-Biphenyl)-6-phenol-benzoxazole
3 p-bis-(o-Methylstyryl)-Benzene

cpm at each time interval were then divided by initial plant radioactivity and multiplied by 100 to give % initial internal ^{14}C released.

Experiments were usually performed both in the light and in the dark, so that dark respiration could be accounted for in observations of CO_2 release in the light.

Variation of Dissolved Oxygen Concentration

The first series of experiments was performed using axenic Najas flexilis cultures, with oxygen content of the medium as the experimental variable. Oxygen concentration was kept low in the medium after autoclaving by allowing contact only with N_2 or was raised to higher levels by aerating the medium with O_2.

In three 30-minute analyses, separate light and dark chambers were attached in parallel to the same medium reservoir, with O_2 maintained at <5, 14.98, and 21.0 mg l^{-1}. Two duplicated analyses were performed entirely in the light, one at 3.04 mg O_2 l^{-1}, and the other at 10.85 mg O_2 l^{-1}. Further duplicated analyses were performed, one at 0.56 mg O_2 l^{-1} and one at 25.5 mg O_2 l^{-1}, in which each chamber was in the light for 30 minutes, followed by darkness for another 20 or 25 minutes. Finally, an experiment was performed in which separate light and dark chambers received medium containing 0.98 mg O_2 l^{-1} for 30 minutes immediately followed by medium (from a second reservoir)

containing 23.1 mg O_2 l^{-1} for another 25 minutes. Other experimental conditions were constant and are given in Table 1.

Variation of Total Carbon Dioxide Concentration

The effect of presence of relatively large amounts of total CO_2 in the medium on the ^{14}C photorespiration assay was evaluated in several experiments using axenic cultures of Najas flexilis.

Two chambers were operated in the light for 50 minutes in the first analysis (C-1), one receiving the normal low-CO_2 medium and the other receiving the same medium to which had been added 20 mg Na_2CO_3 l^{-1} and which had been aerated with 0.03% CO_2 giving 9.5 mg total CO_2 l^{-1}. Dissolved oxygen was adjusted to 8.66 mg l^{-1} in both reservoirs.

Two 50-minute replicated assays were performed in the light (C-2) using HA Millipore filtered (pore size 0.45 μm) water from Lawrence Lake, Michigan, described above. CO_2 and carbonates were removed from a portion of the filtered lake water by acidification and N_2 purging (90 minutes); pH was restored with 1N NaOH, facilitated by addition of 150 mg tris buffer l^{-1}. Total CO_2, as determined by alkalinity titrations and calculations as in Golterman (1969), was 185.15 mg l^{-1} in the untreated filtered water. Total CO_2 in the purged water was assumed to be <0.5 mg l^{-1} (i.e. below atmospheric equilibrium), although alkalinity

titration indicated 23.28 mg l^{-1}. Tris buffer interferes with the titration, and its presence in the treated water likely is responsible for the relatively high value obtained. Oxygen was adjusted to 15.9 mg l^{-1} in both media.

Other experiments (C-3) were performed using filtered lake water, in which separate light and dark chambers received CO_2-purged water for 30 minutes, immediately followed by non-purged water (CO_2 and carbonates present) from a second reservoir for another 25 minutes. Alkalinity titrations indicated total CO_2 concentrations similar to those in the previous experiments. Oxygen was adjusted to 12.0 mg l^{-1} in the purged water and to 10.75 mg l^{-1} in the non-purged water.

Synthetic lake water medium was used in further experiments (C-4) in which CO_2 was removed by acidifying and N_2-purging the distilled water prior to adding the salts, chelator, and buffer; final pH was 8.1 with no adjustment necessary. Duplicated assays were performed simultaneously, one with the CO_2-free medium (<0.5 mg CO_2 l^{-1}), and one with the same medium containing 150.5 mg CO_2 l^{-1} added as $NaHCO_3$ and as atmospheric CO_2. Equivalent NaCl was added to the CO_2-free medium to balance Na content. Oxygen was adjusted to 8.14 in both media. All chambers were incubated in the light for 30 minutes, followed by darkness for 25 minutes. Other experimental conditions in the experiments were constant and are given in Table 2.

Comparison of Effects of Presence and Absence of Epiphytic Microflora

Axenic cultures of Najas flexilis were inoculated with axenic Gomphonema parvulum Kutz., an alga which is a common epiphytic diatom in Lawrence Lake, obtained as an axenic culture from the Indiana University algal culture collection. Epiphytic growth of the diatoms was allowed to develop in the N. flexilis cultures in the controlled environmental chamber for about 4 weeks, at which time cultures still bacteria-free (tested by nutrient broth inoculation and microscopic examination) were assayed for photorespiration and organic carbon release, along with axenic N. flexilis control cultures of the same age. Experimental and control chambers, all receiving low CO_2 synthetic medium from a single reservoir, were operated in the light for 30 minutes followed by darkness for 25 minutes.

Axenic N. flexilis cultures also were inoculated with material scraped from the surface of natural plants in Lawrence Lake to establish a mixed microbial epiphytic growth. After about 3 weeks, microscopic examination revealed dense epiphytic growth of bacteria and fungi of many kinds, including motile and non-motile unicells, short chains, and long filaments. No identifications were attempted. Very few diatoms and other algae were present. Experimental cultures and axenic control cultures of the same age were assayed for respiration and organic carbon

release simultaneously in the light for 30 minutes, followed by darkness for 25 minutes. Environmental conditions in both experiments are given in Table 3.

Characterization of Photosynthetic Type

First ^{14}C Fixation Products

Procedures for analysis of the ^{14}C fixation products were essentially those developed by Benson, et al. (1950), with the modification of Laing and Forde (1971) in which chromatographic separation is limited to one solvent system (butan-1-ol-propionic acid-water), which effectively separates the initial C_3 and C_4 ^{14}C products when not confounded by products of subsequent metabolic transformations.

In each analysis, several axenic Najas flexilis cultures were combined, with a total dry weight of about 200 mg, and incubated for 10-15 seconds in 300 ml synthetic growth medium containing 1.5 $\mu Ci\ ml^{-1}$ at 4850 lux (22 C). The plant material was then quickly rinsed in distilled water and placed in 150 ml boiling 80% ethanol for 15 minutes to halt photosynthesis and to extract organic products. The extract was reduced in volume to 2 ml by flash evaporation at 38 C, and spotted in 2, 5, or 10 µl quantities on Whatman No. 1 chromatographic paper adjacent to, and in combination with, ^{14}C marker standards of glucose-6-phosphate, representing the C_3 phosphate esters, and malic

and aspartic acids, representing the first C_4 products. Duplicate chromatograms were prepared for each extract obtained. The paper was subjected to one-way descending chromatography, using a butan-1-ol-propionic acid-water (10:5:7, v/v/v) solvent system. Positions of radioactive materials were identified by autoradiography (Kodak RPR medical X-ray film; two-week exposure).

Several analyses were carried out with N. flexilis cultures. Subsequently, samples of Scirpus subterminalis were collected from Lawrence Lake and analysed similarly, using filtered lake water as the labeling medium. Epiphytic material was stripped from the samples as thoroughly as possible with cheesecloth and cold running tap water.

Analysis of Leaf Cross Sections

Fresh thin cross sections of leaves of axenic and natural N. flexilis and of natural S. subterminalis were obtained with a Hooker plant microtome (Lab-Line/Hooker; prototype unit used). Using starch accumulation, identified by iodine-potassium iodide staining, as an index of C_3 metabolism, the fresh sections were examined in wet mounts for presence or absence of C_4 anatomical system under a Zeiss phase contrast microscope. Photomicrographs were taken concurrently with visual observations.

In situ Field Analyses of Photorespiration and Organic Carbon Release

General Procedures

For *in situ* analyses of respiration and organic carbon release, four flow-through chambers were clamped vertically in parallel on a tubular aluminum frame. Lake water was used directly as the medium; each chamber intake port was equipped with a filter unit containing a disc of Nitex plankton netting (diam. 20 mm; mesh size 75 μm) to exclude potentially clogging plankton and debris. Sufficient lengths of tubing were attached to the outflow ports to reach the pump and collection bottles at the surface of the lake from the experimental depth.

All field experiments were performed in the littoral zone of Lawrence Lake, described above. In summer and fall, a small boat was used; the bow was moored against the shore, and the stern, from which the respiration chambers were lowered, was anchored over 1 meter of water. In winter equipment was operated through the ice.

Plants were carefully removed from the bottom sediment with roots intact, and prelabeled *in situ* in stoppered flasks containing lake water and $NaH^{14}CO_3$. Plants were then thoroughly rinsed in fresh lake water and placed in the flow-through chambers. In all manipulations, exposure of the plants to direct sunlight was avoided. The chambers were lowered to the site of natural growth, and the peristaltic

pump was activated. Electricity (110V AC) was provided by a portable gasoline-powered generator. Five-minute effluent fractions were collected as usual; after 30 minutes the rack was raised briefly to pull the dark jackets into place and returned to depth for the remaining 25 minutes. Plants and fractions then were returned to the laboratory for radioassay.

After the plants were lyophilized, prior to combustion and radioassay, encrusting material composed of $CaCO_3$ and ephiphytic microflora was removed from the plant surfaces. On three occasions this material was weighed and radioassayed in the same manner as were the plants. A portion of the material was exposed to concentrated HCl fumes to remove carbonates, allowing radioassay of residual organic carbon.

During each experiment, environmental conditions were determined as follows: Temperature was measured with a YSI tele-thermometer with underwater probe. Light intensity at depth was measured as per cent intensity at the surface with an underwater photometer designed by Rich and Wetzel (1969); absolute incident light intensity at the surface was measured with a Weston illumination meter (Model 756). Water samples were collected in a Van Dorn sampler for determination of pH, total inorganic carbon, and oxygen (techniques as described above for laboratory experiments).

Najas flexilis

The initial in situ analyses of photorespiration and organic carbon release in N. flexilis were performed in quadruplicate on 28 July 1973 at noon. Plants of similar size and appearance were selected from a healthy bed of N. flexilis at 0.5 m in depth. The weather was warm and clear, with 0 wind and 0% cloud cover.

On 15 September 1972 three additional in situ assays were performed in duplicate; one in mid-morning, one in early afternoon and one in late afternoon. Technical difficulties prevented inclusion of a dark period in the morning assay. Plants were in early stages of senescence, consistent with annuals at this time of year, so that careful handling was necessary to avoid fragmentation. The weather was cool and clear, with a light breeze and intermittent (25%) cloud cover.

Environmental conditions during all analyses are presented in Table 3.

Scirpus subterminalis

Photorespiration and organic carbon release in situ in S. subterminalis were assayed initially in quadruplicate on 14 October 1972 in mid-afternoon. Four plants were selected from a healthy bed at 1 m depth. Epiphytic growth was removed as much as possible from two of them by gently stripping them with wet cheese cloth until the brown diatomaceous material no longer appeared on the cloth; the

remaining two plants were left with epiphytic microflora intact. The weather was cool and clear, with 0 wind and 10% cloud cover.

On 28 February 1973 assays were performed with S. subterminalis in quadruplicate at 1 m depth under 21 cm ice cover. Loosely attached epiphytic material was removed from the plants by hand-stripping. Weather was cloudy-bright with 10% cover.

Photorespiration assays of S. subterminalis were performed with flow-through chambers 30 cm in length and 220 ml in volume.

RESULTS

Light and Dark Respiration and Organic Carbon Release in Axenic Najas flexilis

Influence of Dissolved Oxygen

Cumulative CO_2 release from axenic Najas flexilis generally was enhanced by increased dissolved oxygen concentration in the light. In Figure 3, the lowest rate of CO_2 loss in the light is at < 5 mg O_2 l^{-1}; CO_2 loss in the dark was relatively independent of O_2 concentration, except at 21 mg O_2 l^{-1}, where it appeared somewhat depressed. Figure 4 indicates a similar effect of oxygen on CO_2 loss in the light. Experiments in which each plant was assayed both in the light and dark (Figure 5) also resulted in O_2-enhancement of CO_2 loss in the light but not in the dark. The response of respiration in the light to sudden increase from very low $[O_2]$ to very high $[O_2]$ was inducible within a few minutes (Figure 6).

Cumulative CO_2 release vs. time in each experiment (Figures 3-6) was subjected to linear regression and slopes used to express rate of CO_2 release in terms of % initial internal ^{14}C released per hour (Table 1). Correlation of rates in light and dark with dissolved O_2 concentration (Figure 7; includes data from the O_2 series and from control

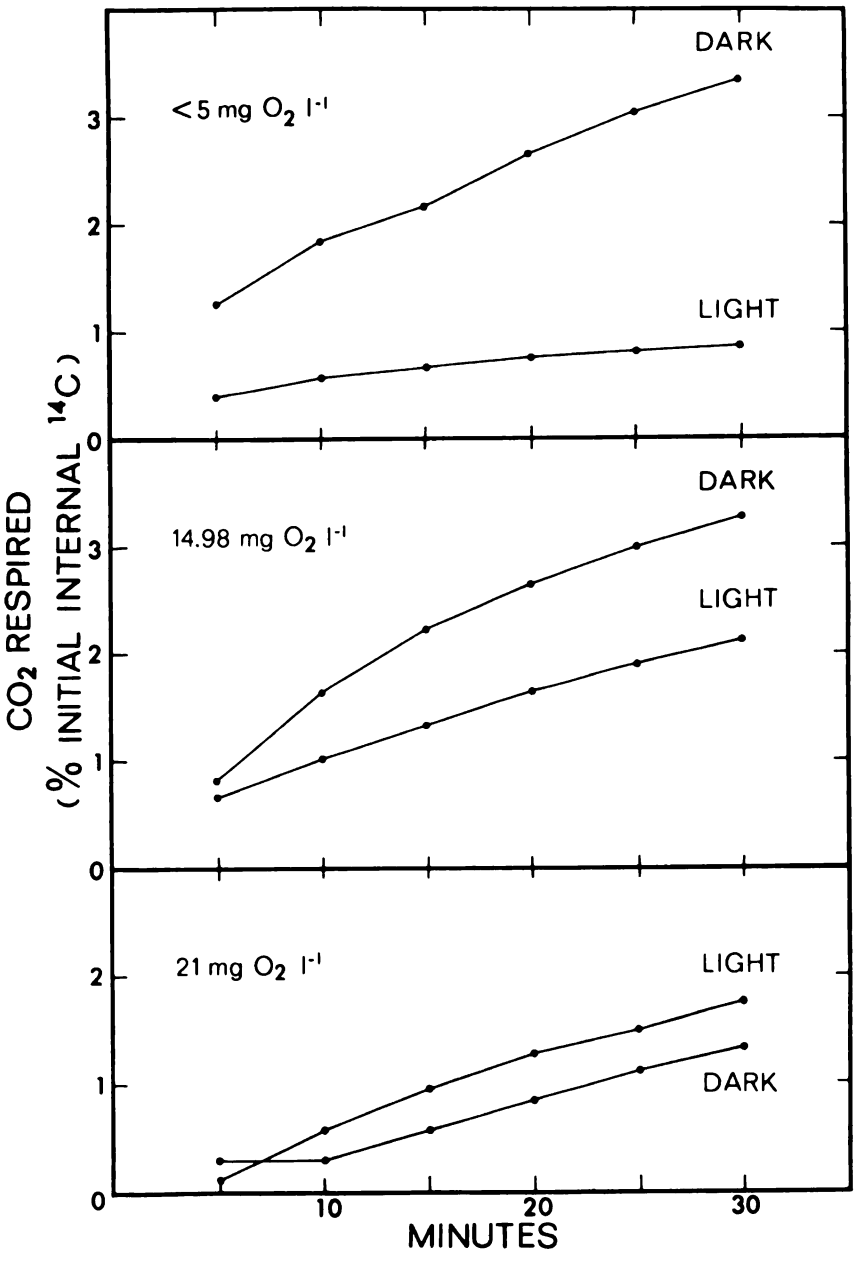


Figure 3. Cumulative carbon dioxide release from axenic Najas flexilis in light and dark in relation to dissolved oxygen concentration. Separate light and dark analyses performed simultaneously at each oxygen concentration.

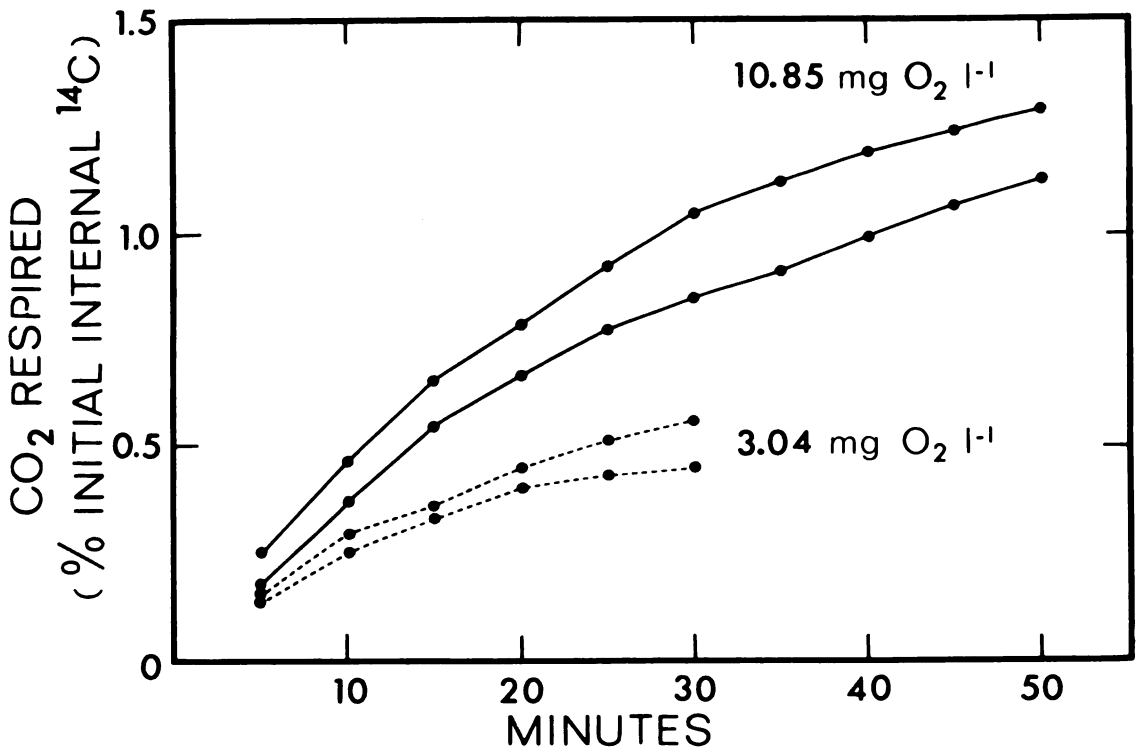


Figure 4. Cumulative carbon dioxide release from axenic Najas flexilis in the light at medium and low dissolved oxygen concentrations. Lines represent duplicate analyses performed simultaneously at each oxygen concentration.

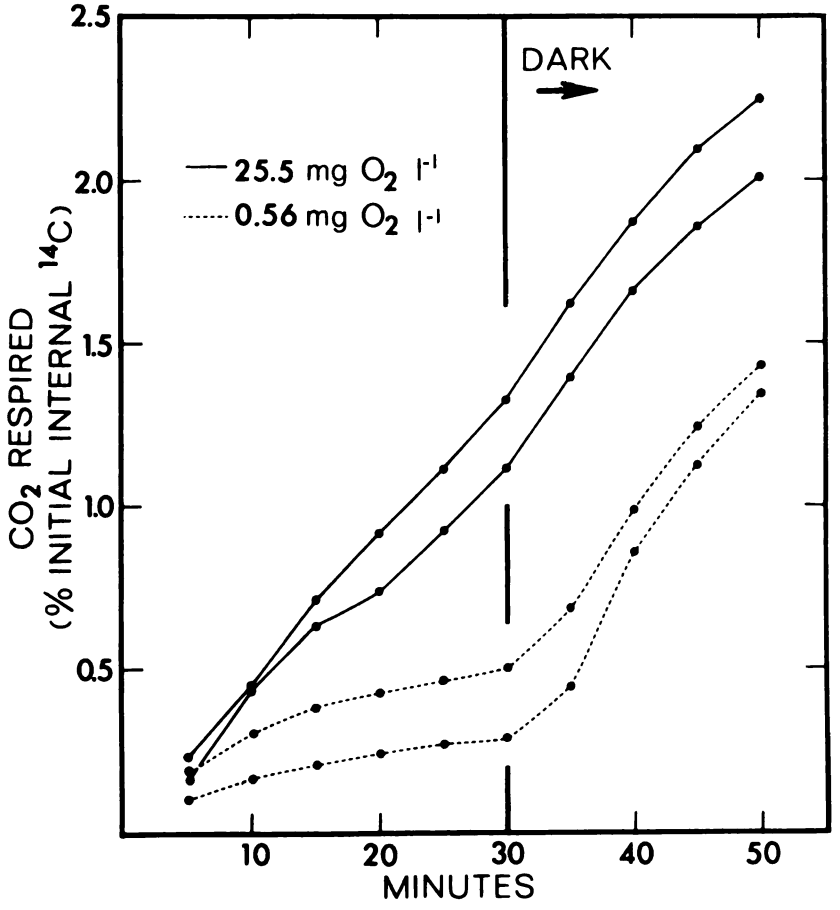


Figure 5. Cumulative carbon dioxide release from axenic Najas flexilis in light and dark at high and low dissolved oxygen concentrations. Lines represent duplicate analyses performed simultaneously at each oxygen concentration; all plants in light and then in dark.

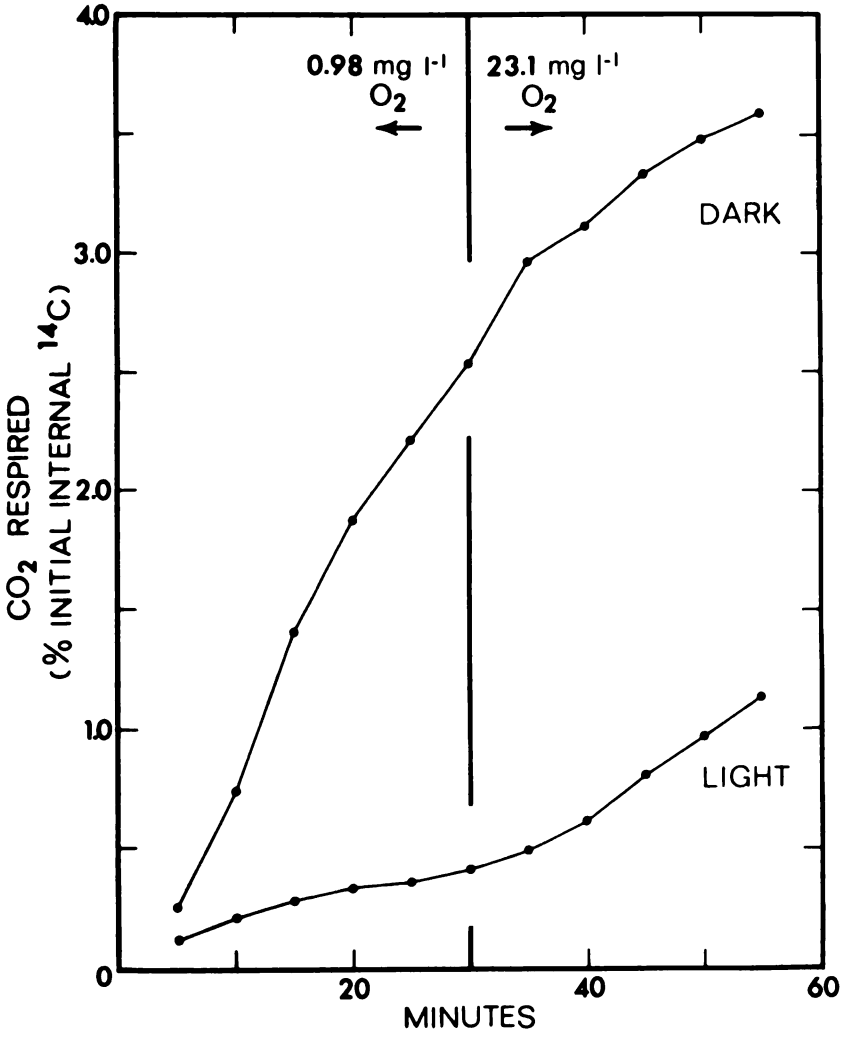


Figure 6. Cumulative carbon dioxide release from axenic Najas flexilis in light and dark at high and low dissolved oxygen concentrations. Separate light and dark analyses performed simultaneously, each at low and then high oxygen concentration.

Table 1. Relationship of net photorespiration, dark respiration, and organic carbon release to dissolved oxygen concentration in axenic Najas flexilis. Determined as % initial internal ^{14}C evolved per hour from prelabeled plants (2150 lux, 22 C, pH 8.1 - 8.2, 0.7 mg CO_2 l^{-1}, flow rate 20 ml min^{-1}).

Plant Dry Wt. mg	Prelabel ^{14}C concn μ Ci ml^{-1}	^{14}C Fix. μ Ci g^{-1} hr^{-1}	O_2 Concn mg l^{-1}	Respiration			Organic Carbon	
				Light % hr^{-1}	Dark % hr^{-1}	L : D ratio	Light % hr^{-1}	Dark % hr^{-1}
60.98	1.181	108.51	0.56	0.48	3.36	0.14	0.46	1.17*
70.21	1.181	83.37	0.56	0.86	2.90	0.29	0.09	0.70*
53.39	1.181	91.15	0.98	0.67	--	0.12	0.14	--
58.16	1.181	118.03	0.98	--	5.59		--	1.35
61.92	0.673	93.42	<5	1.12	--	0.22	1.08	--
51.40	0.673	49.98	<5	--	5.01		--	2.13
40.38	0.531	35.47	3.04	0.98	--	--	1.20	--
35.36	0.531	38.47	3.04	0.71	--	--	1.38	--
82.72	1.181	113.04	10.85	1.22	--	--	0.09	--
68.45	1.181	112.28	10.85	1.91	--	--	0.12	--
60.36	0.531	25.63	14.90	3.53	--	0.61	3.33	--
71.31	0.531	69.58	14.90	--	5.74		--	2.34
65.84	0.531	40.32	21.12	3.91	--	1.07	1.90	--
50.19	0.531	29.80	21.12	--	3.16		--	3.12
53.39	1.181	91.15	23.12	1.94	--	1.04	0.04	--
58.16	1.181	118.03	23.12	--	1.87		--	0.16
63.08	1.181	96.80	25.50	2.66	2.79	0.90	0.24	1.60*
70.61	1.181	86.17	25.50	2.16	2.71	0.80	0.23	1.58*

* First 10 minutes in dark.

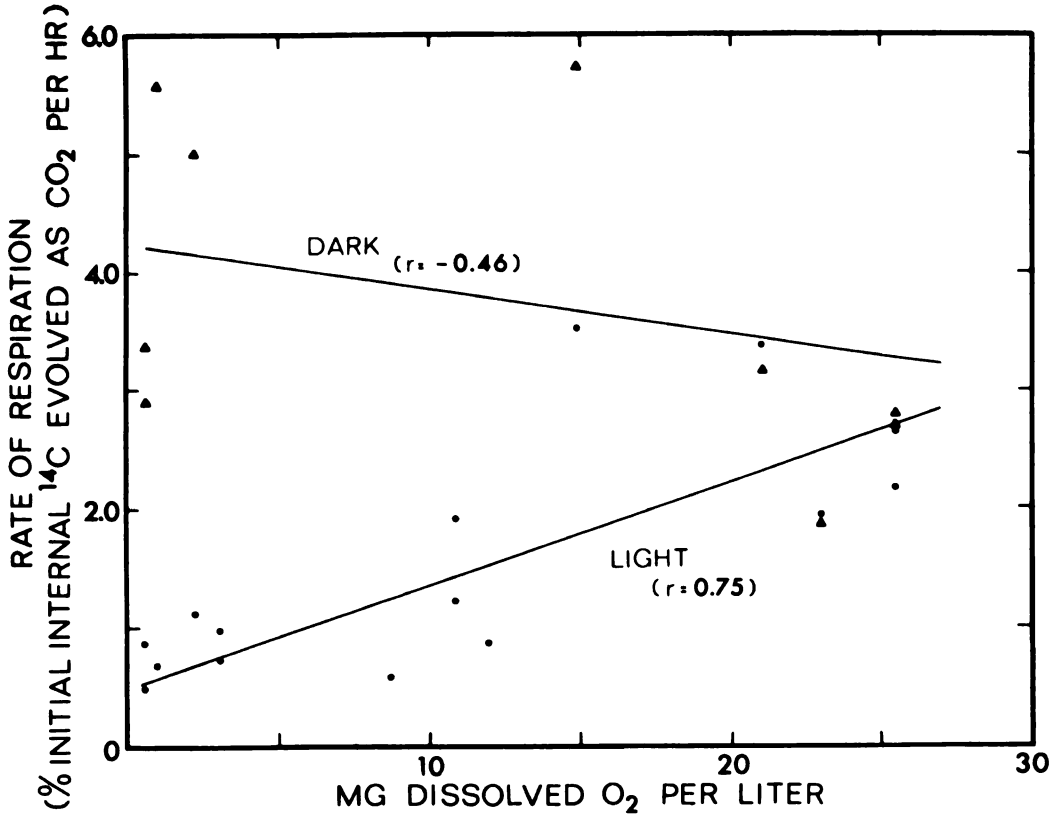


Figure 7. Relationship of rate of respiration in axenic Najas flexilis in light and dark to dissolved oxygen concentration (● = rates in light; ▲ = rates in dark; r = correlation coefficient).

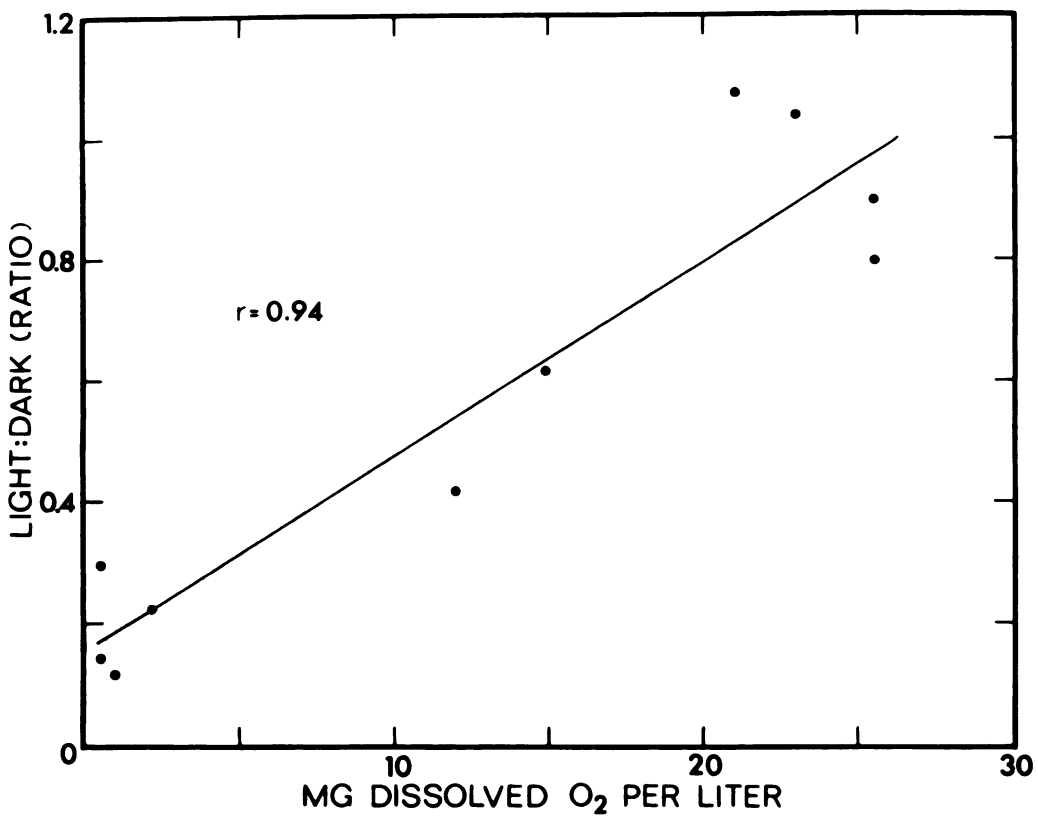


Figure 8. Relationship of light:dark respiration ratio in axenic Najas flexilis to dissolved oxygen concentration (r = correlation coefficient).

portions of subsequent experiments in which experimental conditions were identical to those in the O_2 series) indicated that respiration in the light was positively related to O_2 (r significant at $P<0.01$), with approximately 2-fold increase in respiration rate with 10-fold increase in O_2 concentration, whereas respiration in the dark was relatively unaffected by O_2; the appearance of a slight inverse correlation with O_2 was not significant.

Except at very high O_2 concentrations, rates of CO_2 release in the light were lower than in the dark, as reflected in the light to dark (L:D) ratios (Table 1). Light to dark ratios correlated positively with O_2 concentration (Figure 8; r significant at $P<0.01$), with maxima of about unity at highest O_2 concentrations.

Rates of release of organic carbon were calculated from regression slopes in the same manner as those of CO_2 release. Rates of release of organic carbon in each experiment generally were lower than rates of CO_2 release but varied widely from just slightly lower to over 10-fold lower than corresponding CO_2 release rates (Figures 9-13; Table 1). Organic carbon release in the dark tended to be more rapid than in the light. In particular, when plants were subjected to sudden darkness after an initial light period (Figure 12), rates of organic release increased immediately by more than 2-fold followed by a reduction to rates slightly greater than those in the light.

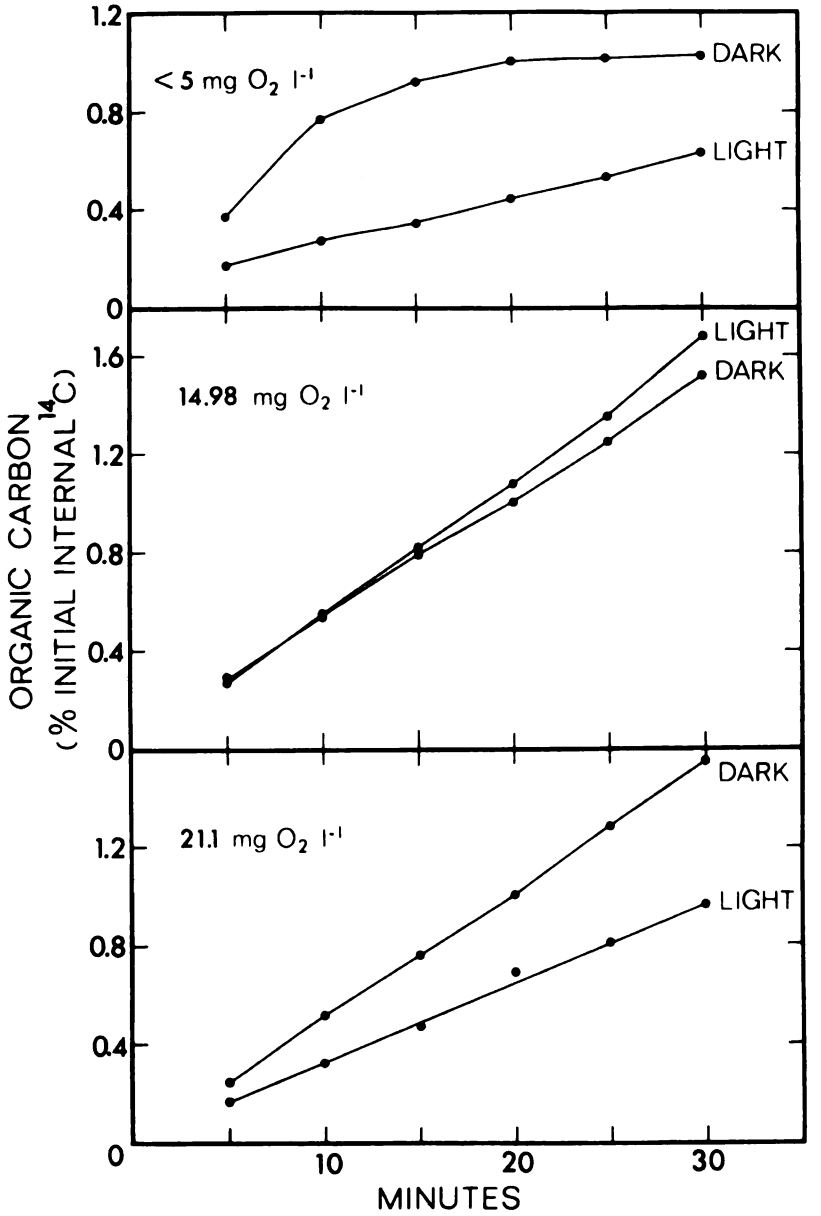


Figure 9. Cumulative organic carbon release from axenic Najas flexilis in light and dark in relation to dissolved oxygen concentration. Separate light and dark analyses performed simultaneously at each oxygen concentration.

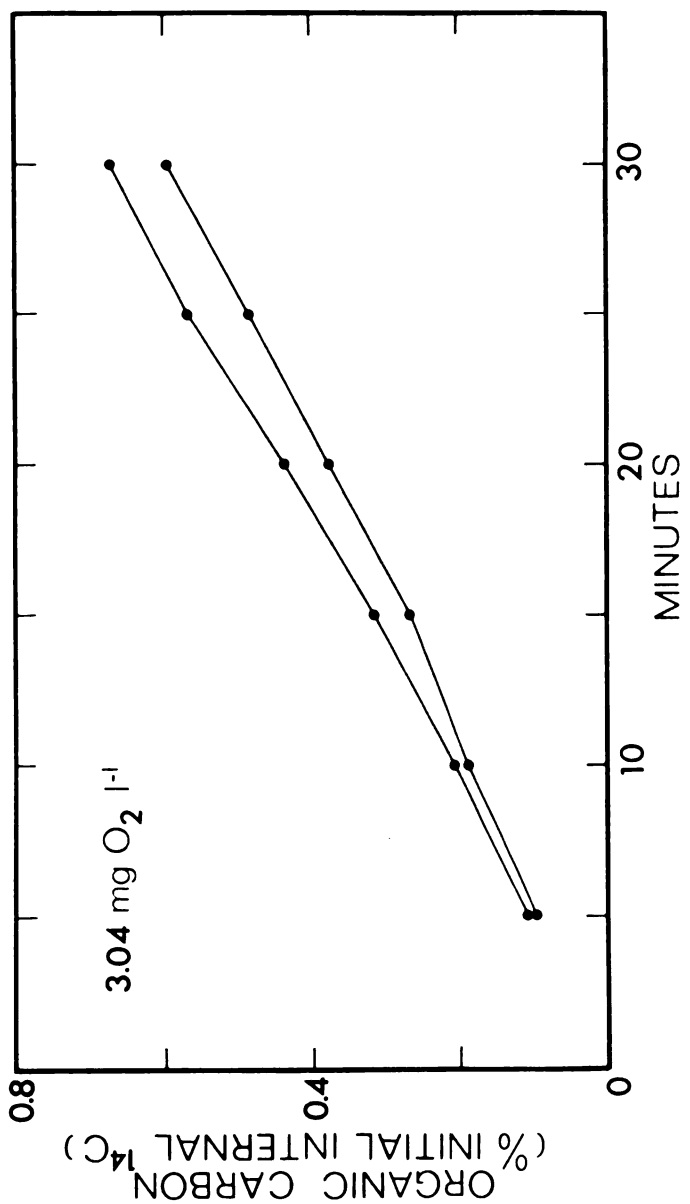


Figure 10. Cumulative organic carbon release from axenic Najas flexilis in the light at low dissolved oxygen concentration. Duplicate analyses performed simultaneously.

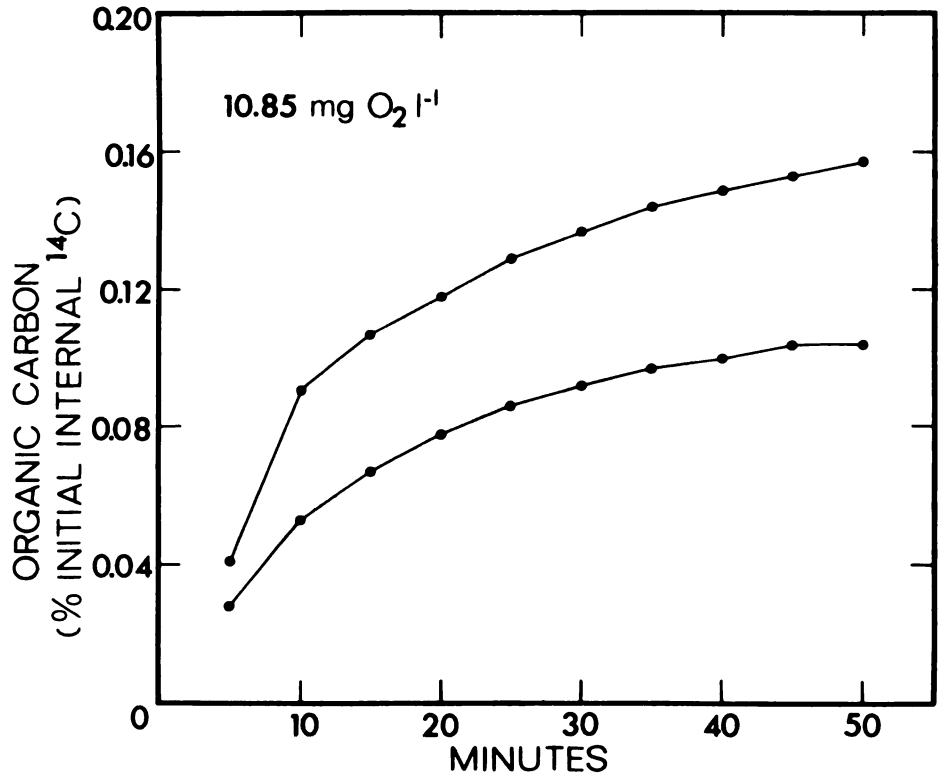


Figure 11. Cumulative organic carbon release from axenic Najas flexilis in the light at medium dissolved oxygen concentration. Duplicate analyses performed simultaneously.

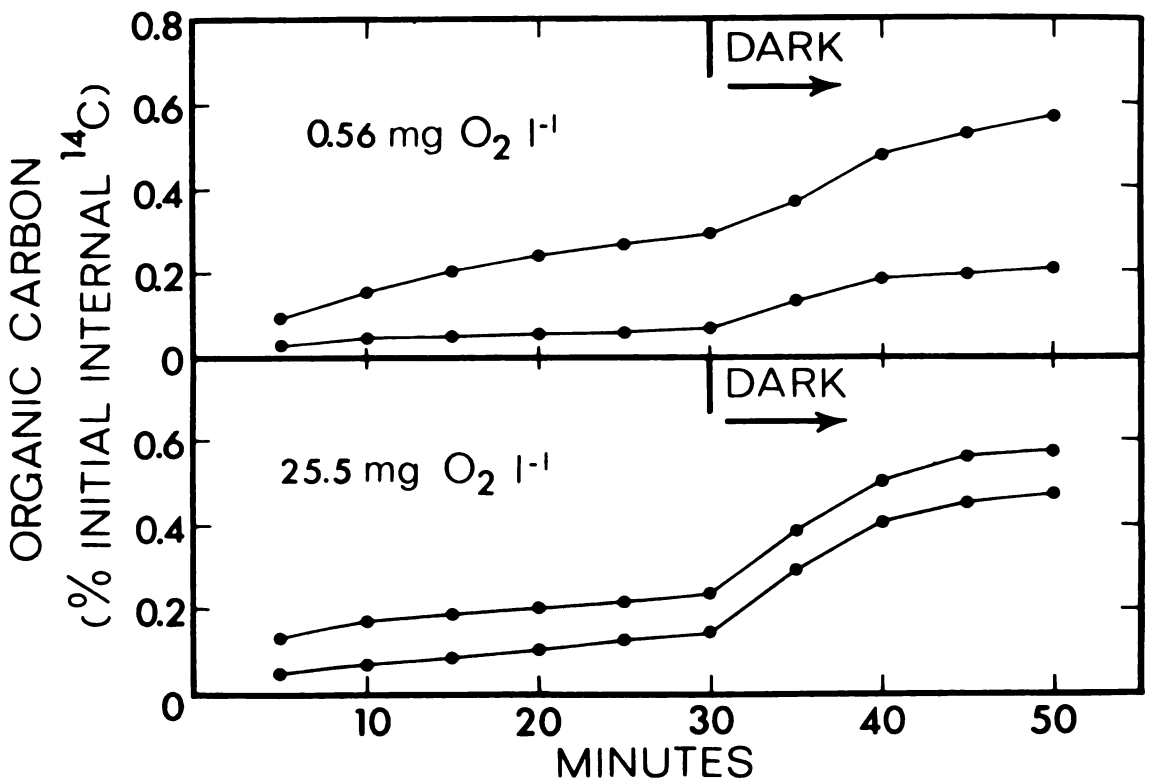


Figure 12. Cumulative organic carbon release from axenic Najas flexilis in light and dark at high and low dissolved oxygen concentrations. Duplicate analyses performed simultaneously at each oxygen concentration; all plants subjected to both light and dark.

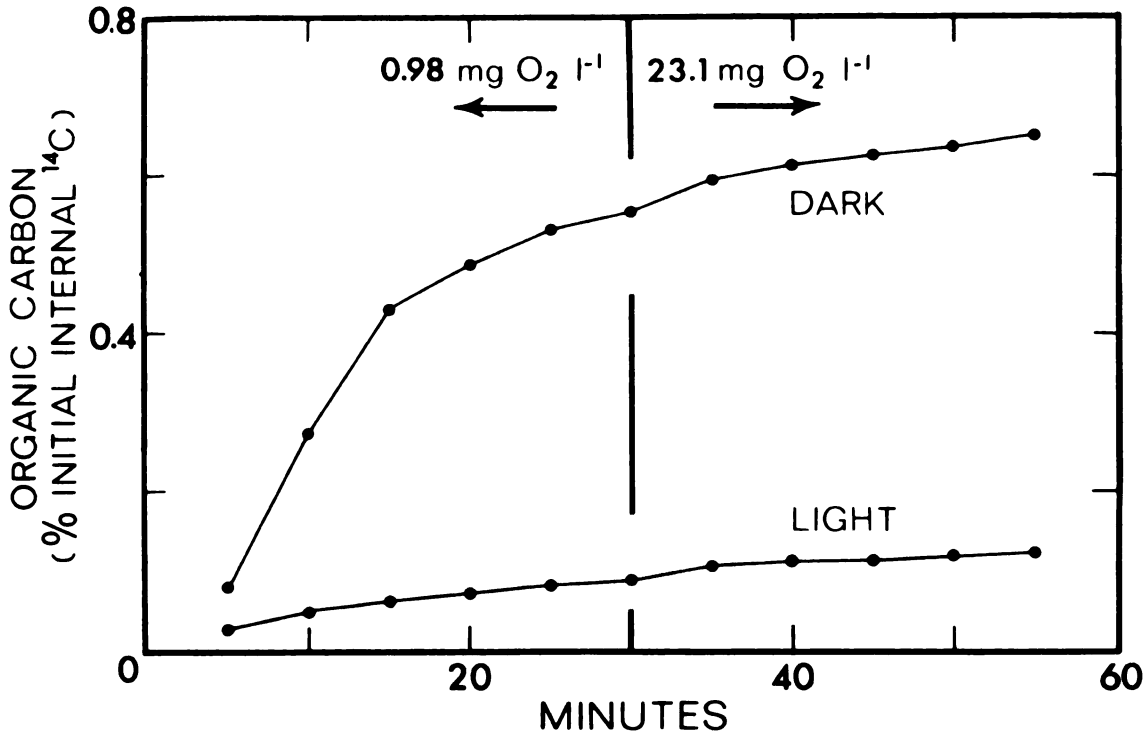


Figure 13. Cumulative organic carbon release from axenic Najas flexilis in light and dark at high and low dissolved oxygen concentrations. Separate light and dark analyses performed simultaneously, each at low and then high oxygen concentration.

Neither rate of release in light nor that in dark corresponded consistently to dissolved oxygen concentration in the experiments (Table 1), although the transient rapid increase in sudden darkness was somewhat greater at 25 mg O_2 l^{-1} than at 0.56 mg O_2 l^{-1} (Figure 12, Table 1).

Influence of Carbon Dioxide

In the first analysis (C-1) using synthetic media, respiration in the light was similar at low and high CO_2 concentrations (Figure 14; Table 2).

Respiration in the light was 30-40% lower at high $[CO_2]$ than at low $[CO_2]$ in experiments using filtered lake water (C-2 and C-3, Table 2); the slower rates appeared to develop 10-15 minutes after exposure to high $[CO_2]$ (Figures 15, 16). Dark respiration was not affected by change in $[CO_2]$.

In the final analyses (C-4), using synthetic medium, mean light respiration rates at high $[CO_2]$ were slightly lower than at low $[CO_2]$, but the same occurred in the dark, and L:D ratios were similar at both levels (Figure 17; Table 2).

Rates of release of organic carbon (Table 2) did not vary consistently with CO_2 concentration, showing contradictory responses including enhancement in high CO_2 (C-2 and C-4; Figures 19 and 21), decrease in high $[CO_2]$ (C-3; Figure 20), and little or no effect (C-1; Figure 18).

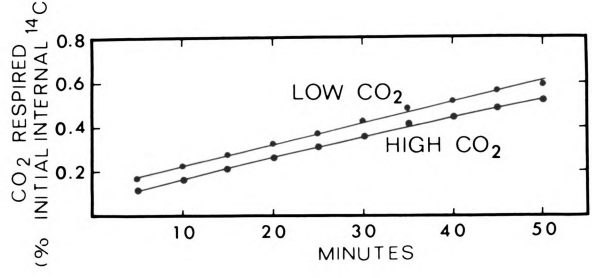


Figure 14. Cumulative carbon dioxide release from axenic Najas flexilis in the light in relation to total carbon dioxide concentration (high CO_2 = 9.5 mg l^{-1}; low CO_2 = 0.7 mg l^{-1}).

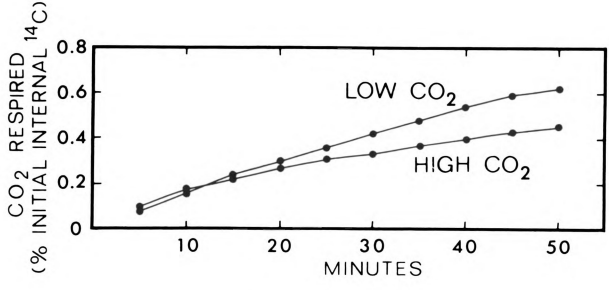


Figure 15. Cumulative carbon dioxide release from axenic Najas flexilis in the light in relation to total carbon dioxide concentration (high CO_2 = 185.15 mg l^{-1}; low CO_2 = <0.5 mg l^{-1}).

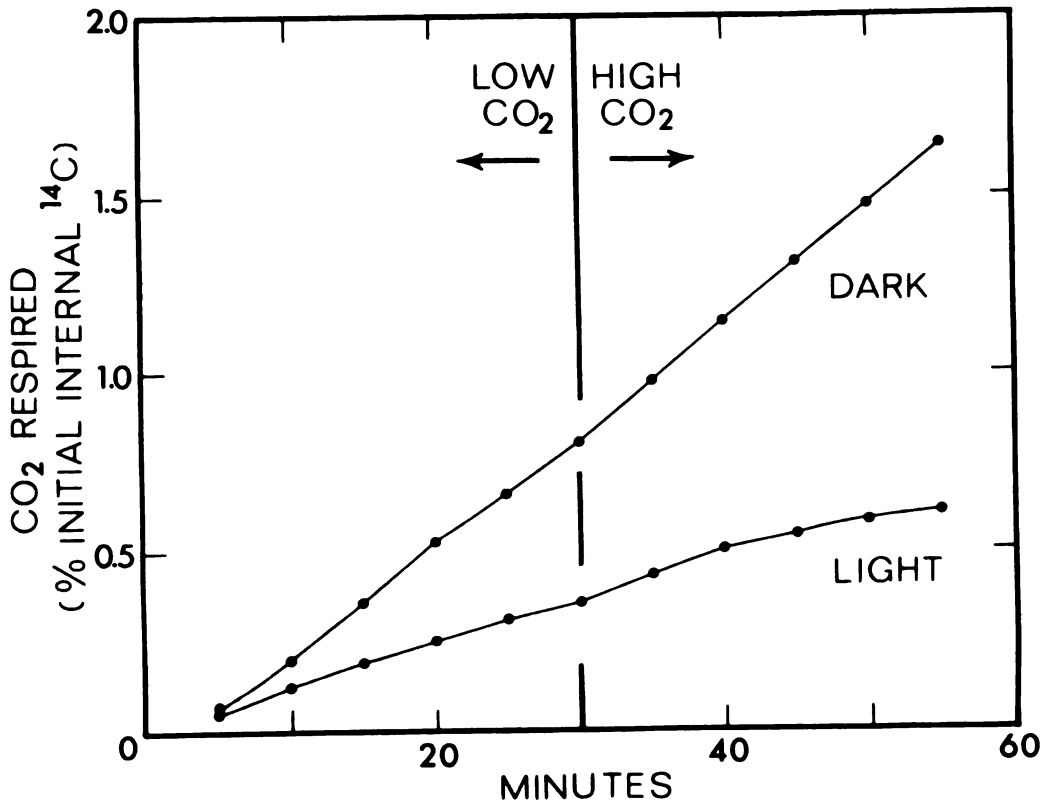


Figure 16. Cumulative carbon dioxide release from axenic Najas flexilis in light and dark in relation to total carbon dioxide concentration. Light and dark analyses performed separately, each at low and then high carbon dioxide concentration (high CO_2 = 186.74 mg l^{-1}; low CO_2= <0.5 mg l^{-1}).

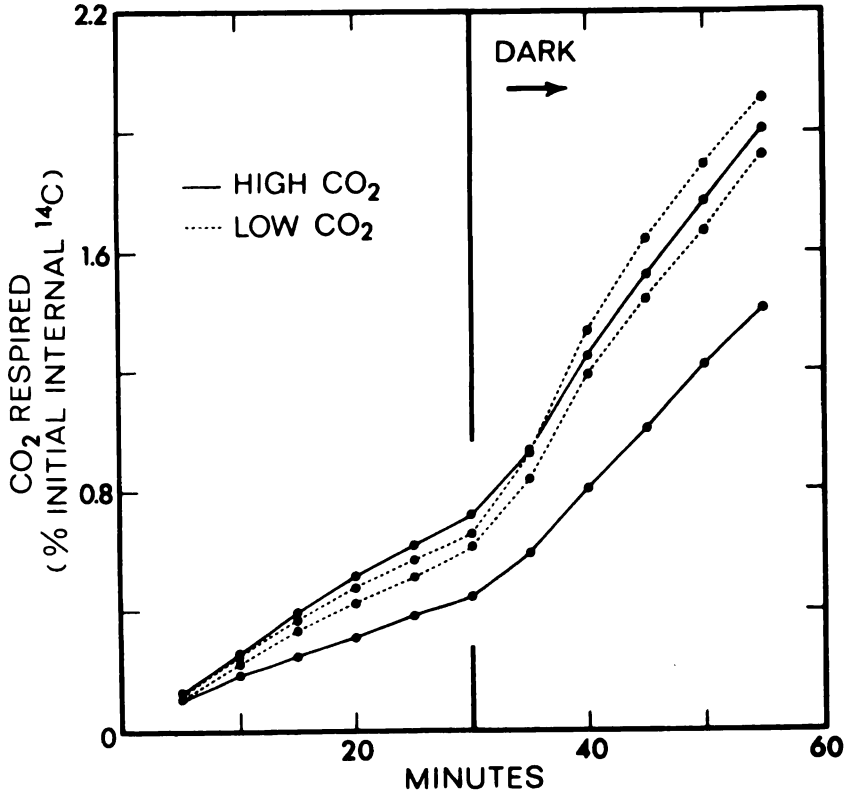


Figure 17. Cumulative carbon dioxide release from axenic Najas flexilis in light and dark in relation to carbon dioxide concentration. Duplicate analyses performed simultaneously at high and low carbon dioxide concentrations; all plants in light and then dark (high CO_2 = 150.5 mg l^{-1}; low CO_2 = <0.5 mg l^{-1}).

Table 2. Relationship of net photorespiration, dark respiration, and organic carbon release to total CO_2 concentration in axenic Najas flexilis. Determined as % initial internal ^{14}C evolved per hour from prelabeled plants, with mean ± SD of duplicate data (2150 lux*, 22 C, pH 8.9 - 8.2, flow rate 14-16 ml min^{-1}).

Experiment	Plant Dry Wt. mg	^{14}Fix.§ μ Ci g^{-1} hr^{-1}	O_2 Concn mg l^{-1}	CO_2 Concn mg l^{-1}	Respiration Light % hr^{-1}	Respiration Dark % hr^{-1}	Respiration L : D ratio	Organic Carbon Light % hr^{-1}	Organic Carbon Dark % hr^{-1}
C-1	64.26	49.00	8.66	0.7	0.57	--	--	0.14	--
	71.91	57.79	8.66	9.5	0.55	--	--	0.10	--
C-2	52.74	42.30	15.90	<0.5	0.74	--	--	0.05	--
	53.74	44.63	15.90	185.15	0.45	--	--	0.12	--
C-3	80.26	47.42	11.98	<0.5	0.76	--	0.40	0.29	--
	90.60	63.22	11.98	<0.5	--	1.81		--	0.35
	80.26	47.42	10.75	186.74	0.53	--	0.27	0.10	--
	90.60	63.22	10.74	186.74	--	1.99		--	0.09
C-4	58.24	26.70	19.60	<0.5	1.28	3.47	0.37	0.38	0.26
	54.84	21.74	19.60	<0.5	1.20	3.18	0.38	0.44	0.33
					1.24±.06	3.32±.20	0.37±.01	0.41±.04	0.29±.05
	49.47	39.41	19.94	150.5	0.82	2.47	0.33	0.61	0.35
	50.63	34.76	19.94	150.5	1.43	3.22	0.44	0.58	0.28
					1.12±.43	2.84±.53	0.39±.08	0.58±.02	0.31±.05

* 4842 lux in Expt. C-4.

§ Prelabel ^{14}C concn 0.587 μCi ml^{-1} in Expts. C-1, -2, -3; 0.729 μCi ml^{-1} in Expt. C-4.

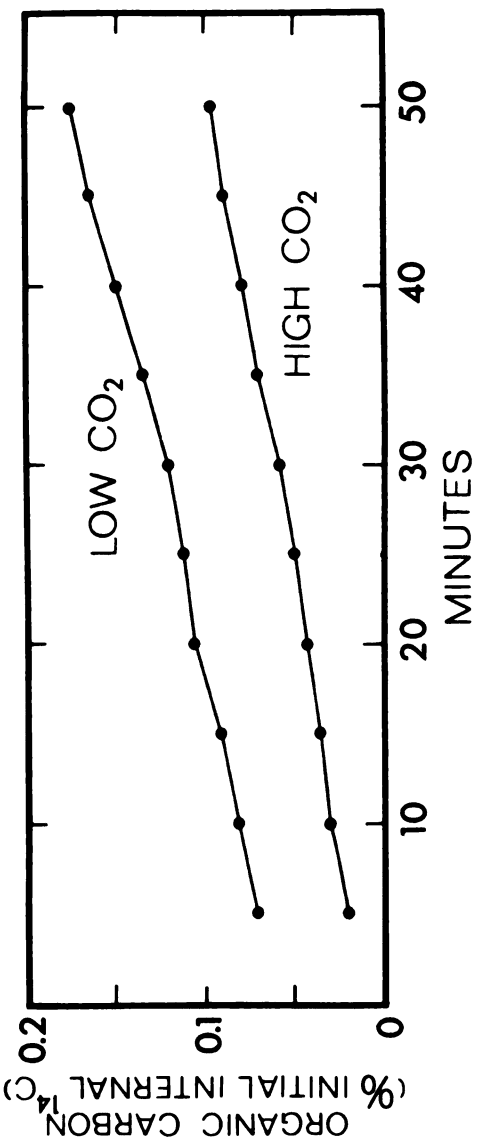


Figure 18. Cumulative organic carbon release from axenic Najas flexilis in the light in relation to total carbon dioxide concentration (high CO_2 = 9.5 mg l^{-1}; low CO_2 = 0.7 mg l^{-1}).

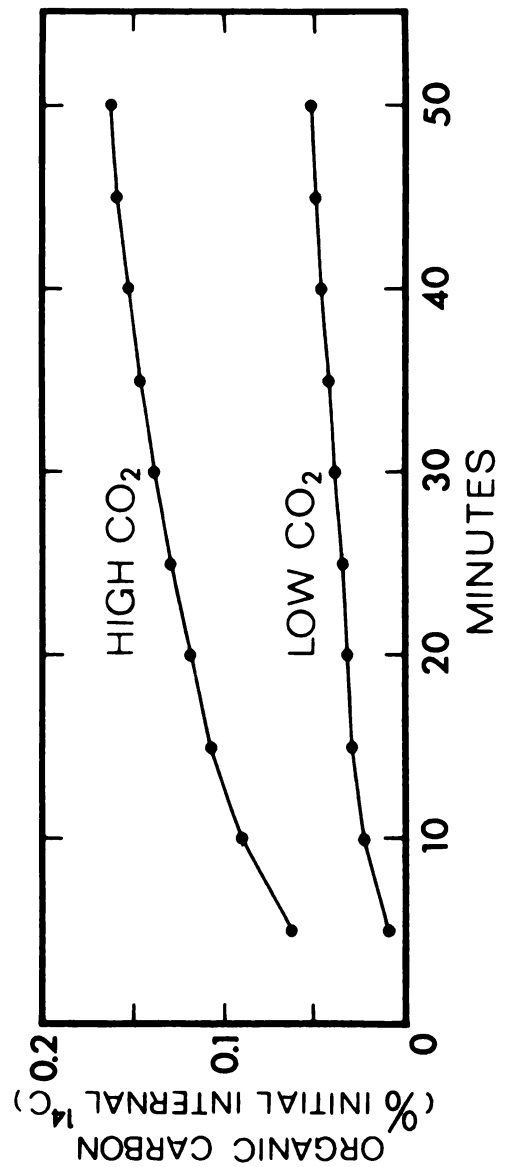


Figure 19. Cumulative organic carbon release from axenic Najas flexilis in the light in relation to total carbon dioxide concentration (high CO_2 = 185.15 mg l^{-1}; low CO_2 = <0.5 mg l^{-1}).

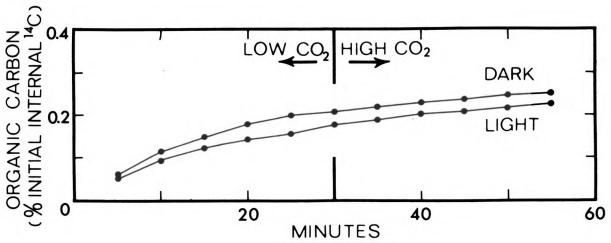


Figure 20. Cumulative organic carbon release from axenic Najas flexilis in light and dark in relation to total carbon dioxide concentration. Light and dark analyses performed separately, each at low and then high carbon dioxide concentration (high CO_2 = 186.74 mg l^{-1}; low CO_2 = <0.5 mg l^{-1}).

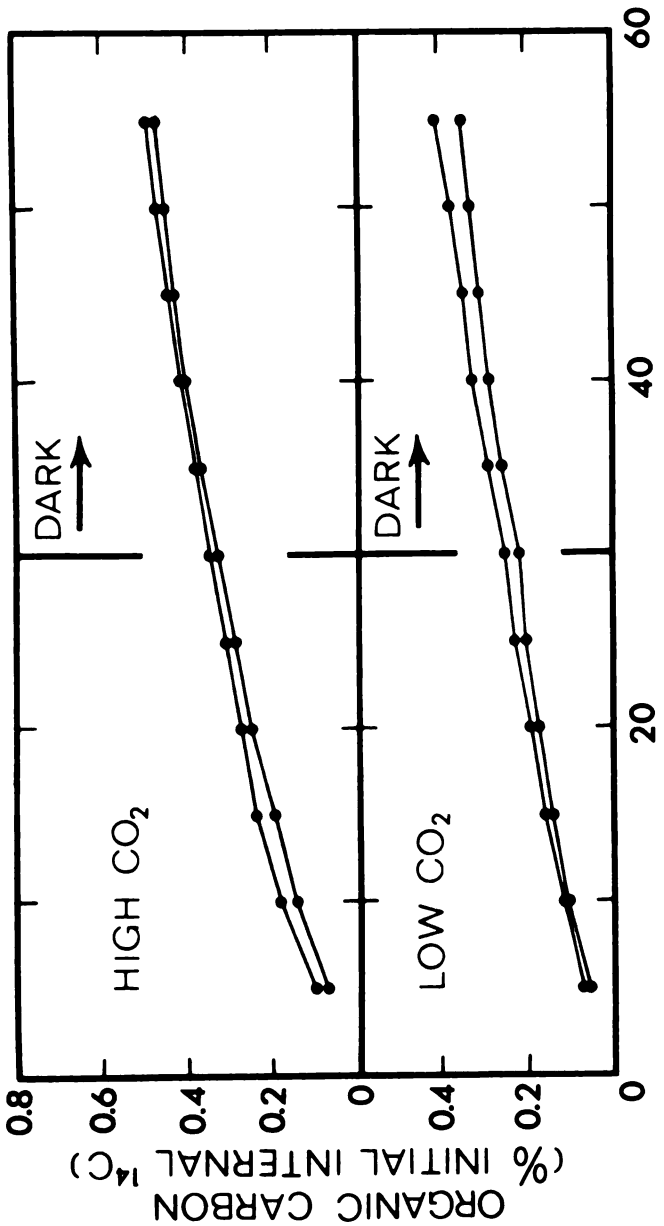


Figure 21. Cumulative organic carbon release from axenic Najas flexilis in light and dark in relation to total carbon dioxide concentration. Duplicate analyses performed simultaneously at high and low carbon dioxide concentrations; all plants in light and then dark (high CO_2 = 150.5 mg l^{-1}; low CO_2 = <0.5 mg l^{-1}).

Epiphytic Microflora

Mean rates of light respiration in plants with epiphytic diatoms (bacteria-free) were slightly higher than those in axenic controls; dark rates were slightly lower in presence of the diatoms than in controls. Consequently, mean L:D ratio in presence of diatoms was 2-fold greater than in axenic controls (Figure 22; Table 3).

Mean rates of light respiration in plants with a mixed epiphytic microflora (fungi, bacteria) were slightly higher than in axenic controls. Dark rates were also higher in presence of the microflora than in controls, with resulting identical L:D ratios (Figure 23; Table 3).

Mean rates of organic carbon release were about 2-fold higher in presence of epiphytic diatoms than in axenic controls both in light and in dark (Figure 24: Table 3).

Mean rates of organic carbon release in plants with a mixed epiphytic microflora were similar to those in axenic controls both in light and in dark, except for the first ten minutes of darkness, during which release rate was slightly lower in presence of microorganisms than in controls (Figure 25; Table 3).

Characterization of Photosynthetic Type

First ^{14}C Fixation Products

In radiochromatograms of 10-15 second ^{14}C fixation products of Najas flexilis, no detectable labeling appeared

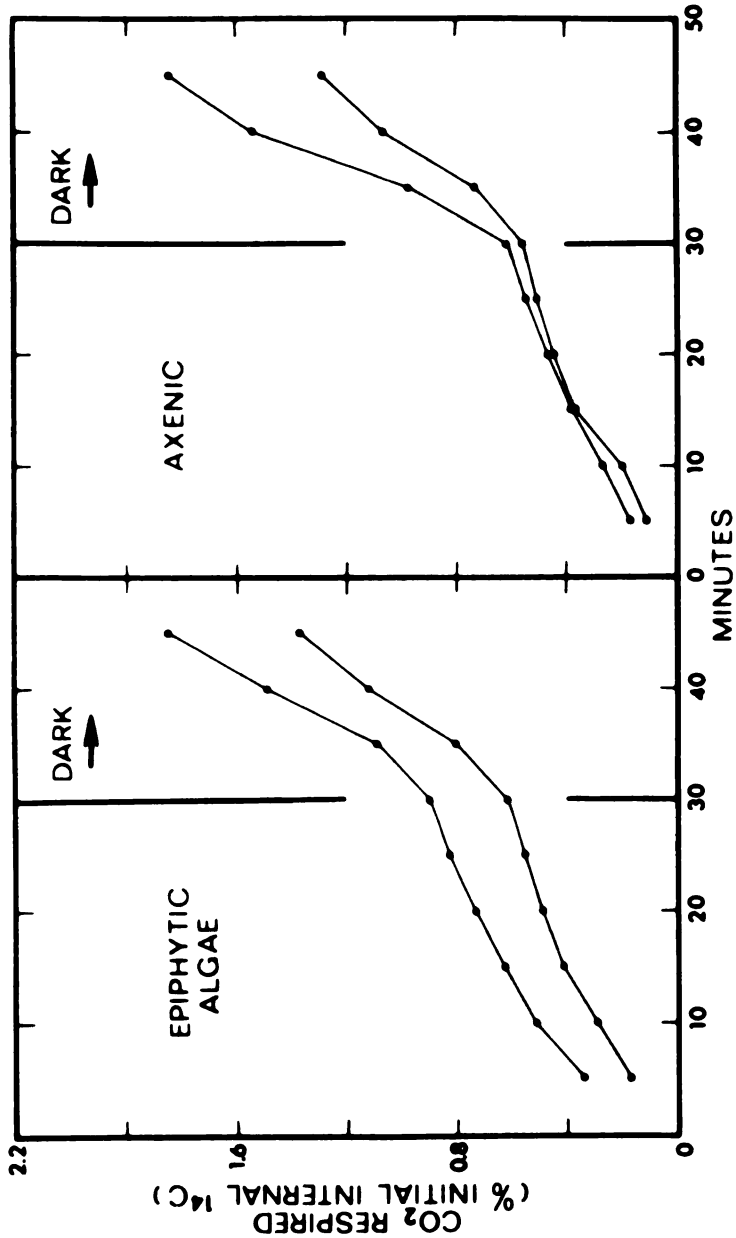


Figure 22. Cumulative carbon dioxide release from Najas flexilis in the light and dark in presence and absence of epiphytic diatom populations (bacteria-free Gomphonema parvulum).

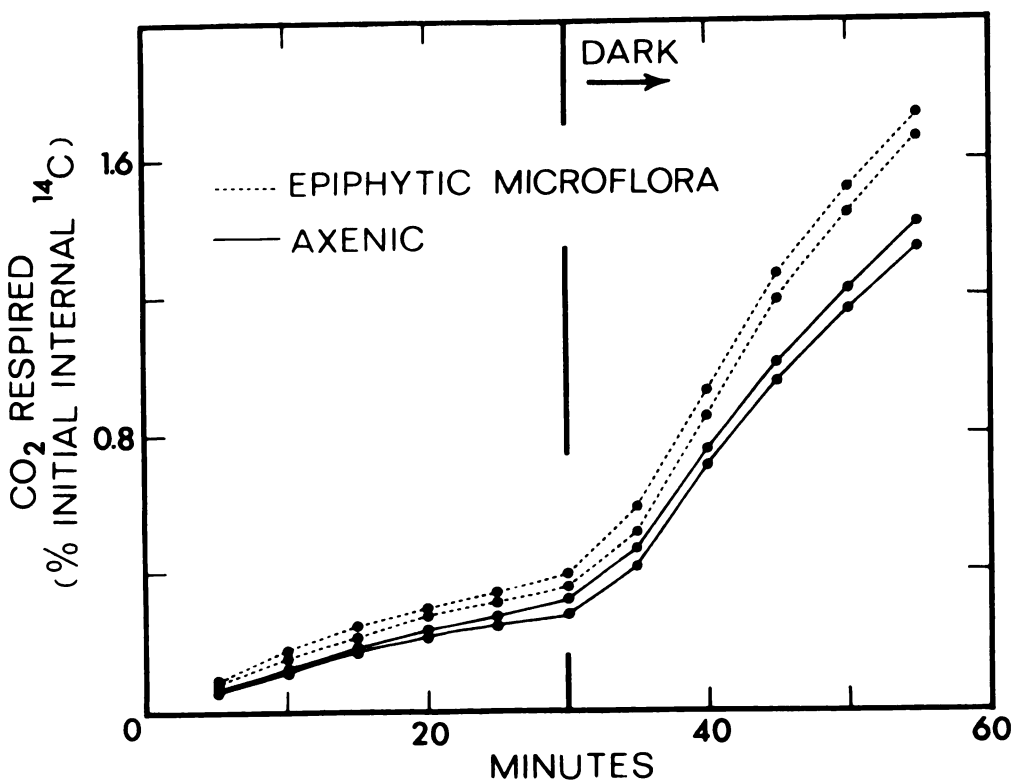


Figure 23. Cumulative carbon dioxide release from Najas flexilis in the light and dark in presence and absence of mixed epiphytic microflora (bacteria, fungi, algae).

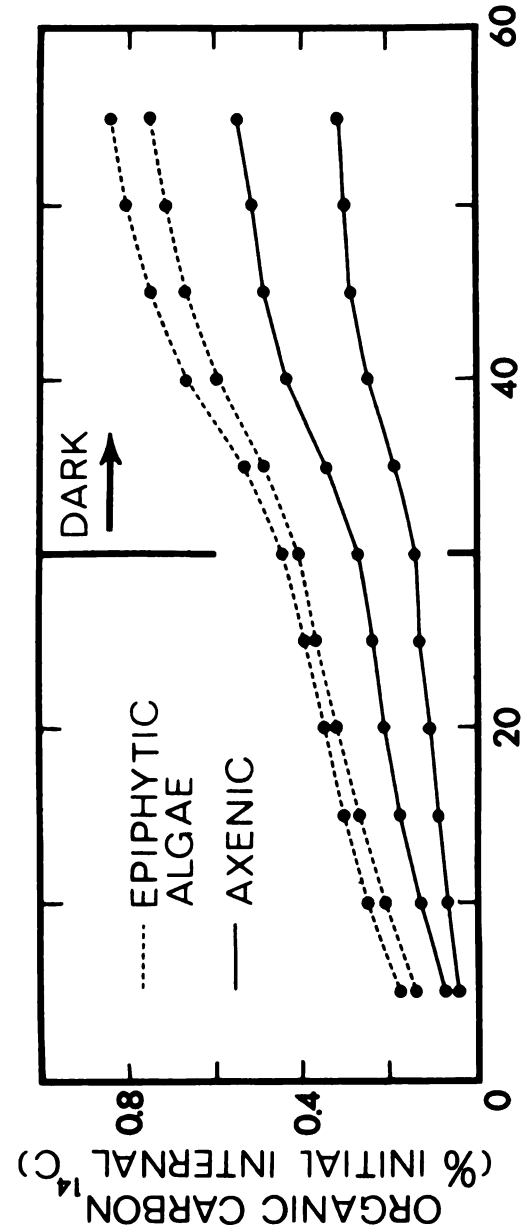


Figure 24. Cumulative organic carbon release from Najas flexilis in the light and dark in presence and absence of epiphytic diatom populations (bacteria-free Gomphonema parvulum).

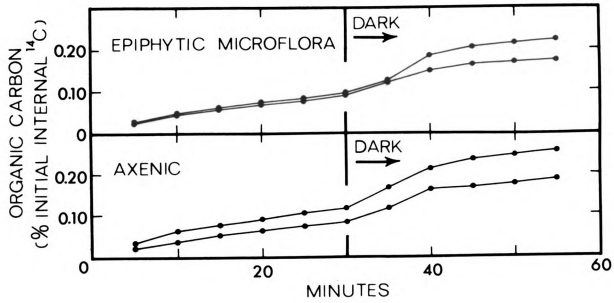


Figure 25. Cumulative organic carbon release from Najas flexilis in the light and dark in presence and absence of mixed epiphytic microflora (bacteria, fungi, algae).

Table 3. Net photorespiration, dark respiration, and organic carbon release in Najas flexilis in presence and absence of epiphytic microflora. Carbon dioxide and organic carbon release in light and dark expressed as % initial internal ^{14}C evolved per hour from prelabeled plants, with mean ± SD of duplicates (4842 lux, 23 C, pH 8.1 - 8.2, 0.7 mg CO_2 l^{-1}, flow rate 13-15 ml min^{-1}).

Plant Condition	Plant Dry Wt. mg	^{14}C Fix. μ Ci g^{-1} hr^{-1}	Respiration			Organic Carbon		
			Light % hr^{-1}	Dark % hr^{-1}	L : D ratio	Light % hr^{-1}	Dark[1] % hr^{-1}	Dark[2] % hr^{-1}
Axenic	31.48	14.41	1.09	5.24	0.21	0.46	1.06	0.41
	29.93	18.17	1.12	3.36	0.33	0.23	0.65	0.28
			1.10± .02	4.30±1.32	0.27± .09	0.34± .16	0.85± .29	0.34± .09
w/epi-phytes[3]	43.83	15.65	1.91	2.83	0.68	0.64	1.34	0.68
	40.18	18.13	1.07	2.56	0.42	0.64	1.14	0.64
			1.49± .59	2.69± .19	0.54± .18	0.64± .00	1.24± .14	0.66± .03
Axenic	54.50	24.56	0.52	2.78	0.19	0.19	0.57	0.17
	74.34	23.20	0.62	2.81	0.22	0.14	0.53	0.11
			0.57± .07	2.80± .02	0.20± .02	0.16± .04	0.55± .03	0.14± .04
w/epi-phytes[4]	61.10	26.95	0.71	3.44	0.21	0.16	0.37	0.08
	50.95	26.90	0.69	3.46	0.20	0.16	0.56	0.15
			0.70± .01	3.45± .01	0.20± .01	0.16± .00	0.46± .13	0.11± .05

[1] First 10 minutes in dark.
[2] After first 10 minutes in dark.
[3] Epiphytic diatom Gomphonema parvulum (bacteria free). Prelabel ^{14}C concn 0.587 μCi ml^{-1}.
[4] Bacteria, fungi, and algae. Prelabel ^{14}C concn 0.443 μCi ml^{-1}.

in the positions of malate and aspartate; all activity appeared as single or closely connected double spots of low R_f corresponding to positions of the C_3 phosphate-esters (Table 4). Proper separation of products was checked by co-chromatography with marker standards and a marker dye with samples.

Table 4. First ^{14}C fixation products of photosynthesis in Najas flexilis and Scirpus subterminalis. Radioactivity is expressed as a percentage of the total in all products.

	N. flexilis	S. subterminalis
C_3 P-esters	> 99%	> 90%
C4 Acids	- -	0-10% ?
Other	- -	0-10% ?

Scirpus subterminalis also initially fixed ^{14}C predominantly in compounds corresponding in R_f to C_3 phosphate-esters. A small amount of activity moved further to one or two positions of higher R_f; the identity of this material was not evident in the one-way system, but further characterization was considered unnecessary inasmuch as it was clearly not among the major first fixation products.

Analysis of Leaf Cross Sections

Cross sections of Najas flexilis (Figure 26) revealed a single, centrally located vascular bundle, surrounded by

WATCH for LHP

from

here on

Figure 26. Cross sections of leaves of natural Najas flexilis. a: Central portion of leaf section with single vascular bundle (v) and adjacent gas lacunae (l). b: Leaf section with iodine stained starch appearing as black bodies (left lateral portion of leaf folded under central portion as a result of sectioning process).

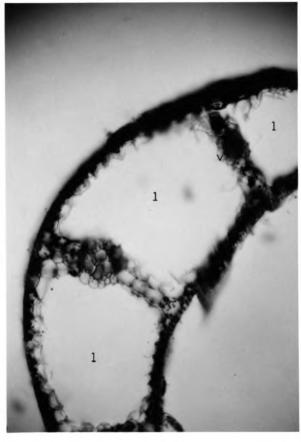
Figure 26.

a.

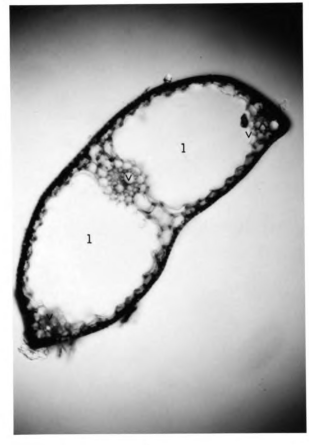
b.

Figure 27. Cross sections of leaves of Scirpus subterminalis. a: Section from mid-length of leaf. b: Section from near distal end of leaf. Both sections: gas lacunae (l) partitioned by septa containing vascular bundles (v). Iodine stained starch appears as black bodies.

Figure 27.



a.



b.

a single layer of large cells. Two moderately large gas lacunae are present adjacent to the vascular bundle, each also surrounded by a single layer of large cells. The remainder of the leaf consists of a double layer of small cells extending laterally from each gas lacuna. The cells surrounding the vascular bundle are not surrounded by extensive mesophyll tissue, nor do they resemble the highly developed bundle sheath cells of C_4 plants. Furthermore, starch production appears to occur substantially in all cells and is not largely restricted to bundle sheath cells as in C_4 plants.

Scirpus subterminalis has 3 to 5 vascular bundles Figure 27), also surrounded by a single layer of cells, and separated by large gas lacunae. The remainder of tissue consists of a single layer of small epidermal cells with an inner layer of large cells. As in Najas flexilis, the cells surrounding the vascular bundles are not highly differentiated, nor are they surrounded by mesophyll tissue. Also, starch production is almost entirely restricted to the epidermal cells.

In situ Studies

Najas flexilis

In situ rates of respiration in Najas flexilis at midday in July (Figure 28) were lower in the light than in dark, with rates and L:D ratios (Table 5) similar to those found

in axenic laboratory cultures at medium oxygen concentrations. Rates of organic carbon release (Figure 29: Table 5) were 1/3 to 1/6 respiration rates, generally similar to rates of organic release in axenic cultures, but were somewhat greater in light than in dark, contrary to results in axenic cultures.

In September, respiration rates in the light were greater than in the dark (Figure 30), with corresponding L:D ratios greatly exceeding unity (Table 5). Both light and dark rates exceeded those in July about 10-fold. The mean rate in the light in September was lowest in the morning and highest in the late afternoon (Figure 31). Mean rate of net carbon fixation was highest in the morning and lowest in the late afternoon. Dissolved oxygen concentration was lowest in the morning and rose slightly through the day, as did temperature. Light intensity was highest in early afternoon.

Rates of release of organic carbon in September (Figure 32) were about 3 to 6-fold those in July (Table 5). Rates were highest in early afternoon.

Epiphytic encrustation on Najas flexilis increased in dry weight by 2-fold from July to September (Table 6). Microscopic examination revealed diatoms and other algae and microorganisms in an amorphous matrix. Radioactivity in the material in September was also nearly 2-fold that in July. The relative distribution of ^{14}C in organic matter

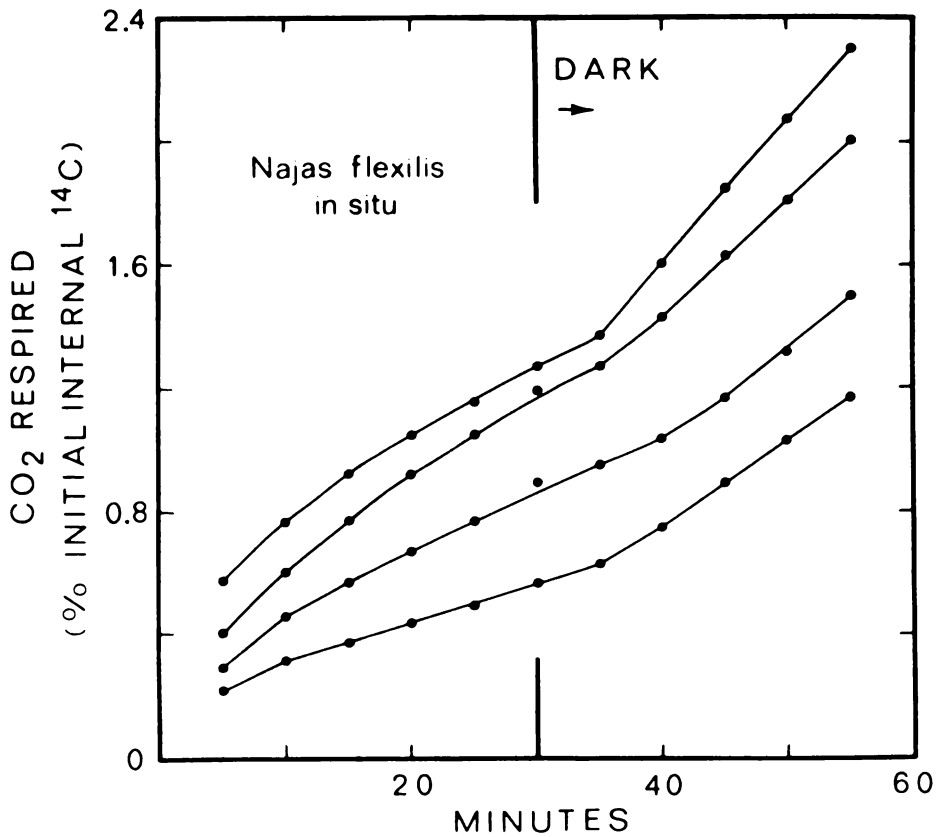


Figure 28. Cumulative carbon dioxide release from natural Najas flexilis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, at 1230 hrs on 28 July 72, 0.5 m depth (simultaneous quadruplicate analyses).

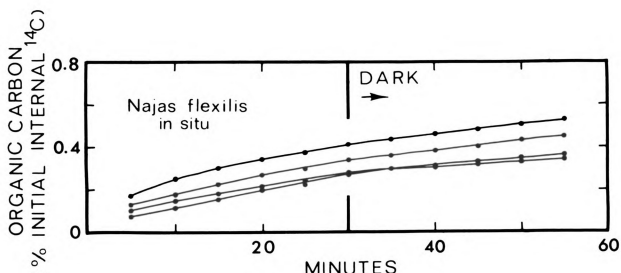


Figure 29. Cumulative organic carbon release from natural Najas flexilis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, at 1230 hrs on 28 July 72, 0.5 m depth (simultaneous quadruplicate analyses).

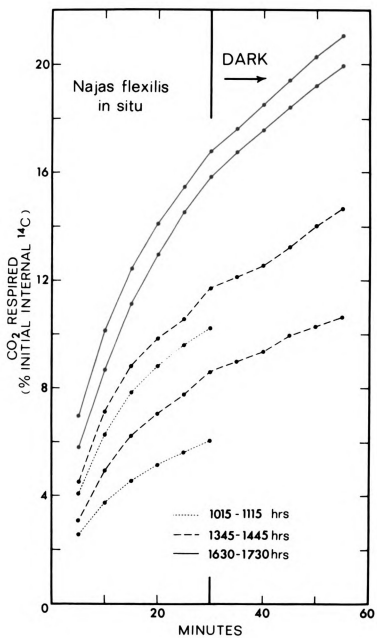


Figure 30. Cumulative carbon dioxide release from natural Najas flexilis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, 0.5 m depth. Duplicate analyses in mid-morning, mid-afternoon, and late afternoon on 15 September 72.

Table 5. *In situ* net photorespiration, dark respiration, and organic carbon release in natural *Najas flexilis* (0.5 m depth) and *Scirpus subterminalis* (1 m depth) in Lawrence Lake, Michigan. Carbon dioxide and organic carbon release in light and dark expressed as % initial internal ^{14}C evolved per hour from prelabeled plants, with mean ± SD of replicate data. Flow rate 15-16 ml min^{-1}.

Plant	Date	Time of Day hrs	Temp C	pH	Light Intens. lux	CO_2 Concn mg l^{-1}	O_2 Concn mg l^{-1}	Plant Dry Wt. mg	^{14}C Fix* μCi g^{-1} hr^{-1}	Respiration Light % hr^{-1}	Respiration Dark % hr^{-1}	Respiration L : D ratio	Organic Carbon Light % hr^{-1}	Organic Carbon Dark % hr^{-1}
Najas	28 Jul 72	1230	25.5	7.96	44590	168.87	8.65	83.69	19.88	1.86	2.56	0.73	0.50	0.28
								89.33	24.92	1.39	1.64	0.85	0.44	0.24
								72.07	40.59	0.81	1.65	0.49	0.38	0.16
								116.46	20.21	1.63	2.80	0.58	0.52	0.28
									26.40±9.74	1.42±0.45	2.16±0.61	0.66±0.16	0.46±0.006	0.24±0.006
Najas	15 Sep 72	1015	20.6	7.99	25153	140.62	8.69	150.60	10.07	14.68	--	--	1.30	--
								172.84	14.99	8.05	--	--	0.73	--
									12.53±3.48	11.36±4.68			1.02±0.40	
		1345	21.3	7.86	43261	138.34	8.88	131.74	9.69	16.25	7.74	2.10	2.13	1.25
								226.74	9.32	12.65	5.14	2.46	2.37	2.45
									9.50±0.26	14.45±2.54	6.44±1.84	2.28±0.26	2.25±0.17	1.85±0.85
		1630	21.6	8.31	7242	145.38	9.09	253.76	7.99	23.83	9.68	2.46	1.38	0.63
								204.18	6.11	22.94	10.05	2.28	1.11	0.52
									7.05±1.33	23.38±0.63	9.86±0.26	2.37±0.13	1.24±0.19	0.58±0.08
Scirpus	14 Oct 72	1440	13.9	8.04	19468	174.59	8.99	165.50	7.75	1.34	1.56	0.86	0.26	0.20
								114.21	9.80	1.45	1.32	1.10	0.30	0.19
								164.92	8.44	3.44	3.09	1.11	0.48	0.26
								157.68	8.30	0.97	0.91	1.07	0.29	0.17
									8.57±0.87	1.80±1.11	1.72±0.95	1.04±0.12	0.33±0.10	0.20±0.04
Scirpus	28 Feb 73	1140	3.3	8.33	6508	197.48	9.05	140.15	2.02	19.36	12.61	1.54	1.16	1.06
								158.56	2.77	14.53	7.43	1.96	1.03	0.54
								156.67	2.83	9.18	6.34	1.45	0.68	0.50
								171.02	3.06	19.64	11.08	1.77	0.74	0.58
									2.67±0.45	15.68±4.92	9.36±2.96	1.68±0.23	0.90±0.23	0.67±0.26

*Prelabel ^{14}C concn 0.660 μCi ml^{-1} in all experiments, except 0.729 μCi ml^{-1} on 28 Feb 73.

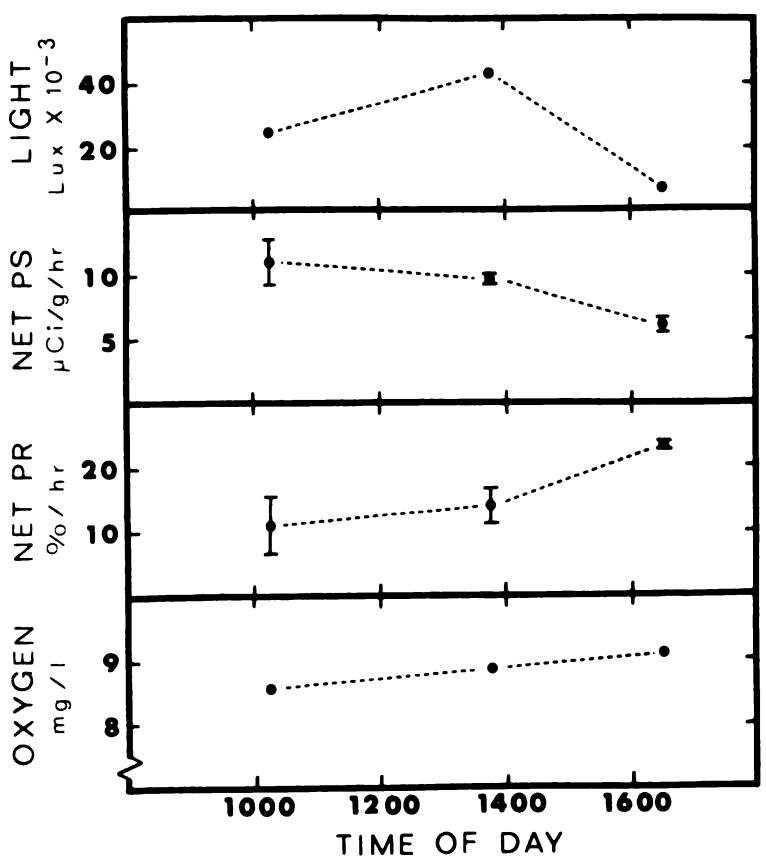


Figure 31. In situ net photosynthesis and net photorespiration in natural Najas flexilis, light intensity, and dissolved oxygen concentration in littoral zone of Lawrence Lake, Michigan, 0.5 m depth, on 15 September 72 (data points: mean ± SD).

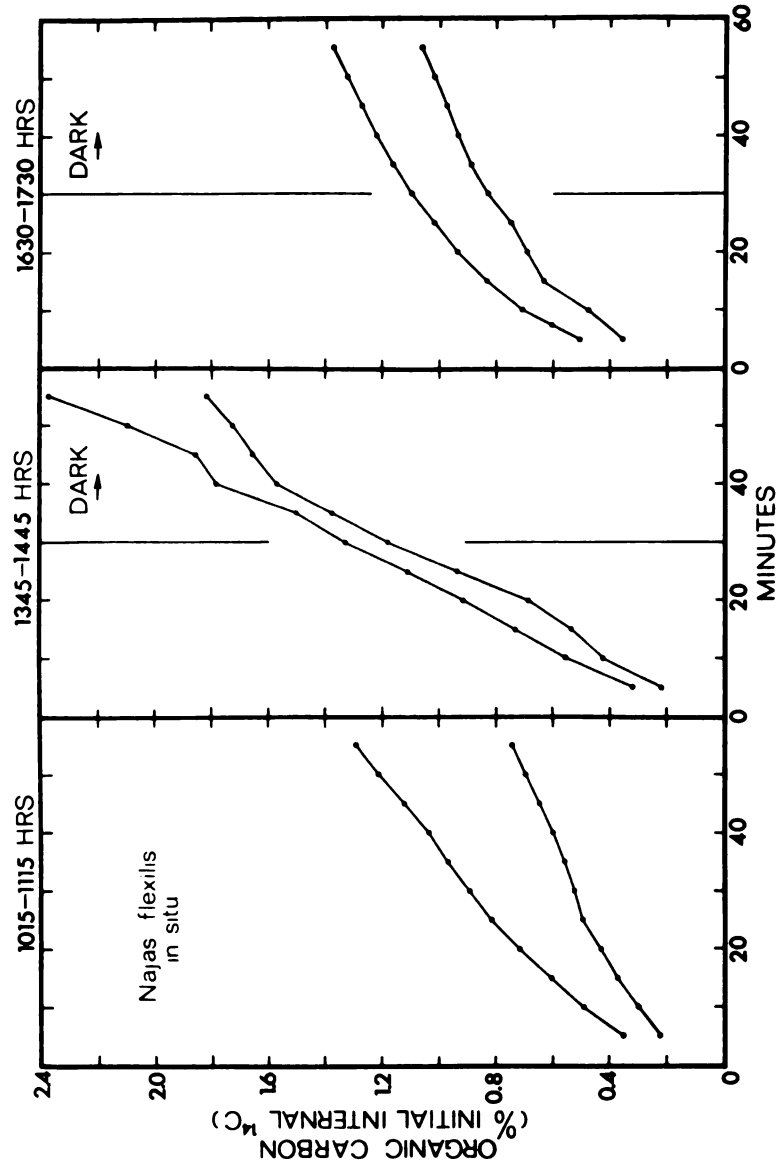


Figure 32. Cumulative organic carbon release from natural Najas flexilis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, 0.5 m depth. Duplicate analyses in mid-morning, mid-afternoon, and late afternoon on 15 September 72.

and carbonates in the material reversed from July to September, with a relative increase in organic ^{14}C in September.

Table 6. Characterization of encrusting material removed from lyophylized ^{14}C-labeled natural Najas flexilis following in situ experiments.

	Dry Wt. % plant wt.	Radioactivity % plant activ.	^{14}C Distribution %organic	% inorganic
July	55	34	27	73
Sept.	113	50	67	33

Scirpus subterminalis

In situ rates of respiration in Scirpus subterminalis in early afternoon in October (Figure 33; Table 5) were similar to those in Najas flexilis in July except that rates were the same in light as in dark in Scirpus subterminalis, with corresponding L:D ratios of about unity. Variance in rates was least in plants from which epiphytic material had been removed (Figure 33). Rates of organic carbon release (Figure 34; Table 5) were similar to those in Najas flexilis in July, and similarly were 1/3 to 1/6 respiration rates. Rates were slightly greater in light than dark.

Dry weight of epiphytic encrustation on Scirpus subterminalis in October was equivalent to 58% of plant dry weight. Radioactivity of the material ($\mu Ci\ g^{-1}$) was

equivalent to 34% of plant radioactivity. Forty % of the activity in the material was in organic carbon, and 60% in carbonates.

In February, respiration rates were greater in light than in the dark (Figure 35). Variance in rates between plants again was high, but L:D ratios were relatively uniform (Table 5). Rates in the light in February were nearly 10-fold those in October; rates in the dark in February were about 5-fold those in October. Rates of organic carbon release in February were 3-fold those in October, and were somewhat greater in light than in dark (Figure 36).

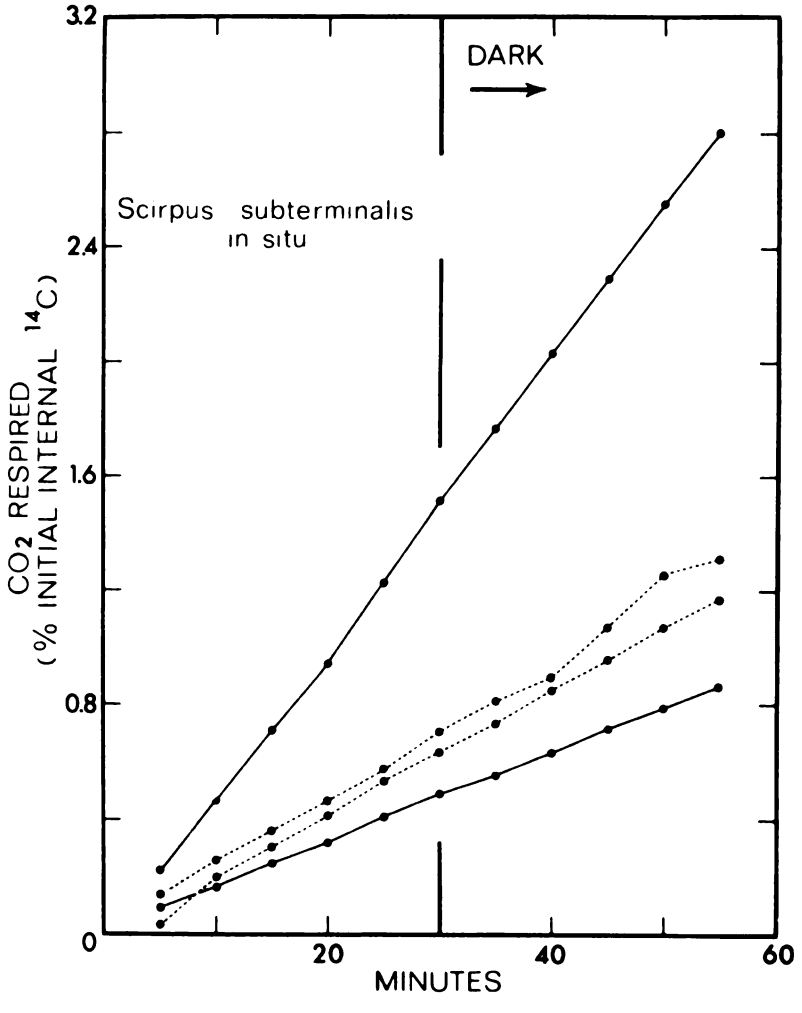


Figure 33. Cumulative carbon dioxide release from natural Scirpus subterminalis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, at 1440 hrs on 14 October 72, 1 m depth. Duplicate analyses with epiphytic microflora removed from plants (dashed lines); duplicate analyses with untreated plants (solid lines).

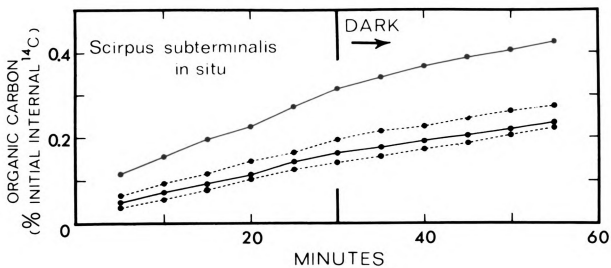


Figure 34. Cumulative organic carbon release from natural Scirpus subterminalis in light and dark in situ in littoral zone of Lawrence Lake, Michigan, at 1440 hrs on 14 October 72, 1 m depth. Duplicate analyses with epiphytic microflora removed from plants (dashed lines); duplicate analyses with untreated plants (solid lines).

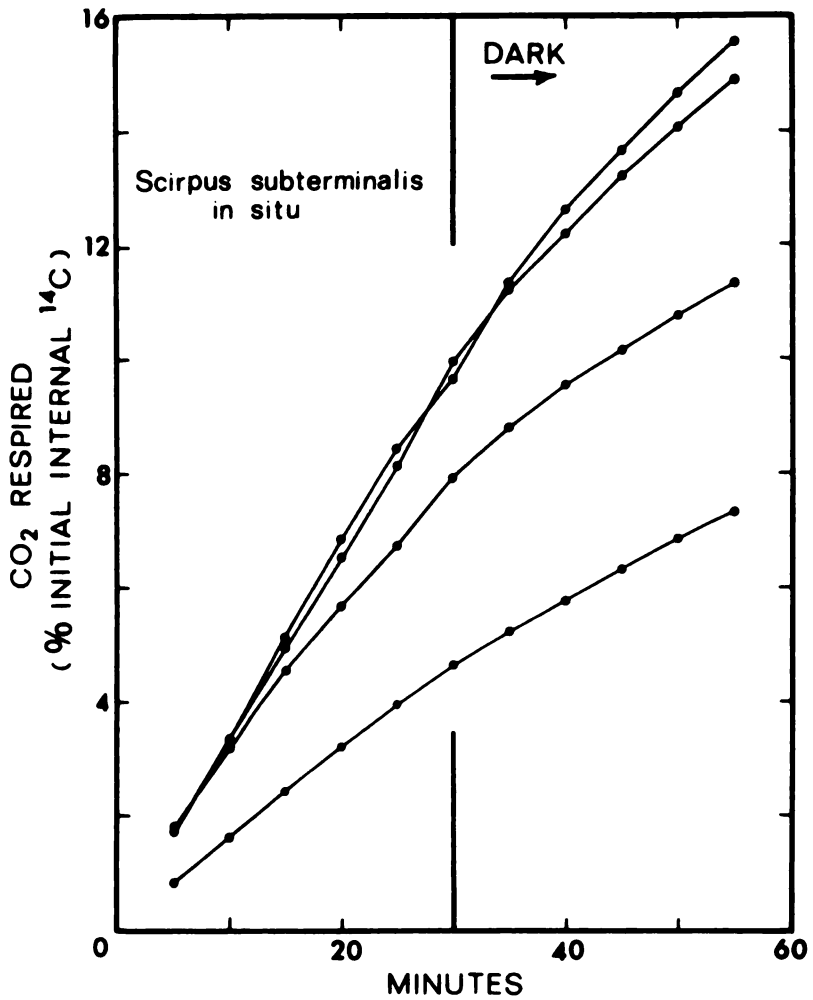


Figure 35. Cumulative carbon dioxide release from natural Scirpus subterminalis in light and dark in situ under ice in littoral zone of Lawrence Lake, Michigan, at 1140 hrs on 28 February 73, 1 m depth (simultaneous quadruplicate analyses).

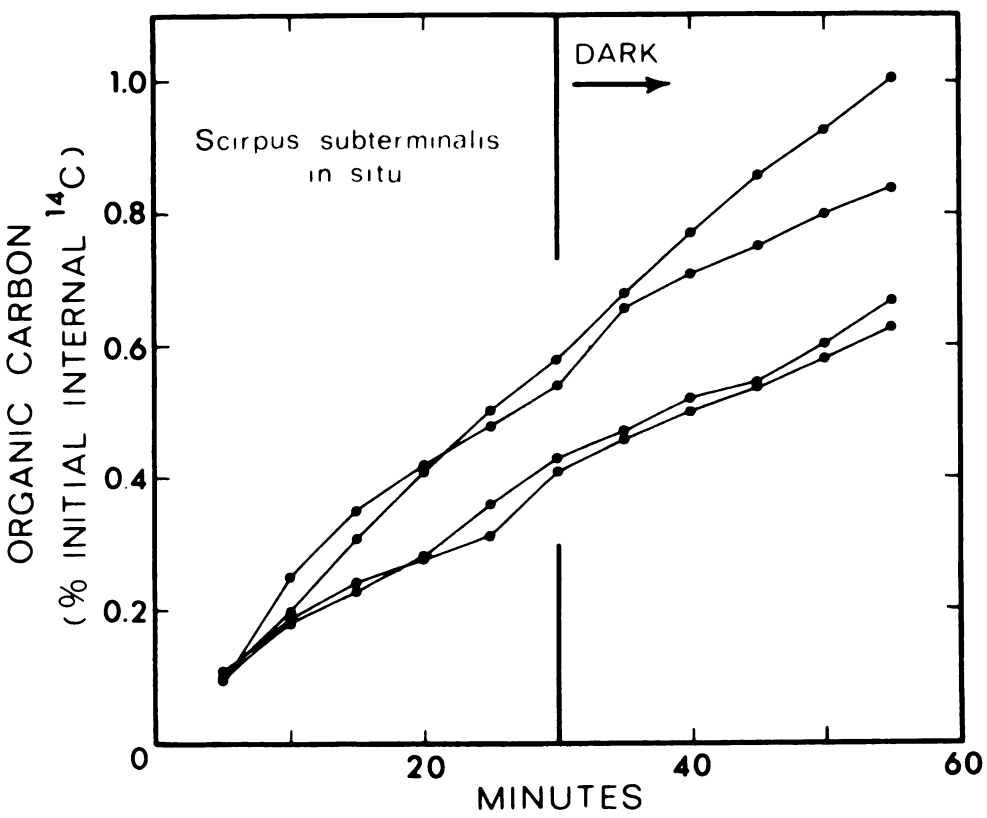


Figure 36. Cumulative organic carbon release from natural Scirpus subterminalis in light and dark in situ under ice in littoral zone of Lawrence Lake, Michigan, at 1140 hrs on 28 February 73, 1 m depth (simultaneous quadruplicate analyses).

DISCUSSION

Net Photorespiration and Dissolved Oxygen

While the initial laboratory experiments indicated that loss of CO_2 was measurable in the light using the ^{14}C-assay, it was striking that the rates in the light were never appreciably greater than in the dark, contrary to results of Goldsworthy (1966) and Zelitch (1968) with terrestrial plants in which rates in the light were 2 to 5-fold those in dark. Indeed, CO_2 loss in the light in axenic Najas flexilis was less than in dark (except at very high O_2 concentrations), which indicated extensive refixation of respiratory CO_2 in the light. There is a possibility that dark respiration was inhibited somewhat in the light, although such an inhibition was not observed at high O_2 concentrations. Some evidence exists for low-level light inhibition of glycolysis in green plants (e.g. Kok, 1947; Hoch, et al., 1963; Hirt, et al., 1971), but this effect is apparently saturated at very low light intensities, and is discounted by other workers (e.g. Gessner, 1937; Warburg, et al., 1949; Zelitch, 1971). Refixation of CO_2 in the light is likely of greater significance here, especially in view of the diffusive resistance of water to CO_2, which is 10^5 times greater than that of air (Hutchinson, 1957; Raven, 1970).

The extensive CO_2 refixation by Najas flexilis is reminiscent of C_4 plants. However, the analyses of leaf cross section anatomy and first ^{14}C fixation products indicate that it is not a C_4 plant; Donaldson and Tolbert (unpublished) have more detailed data on fixation products of N. flexilis, also indicating C_3 metabolism. Furthermore, the enhancement of CO_2 loss in the light by increased O_2 concentration is characteristic only of C_3 plants.

Evidence for refixation of respiratory CO_2 in the light in aquatic plants also appeared in studies of $^{14}CO_2$ loss in the submersed angiosperm Ceratophyllum demersum (Carr, 1969) and Myriophyllum amphibium (Steemann Nielsen, 1955), and in the marine flagellate Dunaliella euchlora (Ryther, 1956). Refixation of CO_2 in vascular hydrophytes is probably enhanced by internal gas lacunae, often of massive size, present in nearly all species (Sculthorpe, 1967). Oxygen produced in photosynthesis builds up in these spaces, and respiratory and photorespiratory CO_2 undoubtedly diffuses into them as well, perhaps more readily than into the water. The evidence for CO_2 refixation casts some doubt on the assumption (Steeman Nielsen, 1955; Wetzel, 1964) that, in short-term ^{14}C primary productivity measurements for macrophytes, incorporated ^{14}C would not be respired and recycled appreciably. The possibility of recycling was subsequently reconsidered (Wetzel, 1965), and it is now clear that the ^{14}C technique for primary

productivity in aquatic plants underestimates photosynthesis to some extent.

Refixation of CO_2 in the light also causes underestimation of true photorespiration as measured by CO_2 evolution, which is a major criticism of the ^{14}C photorespiration assay (Zelitch, 1971); the assay thus measures apparent or net photorespiration. Zelitch (1971) estimates that 50 to 67% of photorespired CO_2 must be recycled and missed by the ^{14}C-assay. A similar underestimation of photorespiration likely occurred in the present study. Apparent photorespiration in axenic Najas flexilis increased only 2-fold with 10-fold increase in O_2 concentration. True photorespiration may have increased by 10-fold internally, inasmuch as glycolate metabolism is directly proportional to O_2 concentration, especially at relatively low concentrations (Andrews, et al., 1973). If so, true photorespiration may have been underestimated by as much as 80%, and corrected rates (8 to 10% hr^{-1} at medium, i.e. normal aquatic, O_2 concentrations, rather than 2% hr^{-1}) and resulting new L:D ratios (3 to 5, rather than 0.5), are similar to those found by Zelitch (1968) and Laing and Forde (1971) in terrestrial C_3 plants. While accurate estimates of true photorespiration cannot be made from these data, the O_2 enhancement of CO_2 evolution in the light, but not in dark, constitutes evidence for presence of photorespiration in N. flexilis. A similar O_2 enhancement of

CO_2 evolution in the light (but not in dark) was demonstrated with the ^{14}C-assay in the marine angiosperms Cymodocea sp. (angustata?) and Halophila sp. (ovalis?) in the Barrier Reef region of Australia (Hough, 1973).

The O_2 enhancement of photorespiration parallels and is assumed to cause the Warburg effect, the O_2 inhibition of net photosynthesis. Oxygen inhibition of net photosynthesis has been demonstrated in several submersed aquatic angiosperms, including Elodea sp. and Sagittaria sp. (Donaldson and Tolbert, unpublished), Elodea canadensis (Kutyurin, et al., 1964), Sagittaria sp. (Bjorkman, 1966), Ranunculus pseudofluitans (Westlake, 1967), and the marine plant Cymodocea sp. (W. J. S. Downton, personal commun.). Downton also found the Warburg effect in the marine macroalga Halimeda cylindracea. Hough (1973) did not find a corresponding O_2 enhancement of CO_2 evolution in the light in H. cylindracea; glycolate may have been excreted rather than oxidized (although this was not reflected in the organic carbon data), or much of the excess CO_2 was bound into the $CaCO_3$ continuously formed internally in this plant.

Normal concentrations of dissolved O_2 in freely mixing natural waters are 10^4 times lower than in air (8-10 ppm compared with 21%) and in themselves are unlikely to support rates of photorespiration as high as commonly found in terrestrial plants. However, buildup of O_2 within the submersed plants during active photosynthesis likely

forces considerable additional photorespiration (cf. discussion below).

The lack of enhancement of dark respiration in Najas flexilis, Cymodocea, and Halophila by increased O_2 concentration is contrary to results of other workers, who have demonstrated an apparent O_2 enhancement of dark respiration in a variety of other aquatic macrophytes (Gessner and Pannier, 1958; Kutyurin, et al., 1964; McIntire, 1966; McDonnell, 1971; McDonnell and Weeter, 1971; Owens and Maris, 1964; Pannier, 1957, 1958; Westlake, 1967). However, none of the latter studies were done using axenic plants, and all of them were done using measurements of O_2 uptake. The O_2 technique measures respiration of the entire community of macrophyte and epiphytic algae, bacteria, and fungi. The epiphytic community can cause greater O_2 fluxes than the macrophytes themselves (Sculthorpe, 1967), and it is possible that much of the increased O_2 uptake occurred in the epiphytic microflora. Gessner (1959) and Gessner and Pannier (1958) found that dark O_2 consumption in several algae was influenced by O_2 concentration; McIntire (1966) demonstrated O_2 enhancement of respiration in benthic periphyton communities (microflora attached to rocks, etc.). Dark respiration in higher terrestrial plants generally is not affected by O_2 concentrations above 2% (Goldsworthy, 1966; Jackson and Volk, 1970; Martin, et al., 1972).

Carbon Dioxide and pH

As reviewed extensively in Hutchinson (1957), Raven (1970), and Stumm and Morgan (1970), CO_2 exists in water in a complex, dynamic equilibrium system composed of free dissolved CO_2, hydrated CO_2 (carbonic acid), bicarbonate, and carbonate. Total amounts of inorganic carbon in a lake depend on the magnitude of dissolution of atmospheric CO_2, the amounts of bicarbonates and carbonates entering the water from the terrestrial watershed, and biotic respiratory CO_2 production. The relative concentrations of the components of the equilibrium system depend mostly on pH, as is well known, with free CO_2 favored by low pH. In freely mixing surface waters, the partial pressure of total free and hydrated CO_2 is in equilibrium with the atmosphere, at a concentration of about 10 μM or 0.5 mg l^{-1}, and is independent of pH. Lakes containing very high concentrations of bicarbonate and carbonate (>150 mg l^{-1}) are often supersaturated with free CO_2 (Hutchinson, 1957), as is true of Lawrence Lake (Otsuki and Wetzel, 1973).

Several aquatic plants appear to require free (dissolved) CO_2 for photosynthesis (James, 1928; Steemann Nielsen, 1947, 1951, 1952b; Osterlind, 1950; Briggs and Whittingham, 1952; reviewed in Raven, 1970), on the basis of CO_2 fixation experiments in which pH and total CO_2 content are varied, with low pH favoring photosynthesis. Using this technique, Wetzel (1969) demonstrated such an apparent

affinity for free CO_2 in Najas flexilis at several total CO_2 concentrations, including those comparable to levels in hardwater lakes, both in cultures exposed to air and in those sealed from atmospheric contact. Argument has been made (Raven, 1970; Wetzel, 1972) that the dehydration of H_2CO_3 within the CO_2-carbonate equilibrium complex may be slow enough to limit the availability of CO_2 for photosynthesis, but this should not be relevant in water at equilibrium with the atmosphere or supersaturated with free CO_2. Strictly from the standpoint that the partial pressure of free dissolved CO_2 in equilibrium with air is independent of pH, and is supersaturated in presence of high concentrations of bicarbonates and carbonates, photosynthesis should not be affected by varying pH; the results of Wetzel and others suggest that not all of the free CO_2 is instantaneously available at the plant surface, perhaps as a result of the low diffusion rate of CO_2 in water. Raven (1970) suggests that diffusion resistance of water should be added to that of cell wall and cytoplasm in estimates of diffusive resistance to CO_2 fixation in aquatic plants. It is not clear how pH would affect diffusion rate in the water. Alternatively, the pH effect on photosynthesis can be explained in that the pH optimum for RuDP carboxylase activity (7.8) is lower than that for RuDP oxygenase activity (9.3-9.5) (Andrews, et al., 1973), with lower pH favoring photosynthesis and higher pH favoring

photorespiration. Within a pH range of 7.3 to 8.8, net photosynthesis in axenic N. flexilis is greatest at pH 7.3-7.9 and lowest at 8.5-8.8 over a wide range of total CO_2 concentrations (Wetzel, 1969). A causal relationship between the enzymatic pH optima cited above and the response of net photosynthesis in N. flexilis can be assumed only if pH of the water significantly influences pH in the chloroplasts of intact submersed plants, which is unknown. In any case, it remains unresolved whether, over the range of pH and carbonate alkalinity in freshwaters, photosynthesis in aquatic plants is CO_2-limited to a greater or lesser extent than it is in terrestrial plants.

In view of the above, it is difficult to predict the influence of ambient CO_2 on photorespiration in aquatic plants in terms of what is known of terrestrial plants. Effects of CO_2 on the ^{14}C-assay were tested in the laboratory partly to facilitate interpretation of in situ studies in Lawrence Lake. The assay normally is performed under CO_2-free conditions, to avoid dilution of the ^{14}C labeled organic carbon pool by fixation of ^{12}C during the experiment (Goldsworthy, 1966; Zelitch, 1968). The removal of CO_2 (including bicarbonate and carbonate) from lake water involves acidification, gas purging, and reinstatement of original pH with the aid of a buffer, which drastically alters water chemistry and is not desirable ecologically. Influence of CO_2 was tested also because photorespiration itself is highly influenced by CO_2, presumably as a result

of the direct competition of CO_2 and O_2 for reaction with RuDP. CO_2-free conditions thus would favor unusually high rates of photorespiration by allowing unrestricted oxygenation of RuDP and glycolate synthesis.

Presence of large amounts of $^{12}CO_2$ in the ^{14}C-assay would be expected to lower the apparent rate of photorespiration both by decreasing the rate of glycolate synthesis and by diluting the ^{14}C organic substrate pool. The relative magnitudes of these effects would be difficult to determine, but they probably are similar since they are part of the same process (CO_2 fixation). Thus in performing the assay in presence of $^{12}CO_2$, the ^{12}C dilution effect (causing some degree of underestimation of photorespiration) replaces the unnatural effect of zero CO_2 (causing some degree of overestimation of photorespiration), and the former is probably no more serious than the latter.

The results with changing $[CO_2]$ were variable, depending on experimental conditions. Presence of CO_2 in its natural forms in lake water did appear to cause lower net photorespiration rates relative to CO_2 purged lake water, although water chemistry other than CO_2 concentration was unavoidably different in the two treatments. Also, the axenic plants had not been grown in lake water prior to the experiments. The apparent lag-time in the reduction of rates after exposure to high CO_2 may reflect extensive recycling of internal CO_2, which would reduce the rate of ^{12}C dilution of the ^{14}C pool. In synthetic media, to which the axenic

plants were acclimated, additions of bicarbonate or carbonate to levels similar to those in Lawrence Lake water did not affect the results of the assay appreciably. In general the results indicated that the assay could be performed *in situ* without drastic complications resulting from ambient CO_2.

Carbon dioxide concentration also is important with respect to plant CO_2 compensation point, or the minimum CO_2 concentration at which the plant can continue to remove CO_2 from the environment. Rapidly photorespiring plants have high CO_2 compensation points. The compensation point of *Najas flexilis* is rather low (Donaldson and Tolbert, unpublished). The same is true of *Myriophyllum spicatum* L. (Stanley and Naylor, 1972). *Myriophyllum spicatum* was characterized in terms of fixation products as a C_3 plant, as is *N. flexilis*. Stanley and Naylor assumed that the low compensation point was the result of extensive CO_2 refixation, regardless of the lack of C_4 carboxylation. This argument may be inaccurate: if the plant were rapidly photorespiring, the compensation point would be high regardless of extensive refixation, since refixation of a given amount of photorespired CO_2 would preclude fixation of a similar amount of external CO_2. Thus, while refixation is certainly extensive in these plants, low CO_2 compensation point probably should be regarded as evidence for relatively low rates of photorespiration.

Epiphytic Microflora

The community of epiphytic microflora on naturally growing aquatic macrophytes is a highly active, and often major, component of the metabolism of the littoral and overall lake systems (Allen, 1971; Wetzel, 1964; Wetzel and Allen, 1972; Wetzel, et al., 1972). The intimate association of macrophyte surface and the epiphytic biota undoubtedly provides for extensive exchange of CO_2 and organic carbon between the two. Any CO_2 photorespired by the macrophyte would be available for use by epiphytic algae, and any of this CO_2 fixed by the algae would not be measured by the ^{14}C photorespiration assay. On the other hand, the epiphytic algae are prelabeled along with the macrophyte, and of course are respiring and releasing organic carbon themselves during the assay. Epiphytic bacteria and fungi likely utilize some of the organic carbon released by the macrophyte, and respire some of it as well. All of these processes potentially confound the results of the ^{14}C-assay when applied to natural plants.

Effects of epiphytic diatoms and mixed epiphytic microflora generally were minimal in the laboratory experiments, which would suggest that *in situ* ^{14}C-assays would not be confounded seriously by the epiphytic community. However, the dense epiphytic growth common on naturally growing plants was not obtainable on the axenic *Najas flexilis* seedlings without deterioration of the plants and

resulting incomparability with controls, especially in the case of the axenic diatoms, and experiments were performed with less than maximal epiphytic growth.

Net photorespiration and organic carbon release were enhanced somewhat in presence of the diatoms, which apparently were releasing ^{14}C in excess of any that they were obtaining from the host plants.

The effect of the mixed epiphytic community was largely an enhancement of dark respiration (Figure 23), which may reflect mineralization of labeled organic substrate released from the host, although total release of organic carbon (Figure 25) was not reduced in presence of the epiphytes except possibly during the first 10 minutes of darkness.

In situ Photorespiration

Najas flexilis

The relatively low rates of net photorespiration in natural Najas flexilis in July were unexpected, in view of the high light intensity. In this context, light intensities in the laboratory epiphyte experiments were over 2-fold those in the O_2 and CO_2 experiments, but at similar O_2 concentrations there were no appreciable differences in net photorespiration. The light intensity in Lawrence Lake during the July in situ assays was over 10-fold those in the laboratory; if this intensity was actually causing high rates

of true photorespiration, then CO_2 refixation rates must have been high as well.

In September, net photorespiration exceeded dark respiration for the first time in the study, even though light intensity was lower than in July. However, the plants were beginning to undergo senescence, and were losing 10-fold more carbon than in July, both in light and in dark. Capability of refixing CO_2 likely was reduced, allowing more photorespired carbon to be released. Carboxylation may be diminished relative to oxygenation of RuDP as a result of changes in chloroplast chemistry (including loss of chlorophyll) during senescence, enhancing photorespiration.

The variation of net photorespiration during the day in September was consistent with the hypothesis that afternoon depression of net photosynthesis in aquatic plants is associated with increase in photorespiration. The afternoon depression in net photosynthesis, depicted schematically in Figure 37, has been demonstrated consistently in several submersed angiosperms and phytoplankton populations (Doty and Oguri, 1957; Hartman and Brown, 1967; Hartman, et al., 1965; Meyer, 1939; Steeman Nielsen and Wium-Andersen, 1972, and Ohle in discussion thereof; Verduin, 1957; K. F. Walker, personal commun.; Wetzel, 1965). Various explanations for the phenomenon have been offered, including narcosis or "sugar glut", photooxidation by surplus light energy, protection against photooxidation by inactivation of the

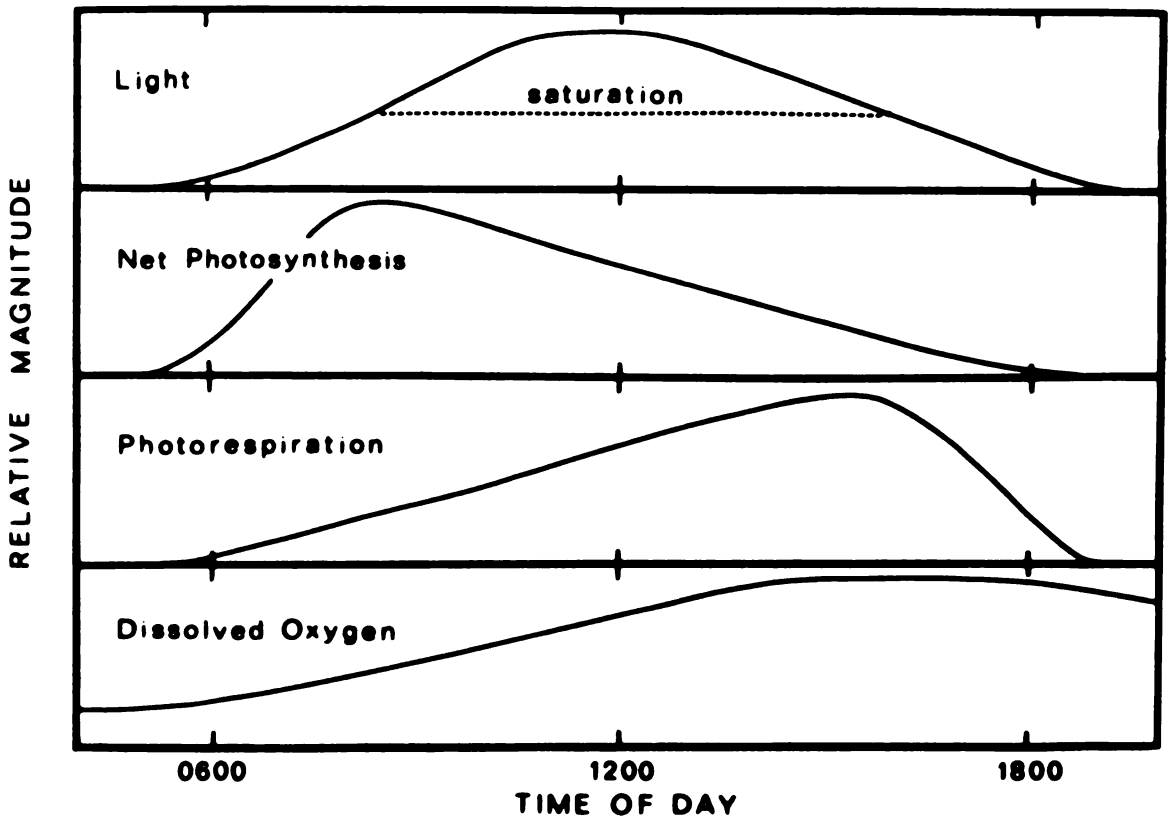


Figure 37. Commonly observed variations in light intensity, dissolved oxygen concentration, net photosynthesis of natural submersed macrophyte and phytoplankton populations, and proposed concurrent variations in photorespiration.

photochemical reaction, or poisoning by Cu^{++} contamination in ^{14}C stocks (Strickland, 1960; Steemann Nielsen, 1962; Steeman Nielsen and Wium-Andersen, 1972); most of these propositions are somewhat vague, and no specific mechanisms have been demonstrated in the natural situation. Photorespiration is an alternative explanation, inasmuch as conditions conducive to it can develop during the day. Indeed, net photorespiration in Najas flexilis in September increased through the day (Figure 31), while net photosynthesis decreased through the day. These processes, as well as light intensity and oxygen, generally followed the patterns predicted in Figure 37. Dissolved oxygen did not increase sufficiently to fully explain the increase in photorespiration. However, the increase of dissolved O_2 may reflect a much greater increase in O_2 tension within the plants. Hartman and Brown (1967) demonstrated that dissolved O_2 in the water surrounding Elodea canadensis fluctuated with internal O_2 tension, and in one case both were greatest in late afternoon; the internal O_2 concentration was generally similar to that in air, i.e. much higher than in water.

Scirpus subterminalis

The leaf cross sections and first ^{14}C fixation products indicated that Scirpus subterminalis is a C_3 plant, and photorespiration was expected. However, the massive gas lacunae were expected to engender extensive internal CO_2

recycling, with resulting lower rates of CO_2 evolution in the light than in the dark. On the contrary, rates in light were the same as in dark. The photosynthetically active epidermal cells are one cell removed from the gas lacunae, and thus CO_2 may diffuse into the water as readily as through the inner cells into the lacunae, especially if the partial pressure of CO_2 in the lacunae is already high relative to the water. The rates of respiration were generally similar to those in Najas flexilis in July, showing no evidence of the senescence reflected in rates in N. flexilis in September. Rates of carbon fixation (Table 5) were lower than in N. flexilis in July, however, similar to those in N. flexilis in September, perhaps because temperature was substantially lower.

Rates of carbon fixation in S. subterminalis were drastically lower under the ice in February than in October, undoubtedly because of very low temperature (3 C) and low light. Surprisingly, rates of respiration (in terms of % of fixed carbon) were high, similar to the September rates in N. flexilis. Extremely low temperature may reduce the rate of carbon fixation more than it does the rate of respiration, with corresponding apparent increase in respiration relative to fixation. CO_2 refixation might then be reduced as well, with resulting relative increase in release of photorespired CO_2, reflected by the L:D ratios of greater than unity.

Release of Organic Carbon

The lack of response of organic carbon release from axenic Najas flexilis to increased O_2 concentration in the light suggests that glycolate is not a major component of the material released, since excretion of glycolate should parallel its synthesis, which was undoubtedly enhanced by high O_2. The apparent post-illumination surge of organic carbon also does not suggest involvement of glycolate, because oxidation vs. excretion of glycolate is not a function of light. The burst is reminiscent of the sudden rise of PGA in chloroplasts upon sudden darkness, as demonstrated in Chlorella pyrenoidosa by Bassham (1971), in which the light-dependent reduction of PGA ceases immediately while PGA synthesis from RuDP carboxylation (a dark reaction) continues briefly until the RuDP pool is exhausted. The excess PGA is gradually metabolized to amino acids and fatty acids in C. pyrenoidosa. Release of PGA in large quantity has not been reported in algae or higher aquatic plants, but this would be a highly transient phenomenon. However, the apparent enhancement of the burst by high oxygen (Figure 12) suggests that PGA is not involved, inasmuch as oxygenation of RuDP produces half as much PGA as does carboxylation, and the PGA buildup would be inhibited by high O_2 rather than enhanced. The data are based on ^{14}C tracing, of course, and it was assumed that the actual amount of organic matter released in this short period

was too small to characterize. Special efforts to obtain relatively large quantities for isolation may be desirable, however.

The ecological significance of the post-illumination excretion is questionable inasmuch as sudden darkness is not likely to be common under natural conditions, although rapidly intermittent cloud cover might have a similar effect. In any case, the data provide additional evidence that excretion of organic carbon is a metabolic alternative in aquatic macrophytes under certain environmental conditions. The post-illumination excretion burst was not observed in any of the *in situ* photorespiration assays; immediate microbial utilization of the excreted carbon is an obvious possibility. The reduction of the burst by mixed epiphytic microbes in the laboratory (Figure 25) was minimal, but these organisms may not have been prepared metabolically for the compound(s) involved, particularly in view of the high concentration of organic buffer in the cultures, providing a plentiful organic substrate.

The rates of organic carbon release in natural *N. flexilis* in July (<1% initial internal ^{14}C hr^{-1}) do not support the estimate of Wetzel, *et al.* (1972) that 4% of photosynthetically fixed carbon in macrophytes is released as dissolved organic carbon. However, rates of release in September were greater (>2% hr^{-1}), especially in early afternoon, and allowing for differences in analytical

methods, the estimate of Wetzel, et al. (as a mean rate of release over a year) is not disputed here. The increased rate of release in September reflects, as does the increased respiration, the senescence of the plants at this time. The relative increase of ^{14}C labeling in the organic fraction of the encrusting material in September (Table 6) may be a result of the increased release of organic matter from the plants, some of which likely was assimilated by micro-organisms or adsorbed by calcium carbonate; the data are confounded by fixation of $^{14}CO_2$ by algae within the material during prelabeling, however. The data indicate that unusually large quantities of soluble organic carbon become directly available to aquatic microorganisms in the fall, without the necessity of microbial decomposition of the plants. Furthermore, since lake circulation reaches a maximum at this time of year, much of this dissolved organic matter is likely to be dispersed in pelagic waters, and subsequently enter the dynamic organic-inorganic-microbial interactions which are among the fundamental controllers of overall lake metabolism (Saunders, 1957; Wetzel, 1968, 1971; Wetzel and Allen, 1971; Wetzel, et al., 1972). In this manner the enhanced release in the fall may contribute to fall phytoplankton blooms. Rates of release of organic carbon from Scirpus subterminalis remained low (<0.5 % hr^{-1}) in the fall, but increased 3-fold in winter. The carbon released in winter may accumulate to some extent as a result

of low temperature (i.e. low microbial metabolism) and then become dispersed during spring lake circulation, with chemical and trophic involvement similar to that in the fall.

SUMMARY AND CONCLUSIONS

A ^{14}C-assay for photorespiration has been developed for use in submersed aquatic plants both in the laboratory and *in situ*. Advantages of the technique include avoidance of problems associated with closed-bottle techniques, ease of manipulation of experimental conditions, and simultaneous evaluation of total released organic carbon. Problems include imposing a current on plants normally growing in relatively still water (less serious than imposing small-volume stagnation), and internal recycling of $^{14}CO_2$, which appears to be more serious in aquatic plants than in terrestrial plants. All methods of measuring photorespiration have inherent difficulties resulting in underestimation of true photorespiration (Zelitch, 1971), and comparisons of methods in the same plant species result in varying estimates (Martin, *et al.*, 1972). Within the limitations of the ^{14}C-assay, the following conclusions emerged from this study:

1. Net photorespiration is low in C_3 submersed aquatic plants in relation to that in terrestrial C_3 plants, but is enhanced by high oxygen concentration, indicating net

photosynthesis can vary with changes in dissolved oxygen in these plants.

2. True photorespiration may be somewhat lower in submersed plants than in terrestrial plants because of lower environmental oxygen concentrations.

3. Much of true photorespiration is not measured by the ^{14}C-assay because of extensive internal recycling of CO_2, partly or largely accounting for low rates of net photorespiration.

4. Net photorespiration varies within a day and seasonally in submersed plants, and may account for afternoon depressions in net photosynthesis.

5. Kinetics of release of dissolved organic carbon do not indicate that glycolate excretion is extensive in Najas flexilis, which is consistent with the evidence for glycolate oxidation, i.e., O_2 enhancement of CO_2 production in the light.

6. Release of organic carbon is relatively low in natural Najas flexilis in summer, but increases in the fall during senescence. Release of organic carbon from Scirpus subterminalis is low in fall and increases in winter. The patterns of release may be associated with seasonal aspects of planktonic productivity.

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