MONTANA STATE UNIVERSITY

Physiological ecology of aquatic macrophytes in the Madison River of Yellowstone National Park, Wyoming

by Duane Andrew Klarich

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Botany
Montana State University
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Abstract:
A distinct decline in free carbon dioxide concentration occurs through a twenty-three kilometer reach of the Madison River in Yellowstone National Park. Several investigators found a corresponding decrease in primary production which was attributed, in part, to this decline in carbon dioxide; positive and significant partial correlation coefficients were obtained between primary production and this variable. A portion of the primary production in the Madison River is due to a relatively luxurious and diverse aquatic macrophyte community in the stream.

Photosynthetic and respiratory rates of twenty-two species of macrophytes (including three species of Musci, several species of Potamogeton and other monocots, Myriophyllum-Berula and other dicots, and Chara) were determined in Madison River water collected at three stations along the carbon dioxide gradient. A photosynthetic rate response to differences in carbon dioxide concentrations was evident in all species. Greater rates and photosynthetic rate/respiratory rate ratios consistently were obtained in the high carbon dioxide water; intermediate and relatively low photosynthetic rates and ratios were obtained in the medium and low carbon dioxide samples respectively. However, respiratory rates for a species were similar in all treatments.

The photosynthetic-carbon dioxide responses of the species were substantiated by artificially removing carbon dioxide or adding it to appropriate water samples. Direct correlations consistently were obtained between these manipulations and photosynthetic activity, similar to those obtained from the unaltered river samples. However, this photosynthetic response to free carbon dioxide varied considerably among species.

Distributional patterns of aquatic macrophytes in the Madison River were determined by previous investigations. One group of species was found to be restricted to the upper, high carbon dioxide reach of the river whereas a second group was found throughout the river. This latter group was quite abundant in the lower, low carbon dioxide reach.

A distinct parallel was evident between species distribution and their photosynthetic sensitivity to carbon dioxide. Species found to be abundant in the low carbon dioxide reach demonstrated relatively small photosynthetic rate responses to variations in carbon dioxide concentrations whereas species restricted to the upper reach of the river demonstrated much more pronounced responses in photosynthetic rates.

These results indicate that carbon dioxide levels have a definite but variable influence on the photosynthetic activities of aquatic macrophytes in the Madison River. The relationships between the magnitude of the species' photosynthetic responses to carbon dioxide and their distributions in the stream indicate that this variable could act as a limiting factor to river production and macrophyte distribution

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by

DUANE ANDREW KLARICH

A thesis submitted in partial fulfillment
of the requirements for the degree

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ABSTRACT

A distinct decline in free carbon dioxide concentration occurs through a twenty-three kilometer reach of the Madison River in Yellowstone National Park. Several investigators found a corresponding decrease in primary production which was attributed, in part, to this decline in carbon dioxide; positive and significant partial correlation coefficients were obtained between primary production and this variable. A portion of the primary production in the Madison River is due to a relatively luxurious and diverse aquatic macrophyte community in the stream.

Photosynthetic and respiratory rates of twenty-two species of macrophytes (including three species of Musci, several species of Potamogeton and other monocots, Myriophyllum-Berula and other dicots, and Chara) were determined in Madison River water collected at three stations along the carbon dioxide gradient. A photosynthetic rate response to differences in carbon dioxide concentrations was evident in all species. Greater rates and photosynthetic rate/respiratory rate ratios consistently were obtained in the high carbon dioxide water; intermediate and relatively low photosynthetic rates and ratios were obtained in the medium and low carbon dioxide samples respectively. However, respiratory rates for a species were similar in all treatments.

The photosynthetic-carbon dioxide responses of the species were substantiated by artificially removing carbon dioxide or adding it to appropriate water samples. Direct correlations consistently were obtained between these manipulations and photosynthetic activity, similar to those obtained from the unaltered river samples. However, this photosynthetic response to free carbon dioxide varied considerably among species.

Distributional patterns of aquatic macrophytes in the Madison River were determined by previous investigations. One group of species was found to be restricted to the upper, high carbon dioxide reach of the river whereas a second group was found throughout the river. This latter group was quite abundant in the lower, low carbon dioxide reach. A distinct parallel was evident between species distribution and their photosynthetic sensitivity to carbon dioxide. Species found to be abundant in the low carbon dioxide reach demonstrated relatively small photosynthetic rate responses to variations in carbon dioxide concentrations whereas species restricted to the upper reach of the river demonstrated much more pronounced responses in photosynthetic rates.

These results indicate that carbon dioxide levels have a definite but variable influence on the photosynthetic activities of aquatic macrophytes in the Madison River. The relationships between the magnitude of the species' photosynthetic responses to carbon dioxide and their distributions in the stream indicate that this variable could act as a limiting factor to river production and macrophyte distribution.

INTRODUCTION

In the past, ecological studies of lentic environments have largely exceeded those on flowing waters. The various studies of Butcher (1932, 1933, 1945, 1948, among others) on the taxonomy and ecology of English rivers provide a major exception. Butcher's attention was focused primarily upon the microflora of these streams. In recent years, with the rising demand for usable water and with the development of suitable and applicable methods, increasing attention has been directed to the lotic side of the limnological spectrum. With the publication of Odum's (1956) paper, methods became available by which to effectively determine the productivity of running water under a variety of conditions. As a result, biological investigations of creeks and rivers, including considerations of the macrophyte component of the lotic ecosystem, have significantly increased in recent years.

Armitage (1958) demonstrated that the average yearly standing crop of riffle insects in the Firehole River of Yellowstone National Park and their relative abundances were correlated with bicarbonate alkalinity and temperature. Similar results were obtained by Vincent (1967) for the Gibbon River. These findings plus the availability of Odum's (1956) paper stimulated additional interest towards studying the Madison, Firehole, and Gibbon Rivers since this particular river "...system offered an ideal situation to study the effects of natural thermal and chemical enrichment on flowing water communities and their productivity

(Wright 1969)."

Chemical analyses by several investigators have demonstrated a definite carbon dioxide gradient in the Madison River of Yellowstone National Park. Work by Todd (1967) and by Wright and Mills (1967) suggested that this factor was the major cause of the downstream decline in primary productivity. Horpestad (1969) found definite relationships between carbon dioxide concentrations and various distributional features of the aquatic macrophytes in the stream. As a result, this study was undertaken to ascertain the effects of the carbon dioxide differences in the Madison River upon the photosynthetic activities of the large aquatic plants. A physiological-ecological approach was adopted for the study.

Phinney (1969) suggested that physiological ecology is a study of physiological responses in relation to potential environmental variables. Billings (1960) defined this field of science as "...the study of plant (and animal) processes under natural or simulated environmental conditions." Examples of these processes include growth, germination, reproduction, respiration, photosynthesis, and movement. Of added value is the demonstration of some correspondence between an induced change in a process and some ecological manifestation such as abundance changes, distributional irregularities, and/or alterations in diversity and community composition. With the demonstration of a quantitative relationship between these three components, some insight might be gained

towards an explanation of "why organisms live where they do" in any particular study area (Phinney 1969).

In this study, the environmental variable of major interest is the carbon dioxide gradient in the Madison River, and the ecological aspects are the differences in species abundance and distribution through the river study area and the downstream decline in primary production. Emphasis is directed primarily towards the photosynthetic rates of the aquatic macrophytes as a function of variable carbon dioxide concentrations in the river, because, if carbon dioxide is an important factor, photosynthesis would be expected to significantly respond to alterations in its availability. However, if carbon dioxide proved to be a noncritical factor for a particular species, then the photosynthetic response to changes of carbon dioxide levels would be expected to be absent or negligible.

Species restricted to different localities in the river might show considerable variations in their photosynthetic responses to carbon dioxide; thus the demonstration of a correlation between the magnitude of these responses and the species' distribution would provide evidence that this variable operates as a limiting factor in the stream. In addition, a predominance of one species type ("responder" or "non-responder") over the other would support or refute the suggestion that carbon dioxide influences primary production. That is, if the case were realized where most of the major taxa in the river were found to be

"non-responders", this would tend to refute the hypothesis that the carbon dioxide gradient in the Madison River is a major factor affecting the decline in primary production downstream.

LITERATURE REVIEW

MADISON RIVER

Initial studies in the Madison River headwaters area considered the periphyton, primarily diatoms, associations in the major rivers of the system (Roeder 1966). Subsequently, attention was directed towards the identification, distribution, abundance, and ecology of the macrophytic flora in the Firehole River (Rasmussen 1968) and in the Madison and lower Gibbon Rivers (Horpestad 1969). Productivities of the Firehole River, lower Nez Perce Creek, the lower Gibbon River (Wright 1969), and the Madison River (Wright and Mills 1967, Todd 1967, and Wright 1969) were studied as were heat budgets of the Madison River (Wright and Horrall 1967). Included as a part of these various investigations were extensive chemical and physical-hydrological analyses. In addition, macrophyte standing crop and chlorophyll accrual assessments were made. Similar data are also available from a more recent study in the area (Klarich and Wright 1974). As a result, a considerable amount of information is available on the aquatic macrophytes of the rivers and on the associated chemical, physical, and environmental factors that might conceivably influence these plants.

Information on the abundance and distribution of aquatic macrophytes in the Madison headwaters is available from several studies. Horpestad (1969) attempted to relate the distribution of these plants in the Madison River and in the lower Firehole and Gibbon Rivers of

Yellowstone National Park to various chemical and physical parameters using multiple regression methods. He determined the percent canopy cover of twenty macrophyte species at eight locations on the Madison River. Rasmussen (1968) did similar studies on the entire length of the Firehole. Todd (1967) utilized harvest methods and obtained standing crop data for various macrophyte taxa at intervals along the Madison River. Wright and Mills (1967) also obtained standing crop and distribution data for plants in the river in addition to productivity estimates of five sequential reaches of the river which were attributable at least in part to the activities of macrophytes.

Horpestad (1969) obtained a positive correlation between carbon dioxide concentrations and the diversity index of aquatic macrophytes and observed a decline in both towards the lower reach of the river. He also found significant correlations between percent canopy cover and carbon dioxide for certain of the species but not for others. In addition, Horpestad (1969) found that total canopy cover was related in part to the soluble inorganic carbon (including carbon dioxide) present within any given reach of the river. Todd (1967) and Klarich and Wright (1974) observed positive correlations between periphyton-chlorophyll accrual rates and free carbon dioxide concentrations. The latter investigators demonstrated that 93% of the variation in chlorophyll accrual rates between eight sites on the river could be accounted for by the associated variations in carbon dioxide and temperature. Similarly,

Wright and Mills (1967) found that downstream decreases in primary productivity in the Madison River were best correlated with downstream declines in carbon dioxide. Similar observations were made by Todd (1967) and Klarich and Wright (1974). All of these results point to the likelihood that free carbon dioxide functions as an important ecological variable in the stream.

The importance of substrate, current, and interspecific competition in regulating the growth, abundance, and distribution of aquatic plants in flowing water have long been recognized by aquatic scientists (Pond 1903, 1918; Pearsall 1920; Butcher 1933; Bourn 1937; Misra 1938; Curtis 1959). The importance of substrate has been noted by several workers such as Pearsall (1920) and Curtis (1959) and as early as 1903 by Pond. Butcher (1933) considered current velocity to be the major factor controlling the distribution of aquatic macrophytes in English streams. He recognized several distinct communities of aquatic plants relative to current and therefore the substrate characteristics of a river reach. These range from a "torrential community" influenced by high velocities and a bedrock-boulder substrate through several intermediate communities to a "littoral-type" characterized by very deep, slow-moving water and a mud substrate. Depth therefore also becomes an important factor in plant distribution, especially in view of the large size of certain of the aquatic plants such as the pondweeds (e.g., Potamogeton natans). The habitat of the "torrential community" is colonized

primarily by mosses while the deeper "littoral-type community" possesses vegetation similar to that of a pond.

Data on depth, current, substrate, and width are available for the Madison River through previous investigations (Wright and Mills 1967, Todd 1967, and Horpestad 1969). Results from these studies indicate a distinct downstream change in the physical nature of the river; this is qualitatively evident through the development of a long riffle that occurs in the lower reaches of the stream. Both Rasmussen (1968) and Horpestad (1969) noted the potential importance of these factors in regulating the occurrence and distribution of aquatic macrophytes in the Firehole and Madison Rivers. Regardless of the correlations between inorganic carbon and macrophyte diversity-canopy cover in the Madison River, Horpestad (1969) concluded that "...the percent canopy cover of this community was primarily determined by current speed and substrate texture."

CARBON DIOXIDE AS A LIMITING FACTOR IN AQUATIC ECOSYSTEMS

In contrast to the acceptance of substrate and current as important environmental factors, the relative roles of carbon dioxide and bicarbonate as photosynthetic carbon sources for aquatic plants and the importance of carbon as a limiting nutrient in aquatic systems have provoked disagreements among scientists since it was first suggested by Anglestein in 1911 that bicarbonate might be utilized in photosynthesis (Sculthorpe 1967). The supply of carbon dioxide, including the

"half-bound" component in bicarbonate reserves, was considered long ago by Birge and Juday (1911) to be probably the chief controlling factor in primary productivity. However, with the general acceptance of bicarbonate as a carbon source for photosynthesis in aquatic systems, the tendency has been to assume that primary productivity is related mainly to an adequate supply of this constituent rather than to free carbon dioxide.

Bicarbonate ions are often in high concentrations in many waters, and as a result, the role of carbon as a factor in eutrophication has often been overlooked; instead, attention has been directed to those nutrients in much smaller concentrations (e.g., the nitrogen and phosphorous species) as limiting factors to primary production. A variety of studies have suggested that an adequate supply of suitable carbon sources probably is not the general case for many aquatic systems. As a result, the concept that phosphorous is the most limiting nutrient to primary production in natural waters has been questioned in recent years (Goldman et al 1972).

Kerr et al (1970) concluded that the addition of phosphorous and nitrogen to waters with little available carbon dioxide and bicarbonate did not stimulate algal growth. Welch (1968) suggested that the high productivities of some lakes must be related to the large amounts of available carbon present in these systems. Waters (1957) found that the addition of lime to bog lakes increased bicarbonate concentrations,

carbon dioxide utilization, and phytoplankton yields. Myers (1947) stressed the importance of carbon dioxide in regulating algal growth. Martin et al (1969) showed that bicarbonate concentrations in Pickwick Reservoir were important in controlling the growth of Najas sp. Bristow (1969) concluded that concentrations of carbon dioxide greater than that at air saturation may be essential for the optimum development and growth of submerged higher plants.

In another study, increased phytoplankton populations were not always obtained with the addition of inorganic nitrogen and phosphorous to fish ponds; carbon dioxide was more directly involved in the control of phytoplankton growth (Thomaston and Zeller 1961). Wright (1960) has suggested that photosynthetic rates become limited by carbon dioxide once phytoplankton populations have fully utilized the nutrient pool in an aquatic system for chlorophyll synthesis. He concluded that the density dependence between photosynthesis and biomass, as observed in Canyon Ferry Reservoir, Montana, was due to decreases in available carbon dioxide concomitant with increases in standing crop.

For streams receiving wastewater effluents, Odum (1956) pointed out that increases in productivity are often common in recovery zones due to the addition of various nutrients such as nitrogen and phosphorous. However, this is also accompanied by enhanced carbon dioxide concentrations due to the oxidation of organic matter. Kerr et al (1970) suggested that the bacterial oxidation of organic matter is the

principle source of carbon dioxide in most aquatic systems. Carbon dioxide enrichment also occurs in the Madison River, but in this case, primarily via point source inputs from a thermal spring. Wright and Mills (1967) indicated that the enrichment of water with photosynthetically available carbon dioxide may be as important as other nutrients in enrichment causing eutrophication. They further suggest that the tendency for streams to become more productive downstream could not continue without a continued accrual of available carbon dioxide (by respiration and organic matter decomposition or by ground water recharge). In the Madison River, carbon dioxide evasion and utilization by aquatic plants below the thermal spring exceeds accrual. Thus, there is not a gain but rather a decline in this constituent, and correspondingly, productivity declines downstream.

CARBON DIOXIDE AS A LIMITING FACTOR TO PHOTOSYNTHESIS IN AQUATIC PLANTS

Various species of algae have been shown to have different carbon source requirements. As classical examples, Osterlind (1949, 1950, 1951) and Steemann Nielsen (1955) demonstrated that bicarbonate was preferred by young Scenedesmus quadricauda cultures, whereas carbon dioxide was the only carbon source available to Chlorella pyrenoidosa. Briggs and Whittingham (1952) also found that Chlorella sp. could utilize only dissolved carbon dioxide. Other examples and the importance of carbon dioxide and bicarbonate in algal photosynthesis have been thoroughly discussed in several review papers (King 1970, Kerr et al

1972, Goldman 1972, Goldman et al 1972, Likens 1972). Goldman et al (1972) concluded that free carbon dioxide, bicarbonate, carbonate "... and organic compounds can all serve as a carbon source for algae under specific conditions. Under normal conditions where free CO_2 is available it is often the preferred form of carbon used by the photoautotrophic algae (Myers 1951)."

Steeman Nielsen (1944, 1946, 1947) studied several species of aquatic angiosperms and an aquatic moss, Fontinalis sp. He concluded that bicarbonate could be utilized photosynthetically by Myriophyllum spicatum and other angiosperms but not by Fontinalis sp.; both the moss and the angiosperms could utilize carbon dioxide whereas neither taxon could use carbonate as a carbon source. Arens (1936) found that bicarbonate was actually absorbed by submerged leaves. A similar observation was made by Steeman Nielsen (1952). However, doubts as to the ability of aquatic macrophytes to utilize bicarbonate in photosynthesis have been expressed by a variety of physiologists, e.g., Wilmott (1921), Rabinowitch (1945), Briggs (1959).

Ruttner (1969) obtained results similar to those of Steeman Nielsen. He found that the aquatic moss Fontinalis antipyretica was able to use free carbon dioxide in photosynthesis at low pH values but that photosynthetic rates markedly declined as the pH of the medium increased to near 8.5 consequent to this carbon dioxide utilization. Carbon dioxide concentrations in water are quite low at this pH. Elodea canadensis

was also able to photosynthesize at low pH-high carbon dioxide levels, but this plant continued to be active photosynthetically even after the pH of the media exceeded 8.5 due to the photosynthetic removal of carbon dioxide. The photosynthetic utilization of bicarbonate at the higher pH's by E. canadensis would account for the continued increase in pH ($HCO_3^- \longrightarrow CO_2 + OH^-$). In contrast, the inability of F. antipyretica to increase pH much over 8.5 and the associated reduction in photosynthetic rates would indicate the reliance of this species upon carbon dioxide as its major carbon source.

Ruttner's (1969) data also indicate that photosynthetic rates in both species become reduced at high pH's in response to the lowered carbon dioxide concentrations regardless of the apparent ability of E. canadensis to utilize bicarbonate as a source of carbon. This is in accord with Burr's (1941) work which showed that aquatic plants are adapted to low light intensities but not to low carbon dioxide tensions. Briggs (1959) pointed to the possibility that carbon dioxide may still be the actual reactant in those systems where bicarbonate is apparently utilized with bicarbonate serving merely to increase the potential concentration of carbon dioxide at the chloroplasts.

Ruttner (1969) also found that the freshwater, red alga Batrachospermum sp. is highly dependent upon free carbon dioxide for active photosynthesis. Tseng and Sweeney (1946) made a similar observation for the red alga Gelidium cartilagineum. Ruttner (1969) further

observed that the requirement of these plants and that of the aquatic mosses for free carbon dioxide corresponded with their distributions in nature--in springs and mountain streams, and in pools and the eulittoral zone of lakes where atmospheric exchange readily takes place. In all of these habitats, adequate amounts of carbon dioxide are consistently found in the water. Bicarbonate users, in contrast, are more typical of the alkaline and relatively CO_2-free littoral zone and epilimnion of lakes. These observations prompted Ruttner (1953) to develop a classification wherein two groups of plants were delineated: (1) land plants and aquatic mosses plus the freshwater red algae able to use only free carbon dioxide (the Fontinalis-Batrachospermum-type); and (2) aquatic reed plants and algae able to use both bicarbonate ion and carbon dioxide (the Elodea-type).

Recent research, however, has revealed exceptions to this classification system. Bristow (1969) reported that the higher aquatic plants Ranunculus flabellaris and Myriophyllum brasilense can use only free carbon dioxide. As noted, some green algae, e.g., Chlorella, are restricted to the use of free carbon dioxide as a carbon source as are aging Scenedesmus cultures (Osterlind 1949). Horpestad (1969) found aquatic bryophytes growing in the Madison River of Yellowstone National Park under relatively low carbon dioxide concentrations and forming a conspicuous element of the flora in this section of the river; however, these plants were found to be quite scarce in an upstream, high carbon

dioxide reach of the stream. In addition, Wright (1969) and Klarich (1969, 1971) present data for two species of aquatic angiosperms in the Firehole and Gibbon Rivers of Yellowstone National Park that illustrate wide discrepancies in their capacity to use bicarbonate and in their reliance upon carbon dioxide for photosynthesis. Apparently the carbon source preferences of at least some aquatic plants cut across the broad taxonomic categories presented in Ruttner's (1953) classification.

Kerr et al (1972) concluded that the "availability and rate of supply of specific molecular forms of carbon (CO_2, HCO_3^-, CO_3^{2-}, and organic) can regulate the extent and rate of biological activity." However, very little specific information is available concerning the carbon source requirements and preferences of most species of aquatic macrophytes, or of the potential for these carbon species to act as limiting nutrients for aquatic macrophytes.

Sculthorpe (1967) has pointed out that most experimenters have directed their attentions primarily to the use of such habitual experimental subjects as Elodea sp. and Myriophyllum sp. which are widely distributed, easy to culture, and easily manipulated. Data for a large proportion of the macrophyte species are therefore not available. In addition, previous research has often involved laboratory experimentation with artificially constituted systems; as a result, relationships to a specific natural situation are often vague. Similar to the case

with the algae, such research has indicated that the carbon source requirements of aquatic macrophytes are quite variable and dependent upon species and conditions. Apparently either or both bicarbonate and free carbon dioxide can be utilized by these plants with varying degrees of efficiency. As stated by Sculthorpe (1967), the "...present state of knowledge of carbon sources is highly unsatisfactory. Previous experimental results conflict, interpretations differ, and numerous perplexing questions remain unanswered...."

DESCRIPTION OF THE STUDY AREA

GENERAL FEATURES

The Madison River is formed by the confluence of the Gibbon and Firehole Rivers at Madison Junction in the west-central portion of Yellowstone National Park, Wyoming, approximately 22.4 km (fourteen miles) from the western border. Unique in this Park area is the large amount of thermal activity that occurs in the form of geysers, hot springs, and mud-pots which ultimately drain into the Firehole and Gibbon Rivers. The Firehole River originates at Madison Lake and flows in a northerly direction receiving thermal discharge primarily in the lower reaches from the three main geyser basins of Yellowstone National Park. The Gibbon River begins as an outlet of Grebe Lake and flows generally in a southwesterly direction until its junction with the Firehole. The thermal discharge to this river is due primarily to the activity of the Norris Geyser Basin.

After its formation, the Madison River flows in a westerly direction and exits Yellowstone National Park near West Yellowstone, Montana. With the exception of the extreme upper reach, the Madison River is devoid of thermal activity. Approximately 1.6 km below the origin of the Madison River, there is a large, warm spring with thermal discharges both on the shore and in the river channel. Through the upper 9.6 km (six miles) of the river, the stream channel meanders through Madison Canyon. Emerging from the canyon, the river then passes through a riffle region that is characteristic of the lower, 8.0 km (five mile)

reach of the stream in Yellowstone National Park. As a result, distinct physical differences are evident in the study area between the upper Madison Canyon reach and the lower, riffle segment.

The discharge of the Madison River varies seasonally and is distinctly greater in May and June corresponding to the increased runoff after snow melt in this mountainous region. Generally, the stream recedes to a constant level by mid-July. No major tributaries enter the Madison within the defined study area.

CHEMISTRY

Ionic Constituents

Since the Madison River is formed by a union of the Firehole and Gibbon Rivers, the chemistry of the river is a reflection of that of the two headwater streams. The lower Firehole River, as the name implies, is a warm stream as the result of thermal discharges. It has a relatively high concentration of dissolved ionic constituents, a high conductivity, a relatively high total alkalinity, but it has a high pH and therefore a low free carbon dioxide content. In contrast, the Gibbon River is typically a colder stream with a lower conductivity. Total alkalinity and therefore bicarbonate concentrations also are lower than those found in the Firehole River. In addition, the sulfate concentration of the lower Gibbon River is relatively high due to a sulfate-water inflow from several thermal drainages along the lower reaches of the river. As a result of these acid drainages, pH values for

the Gibbon River are markedly lower than those of the Firehole with a concomitantly greater free carbon dioxide content. Although the lower Firehole has a greater total concentration of dissolved ionic constituents, Roeder (1966) and Wright and Mills (1967) observed that the lower Gibbon River has a greater concentration of all the major cations except sodium, which is markedly higher in the Firehole (Table 1).

Since the volume of the Firehole River is greater than that of the Gibbon, the chemistry of the Madison River more closely resembles that of the Firehole (Roeder 1966). In addition, the chemistry of the Madison River is influenced by the discharge of the thermal spring that is located immediately below its origin. This spring not only adds quantities of dissolved ions, but also has a high free carbon dioxide content (low pH) which therefore enhances the free carbon dioxide concentration of the upper Madison reaches. Because of the scarcity of calcium-magnesium-containing rocks in the region, concentrations of these cations are quite low. Sodium is by far the most dominant cation in the Madison River (Table 1). Roeder (1966) found silicate concentrations generally to be near saturation. In order of prevalence, bicarbonate, chloride, and fluoride are the predominant anions in the Madison. Runoff from thermal areas tends to enrich the waters in these constituents. As a result of rhyolite decomposition and thermal input, the Madison River and its headwater streams can be chemically classified as sodium-bicarbonate-chloride waters.

Table 1. Chemistry of river water in ppm at selected stations established within the Madison River drainage system. Stations are described by their distances from the confluence of the Firehole River (FH) and Gibbon River (G) to form the Madison River (M) at Madison Junction in Yellowstone National Park. Data taken from Wright and Mills (1967) and from Klarich and Wright (1974).

Station	Distance (km)	Na	K	Ca	Mg	HCO_3	Cl	SO_4	F	B	Fe	CO_2	pH
Wright and Mills													
FH	mouth	187.0	6.3	2.0	0.8	140.3	76.9	2.2	9.3	<0.1	0.06	0.8	8.4
G	mouth	86.3	11.3	3.9	1.0	115.9	59.9	4.3	5.0	<0.1	0.10	14.6	7.1
M1	1.6	103.5	10.2	2.7	1.0	137.3	73.8	2.8	7.6	0.4	0.20	5.5	7.6
M2	4.9	96.6	10.2	2.7	1.0	146.4	73.8	2.9	7.8	0.5	0.12	7.3	7.5
M3	7.5	96.6	10.2	3.9	1.0	152.5	70.2	2.7	7.1	0.4	0.12	4.9	7.7
M4	10.8	96.6	9.8	3.3	1.0	146.4	72.0	2.7	7.3	0.3	0.12	4.7	7.7
M5	15.9	92.0	9.8	2.7	1.0	146.4	72.0	2.8	7.1	0.2	0.10	2.3	8.0
M6	21.0	92.0	9.8	2.7	0.9	146.4	73.8	2.7	8.0	0.2	0.10	1.9	8.1
Klarich and Wright													
FH	3.2	91.5	7.4	5.0	0.1	125.7	59.3	10.1	8.7	---	0.05	0.4	8.7
G	2.4	56.8	10.1	7.8	1.0	93.9	39.4	17.3	3.6	---	0.13	16.3	7.0
M1	1.6	82.6	9.0	6.0	0.6	123.8	53.6	11.5	6.8	---	0.07	5.6	7.5
M2	4.9	83.0	9.4	6.0	0.5	125.7	53.3	12.0	6.8	---	0.07	5.3	7.6
M3	7.5	81.4	9.0	6.0	0.5	126.3	52.2	11.5	6.8	---	0.07	5.8	7.5
M4	10.8	81.0	9.0	6.0	0.5	125.1	50.4	11.0	6.7	---	0.07	3.5	7.8
M5	15.9	78.4	9.0	6.0	0.4	126.3	50.1	11.0	6.5	---	0.07	1.8	8.0
M6	21.0	80.7	9.0	5.8	0.5	126.9	50.4	10.6	6.5	---	0.07	1.1	8.3

Concentrations of dissolved constituents in the river vary seasonally in response to yearly variations in stream discharge. Concentrations are lowest in the spring because of dilution from snowmelt and enhanced runoff at this time. However, during the period of sampling on the river, a stabilization of concentration was evident relative to the attainment of base flow volumes by mid-July.

In addition, fluctuations of various chemical parameters are feasible diurnally and longitudinally through successive reaches of the river. Of importance to this particular study in relation to affects on macrophyte distribution and production was the recognition of any parameter that might distinctly vary longitudinally along the Madison River at any particular season and at any specific time of the day. As indicated in Table 1, apparently none of the major cations and anions fit into this category since their concentrations remain quite constant through all reaches of the river within Yellowstone National Park.

Phosphorus and Nitrogen

Wright and Mills (1967) found concentrations of phosphate much higher than those typically observed in eutrophic waters. However, except for a slight downstream decline, this was the case for all reaches of the Madison River. Roeder (1966) in turn found a slight downstream increase in orthophosphate concentrations as expressed through yearly sampling means and maximum-minimum values. Klarich and

Wright (1974) also found a slight downstream increase in orthophosphate, although this increase was not totally consistent through all sampling dates and sites. In contrast to orthophosphate, nitrate concentrations were found by Wright and Mills (1967) to be quite low, and less than 0.001 ppm at all stations sampled. Similar results were obtained by Klarich and Wright (1974) and would suggest that the Madison River in the Park is more nitrogen than phosphorous limited. Studies of ammonia-nitrogen and organic nitrogen revealed no distinct downstream changes (Wright and Mills 1967, Klarich and Wright 1974).

Thus, concentrations of all the major cations and anions plus orthophosphate and the nitrogen species appear to be, for all practical purposes, constant through the Madison River. As a result, these factors were not expected to markedly influence the longitudinal distribution, abundance, and physiology of aquatic macrophytes.

Free Carbon Dioxide and pH

Free carbon dioxide concentrations and pH seem to be the only major chemical parameters that distinctly change longitudinally through successive reaches of the Madison River (Table 1). The latter tends to increase downstream with a concomitant decrease of the former. These parameters also varied diurnally and therefore provided for a convenient means of monitoring river productivity since oxygen concentrations were constantly in equilibrium with the

atmosphere (Wright and Mills 1967). As observed by Todd (1967), pH values were lowest during the night at all stations. In turn, free carbon dioxide concentrations were the greatest during this period due to respiratory accrual and to a lack of photosynthesis. After sunrise, and continuing until approximately midday, pH increased and free carbon dioxide decreased with the onset of photosynthesic activity. However, at all hours during the days sampled by Todd (May 15 - October 30), pH values were lower in the upper than in the lower reaches of the Madison River; in turn, free carbon dioxide concentrations were greatest upstream and lowest downstream. Similar results were obtained by Klarich and Wright (1974).

TEMPERATURE

Extensive temperature data were obtained by previous workers from the Madison River spanning the interval from the 10th of April to the end of October (Roeder 1966, Todd 1967). As would be expected, temperatures varied seasonally and diurnally: the river was colder in the spring and fall than in the summer, and it was cooler during the evening and night hours than at midday. After early spring fluctuations, river temperatures ultimately reached a fairly stable level of greater than 20 C at midday in the middle of July following the end of spring runoff, and it maintained this relatively high level until the end of August. Wright and Mills (1967) found that mean water temperatures in the river varied between 19C and 22 C from July 21 to

September 1, 1964. From this time to the first week in November, mean temperatures gradually declined to 10 C. Evening temperatures of the river water were typically 3 C to 6 C cooler than daytime through all of the seasons.

The possibility of distinct and consistent temperature differences through successive reaches of the Madison River is important to this study in terms of affecting macrophyte distributional patterns and affecting rates of production. Such differences were evident in previous studies, but the differences were relatively small, and the direction of differences between reaches varied in relation to the season of the year. As observed by Wright and Mills (1967), temperature differences between any two reaches of the river were never more than 2 C. This is generally confirmed by Todd's (1967) extensive analysis. In addition, he noted that during spring warming periods the downstream reaches were generally warmer whereas during the cooling trend of the late summer and fall these reaches were the coolest. A major temperature gradient, therefore, has not been described for the 22.4 km reach of the Madison River in Yellowstone Park.

PHYSICAL FACTORS

Parameters considered as physical factors include turbidity, substrate texture, current velocity, and river depth. Since the turbidity of water from the Madison River was found to be no greater than

that of distilled water during stabilized flow (Wright and Mills 1967), this aspect might be eliminated as a significant ecological-physiological factor in the study area. In addition, since this river is relatively shallow, light penetration would not appear to be critical other than that related to shading from position competition in the densely aggregated, mound-type of growth common in lentic communities (Hynes 1970)--i.e., light retardation by neighboring and over-growing plants.

Depth and substrate in the Madison River are quite variable. Substrates range from large boulders and bedrock through gravel and sand to fine silt. Depths fluctuate seasonally with runoff and vary with river location. Depths in 1965 typically ranged from 0.37 m (1.2 ft) to 1.26 m (4.1 ft) after stabilization of discharge with a maximum depth approaching 1.96 m (6.4 ft) at certain points during spring runoff (Todd 1967).

Several investigators have observed a longitudinal gradation of current velocities, depths, and river widths in the stream (Wright and Mills 1967, Horpestad 1969, and Klarich and Wright 1974). Generally, as depths decrease, the river becomes wider, and current velocities increase downstream. In addition, Todd (1967) noted that the proportion of gravel and large rocks composing the river bottom increase in the lower reaches of the stream with silt rarely evident; upstream, silt and sand were found to be much more prevalent than cobble and bedrock. These observations are supported by quantitative data

from Horpestad (1969). Given the importance of current, substrate, and depth in aquatic plant ecesis as described previously, the differences in these factors among reaches must be kept in mind in relation to any physio-ecological interpretations that might be derived concerning plant distribution in the river.

AQUATIC MACROPHYTES

Floral Diversity

Observations made in this study and those by earlier investigators demonstrated the flora of the Madison River to be diverse and abundant. At least twenty-six species of aquatic plants that obviously might be classified as macrophytes have been observed in the river. These include at least one charophyte, one liverwort, several species of mosses, and a variety of angiosperms, both monocots and dicots.

Three previous investigations provided a great deal of information concerning the productivity, types, abundances, and distributions of various species of aquatic macrophytes in the Madison River. Wright and Mills (1967) compiled a species list of the dominant macrophytes in the river and briefly discussed their distributions through various reaches of the stream in the Park. Todd (1967) harvested several sections of the river, listed the more abundant species, and briefly described their distributions through six sites along the river.

The major floristic and ecological study of aquatic macrophytes in

the Madison River was completed by Horpestad (1969). He expanded the species lists compiled by previous investigators by inclusion of several less common species. In addition, he suggested several nomenclatural changes, especially with respect to the bryophytes. Ultimately, twenty species of a wide taxonomic spectrum of aquatic macrophytes were recorded by Horpestad (1969) as summarized in Table 2.

Observations made on the Madison River in recent years have contributed to a more complete and accurate representation of the flora in the stream (Table 2). These later additions are of evidently quite rare species that were not encountered by Horpestad (1969). In addition, three minor alterations in his species tabulation of aquatic macrophytes became evident in the collections made during this study.

Standing Crop

Todd (1967) determined the standing crop of the common macrophytic taxa in the Madison River; these data were pooled into relatively broad taxonomic categories in some cases, e.g., the Musci and the Potamogeton spp. Todd (1967) found that the mosses accounted for the major proportion of the standing crop in the lower, riffle segment of the Madison whereas the Potamogeton spp. and Chara vulgaris were the most abundant in the upper segment. Myriophyllum exalbescens and Berula erecta were found to be relatively common in all segments of the river. Similar trends were evident in canopy cover data of the species (Horpestad 1969).

Table 2. Species of aquatic macrophytes located in the Madison River of Yellowstone National Park.

Dicotyledonae			
**		Berula erecta (Huds.) Cov.	Umbelliferae
**	(b)	Ceratophyllum demersum L.	Ceratophyllaceae
**	(b)	Hippuris vulgaris L.	Hippuridaceae
**		Mimulus guttatus Fischer	Scrophulariaceae
**		Myriophyllum exalbescens Fern.	Haloragidaceae
	(a)	Ranunculus aquatilis L.	Ranunculaceae
	(c)	Utricularia minor L.	Lentibulariaceae
**		Utricularia vulgaris L.	Lentibulariaceae
Monocotyledonae			
	(b)	Calamogrostis canadensis (Michx.) Beauv.	Gramineae
		Carex rostrata Stokes	Cyperaceae
	(a)	Deschampsia caespitosa (L.) Beauv.	Gramineae
**		Eleocharis acicularis (L.) R. & S.	Cyperaceae
**	(b)	Eleocharis thermalis Rybd.	Cyperaceae
**		Glyceria borealis (Nash) Batchelder	Gramineae
**		Juncus ensifolius Wiks.	Juncaceae
**		Potamogeton filiformis Pers.	Potamogetonaceae
**		Potamogeton gramineus L.	Potamogetonaceae
**		Potamogeton natans L.	Potamogetonaceae
**		Potamogeton nodosus Poiret.	Potamogetonaceae
**	(b)	Potamogeton pectinatus L.	Potamogetonaceae
**		Potamogeton strictifolius Benn.	Potamogetonaceae
**	(b)	Sagittaria cuneata Sheldon	Alismaceae
**		Sparganium angustifolium Michx.	Sparganiaceae
Bryophyta			
**		Fissidens grandifrons Bird.	Musci
**	(b)	Fontinalis neo-mexicanus S. & L.	Musci
		Jungermannia sp.	Hepaticae
**		Oncophorus virens Horp.	Musci
Charophyceae (Chlorophyta)			
**		Chara vulgaris L.	Characeae
	(a)	Nitella sp.	Characeae

(a) Tabulated by Horpestad (1969 but not observed in this study.
(b) Not tabulated by Horpestad (1969) but observed in this study.
(c) Possibly present in the lower Madison River but not positively identified as a distinct species from U. vulgaris.
** Aquatic macrophytes examined experimentally in this study.

The biomass of plants in the river varies with river location, with season, and from year to year. Mean standing crops in the river were greater near the end of summer than in the spring (Todd 1967) and greater in 1965 than in 1964 or 1973 (Todd 1967, Klarich and Wright 1974). In addition, both standing crop and canopy cover were distinctively greater in the upper than in the lower reaches of the river (Todd 1967, Horpestad 1969, Klarich and Wright 1974). Todd (1967) also observed a downstream decline in the coverage of river substrate by vegetation.

Ranges of biomass observed by various investigators for the Madison River are similar to values obtained for other streams, e.g., Silver Springs, Florida (Odum 1957); Cedar Creek, Minnesota (Bray et al 1959); and River Test, England (Owens and Edwards 1962).

METHODS AND MATERIALS

SAMPLING SITE AND REACH LOCATIONS

The study area in this investigation was confined to a 22.4 km (fourteen mile) stretch of the Madison River within the boundaries of Yellowstone National Park, Wyoming. For this study, the Madison River was divided into three sections. A sampling station was located in each section at which the majority of water and tissue collections were made. A station was established in the upper Madison reach (station 1) at a National Park Service pull-out termed "Wild Animals at Home", 2.3 km from Madison Junction. The middle section had a sampling site (station 2 at Sevenmile Bridge, 10.5 km from Madison Junction. The station in the lower reach (station 3) was located at a scenic pull-out 3.4 km from the West Entrance to Yellowstone Park and 19.2 km below the formation of the Madison River. Figure 1 presents a generalized map of the study area indicating these three reaches and the corresponding sampling stations.

The Madison River was divided into three reaches primarily on the basis of pH data from Todd (1967) and other investigators but also on the basis of carbon dioxide concentrations since alkalinities were found to be fairly constant through the three stations at any given date. The upper reach had relatively low pH values (pH 7.25 to 7.65) at midday and therefore relatively high carbon dioxide concentrations in comparison to the lower reach (pH 8.10 to 8.35), and the middle reach typically was intermediate between the two extremes in both parameters

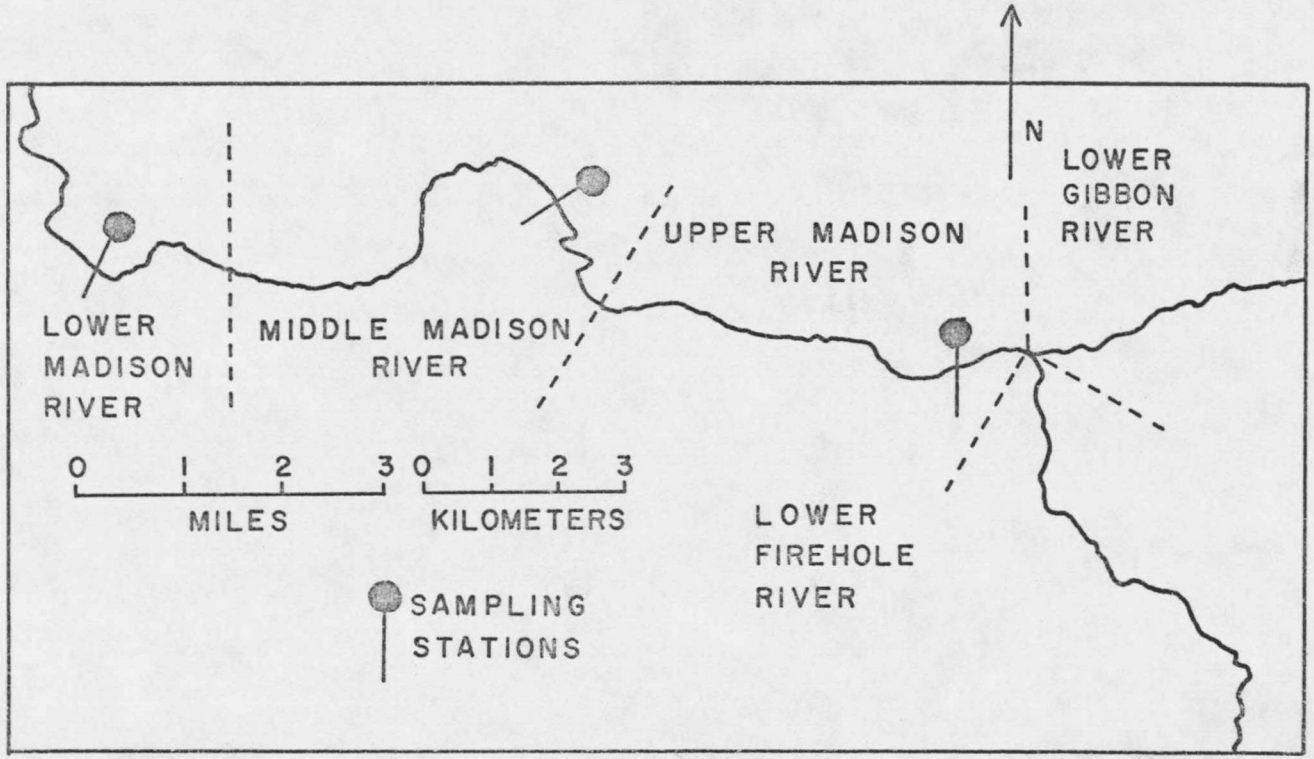


Figure 1. Generalized map of the study area showing the approximate locations of the sampling stations.

(pH 7.65 to 7.90) (Todd 1967).

SAMPLING PERIOD

The Madison study area is largely inaccessible during the late fall, winter, and early spring months due to heavy snowfall and adverse weather that is characteristic of the region during this period. This, coupled with the occurrence of peak flows and high waters in May, June, and July, restricted sampling and experimentation to a four-month time span beginning in mid-July and terminating during the first weeks of November. River discharge had largely stabilized at the start of this sampling period, and weather was not so severe as to preclude effective field work at the end of the time span.

WATER COLLECTION

Water collections were made near midstream at each sampling site using screw-cap, "Nalgene" plastic bottles with volumes of one liter. Care was taken to avoid excessive agitation of the water when filling and to exclude prominent air bubbles which could alter the inorganic carbon chemistry of the sample. The sample bottles were rinsed twice at the sample point prior to collection, refilled, and then tightly capped while still submerged (Rainwater and Thatcher 1960). The water samples were stored in the Nalgene bottles at 6 C without added manipulation and were warmed to room temperature just prior to chemical analyses and experimentation.

TEMPERATURE DETERMINATIONS AND CHEMISTRY

A complete chemical study of the Madison River was not undertaken as a part of this project since extensive chemical analyses of the stream have been completed in other investigations (Roeder 1966, Todd 1967, Wright and Mills 1967, Horpestad 1969, Klarich and Wright 1974). However, alkalinity, pH, free carbon dioxide, and temperature were investigated since these parameters are closely correlated to the central problem of this study.

At the time of sample collection, water temperatures with estimates to 0.1 C were taken with a standard mercury, laboratory thermometer. Midstream temperatures were taken at the three stations on the Madison River at one to two week intervals; individual measurements at the three river locations were generally made within one hour of each other, as a result of travel time between stations, between noon and 1500 hours.

Values of pH for the water samples were determined with a Beckman Expandomatic pH Meter immediately before experimentation. Alkalinities were determined by titrating one-hundred milliliter quantities of the sample water with 0.1N H_2SO_4 to a pH 4.5 endpoint ascertained potentiometrically as described in Standard Methods for the Examination of Water and Wastewater, 11th Edition (1960). The concentration of free carbon dioxide in the sample was then calculated from these pH and alkalinity data according to an equation derived by Rainwater and

Thatcher (1960):

$$\text{ppm } CO_2 = 1.589 \times 10^6 \left[H^+\right] \times (\text{ppm alkalinity as } HCO_3^-).$$

The necessary conversion of pH and alkalinity (as me/l) before substitution is indicated in the equation.

MACROPHYTE COLLECTION, TRANSPORT, AND STORAGE

Twenty-two species of aquatic macrophytes were studied. Most of the plant tissues utilized in the experiment were collected at the major Madison stations. In a few instances, due to scarcity, species of interest could not be found at these locations and other reaches had to be surveyed.

Experience indicated that plastic juice pitchers were the most convenient containers for plant collection and transport in the study area. These were partially filled with river water from the collection site, and the plants, clipped near the substrate level, were transferred to them for transport and temporary storage.

Approximately one hour was required to sample all stations on the Madison River; about two to two and one-half hours were then necessary to transport the water and plant samples from the study area to the laboratory. To avoid excessive heating during travel, samples were transported in a large, styrofoam cooler. Experimentation was conducted as soon as possible after arrival at the laboratory to avoid excessive modification of the plant and water samples due to prolonged storage.

Experiments were often completed on the same day that samples were collected; in some instances, experiments were conducted the following day and in all cases within forty-eight hours. Plants were temporarily stored in the laboratory at room temperature with continual room illumination (fluorescent lights providing near 500 foot candles). Glass battery jars were utilized for storage with river water supplemented by dechlorinated tap water serving as the medium.

MANOMETRY

Manometric methods are readily adaptable to the physiological study of aquatic systems. Artificially constituted waters can be used through the appropriate addition of bicarbonate and/or carbonate salts. Photosynthetic and respiratory rates may then be determined through oxygen changes with time while carbon dioxide concentrations are held constant through the CO_2 - HCO_3^- equilibrium of the enclosed atmosphere and media (Umbreit, et al, 1949). One major advantage is the fact that natural waters can be directly employed in the experiments in the same manner while metabolically induced carbon dioxide changes are largely eliminated through the inherent buffering capacity of in situ bicarbonate.

Thus, suitable plant tissues and water collected from appropriate reaches of the rivers can be directly included in the manometric system for data read-out. This affords an approximation of in situ conditions.

An illuminated, refrigerated, digital read-out Gilson Differential

Respirometer (Model GRP 14) was used in all measurements. Directions for operation are adequately described in the instruction manual for the instrument (Gilson Medical Electronics 1963). This instrument has fourteen outlets. Small reaction vessels with a volume of approximately 16 ml contained the experimental tissue bathed in a 5 to 10 ml aliquot of sample water. Only small sections of tissue cut from the collected macrophytes could be placed in the reaction vessels.

The respirometer contained a built-in light source underneath the plastic-bottomed water tank which provided 780 foot candles at the surface as determined by a Gossen light meter. Both respiratory and photosynthetic data were generally obtained from the various tissue preparations during an individual run. During the respiration phase of a run, a dark sheet was placed over the water bath and submerged reaction vessels to ensure darkness. The photosynthetic and respiratory rates of the tissues were calculated as microliters (ul) of oxygen evolved or consumed per minute per mg dry weight. The respirometer was operated at 21 C for all of the Madison River experiments. This temperature was selected since it is fairly representative of the typical, midday temperature of the river.

TISSUE SAMPLE PREPARATION

Basic materials of the study were the water and plant samples collected from the rivers. Prior to any sectioning of harvested plants all obvious macro-periphyton and large animals were carefully removed

from the tissues with forceps and dissecting neddles. The former included primarily the filamentous algae such as Cladophora, Spirogyra, and Tribonema whereas the latter consisted of such organisms as caddis fly larvae, flatworms, and snails. In this study, the metabolic contributions of micro-periphyton (primarily diatoms and bacteria) and other microorganisms which could not be directly and readily removed from the test tissues were assumed to be insignificant relative to the photosynthetic and respiratory activities of the macrophyte sections. In all cases, the test plants were carefully rinsed before sectioning and insertion into the reaction vessels.

Tissues from the collected plants were selected and sectioned with dissecting scissors. All tissue sections necessary for the various replications of an experiment were cut and pooled into a common container prior to insertion into the reaction vessels. The number of sections that could be included in each vessel was dependent upon the morphology of the particular plant being tested, but typically several tissue sections were included in each sample. Individual sections were randomly taken from the common pool and transferred in no particular sequence to the respirometer vessels which contained an appropriate volume of sample water. This was continued until each reaction vessel had the appropriate amount of tissue.

MORPHOLOGICAL CONSIDERATIONS

A great deal of variation exists in the size and morphology of the various aquatic macrophytes used in this study. At the lower size limit are the minute mosses such as Fissidens grandifrons for which the entire body of an individual gametophyte is less than 10 cm in length. From this minimum, the plants ranged through a series of intermediate sizes (e.g., Berula erecta, Glyceria borealis, and Utricularia vulgaris) to the quite large pondweeds such as Potamogeton natans and P. nodosus which are several decimeters in length. To illustrate the morphological diversity of the plants, the shoot systems of the various species can be divided into several morphological groups (with examples) as follows:

(1) diminutive, compact gametophyte (Fissidens grandifrons);

(2) filamentous gametophyte (Oncophorus virens);

(3) rosette-like (Mimulus guttatus);

(4) filiform (Eleocharis spp.);

(5) stiff, rush-type leaves (Juncus ensifolius);

(6) flexible, ribbon-like leaves (Sagittaria cuneata);

(7) excurrent pattern (central axis with branches, if any, lateral) (Myriophyllum exalbescens);

(8) deliquescent pattern (no distinct, dominant central axis and many branches (Potamogeton spp.).

This variation in size and morphology of the macrophytes posed a variety of individually unique problems and difficulties related to tissue selection, sectioning, and experimental interpretation.

In all instances, attempts were made to select and run the tissue sections so that the data were representative of the major portion of the plant shoot system rather than that of an isolated tissue type. In some cases this was easily attained where the particular size and morphology of a plant allowed the inclusion of all or at least a high proportion of the shoot in the reaction vessels. In other cases, the size and morphology of a species precluded the use of a large fraction of the shoot system. In such cases, estimates of shoot system physiology were therefore made, of necessity, by indirect means.

For the relatively large macrophytes, two to several experiments were conducted using a particular morphological part in each experiment--leaves, stems, and petioles of the deliquescent plants (e.g., Potamogeton natans, and P. nodosus), or the apical or basal regions of excurrent plants such as Hippuris vulgaris. Following experimentation, several other intact plants were fractionated into the particular morphological parts in order to determine what proportion each part contributes to the shoot system weight of the macrophyte. The photosynthetic and respiratory rates obtained for the sample of tissue from each plant part were then adjusted using the corresponding proportional weight

factor. A summation of these values provided a composite rate statistic that estimated the metabolic rates of the entire shoot system of the aquatic macrophyte.

In cases where both the stem and leaf tissue of deliquescent plants could be included in a sample, metabolic rate data representative of the entire shoot system were obtained by first sectioning entire plants (excluding the root systems) into appropriately sized pieces which were then placed in a large container. Several pieces were then randomly added to each of the reaction vessels. It was assumed that all tissue types of the plant would be represented through the treatment replicates and photosynthetic and respiratory rate differences between types ultimately "averaged-out" to afford an estimate of the metabolic rates of the entire shoot system. Photosynthetic and respiratory rates for the smaller deliquescent plants such as Potamogeton filiformis and P. strictifolius were determined through this procedure.

After the manometry was completed, the tissue samples were dried for about four hours at 105 C, and dry weights were determined with a Mettler balance.

EXPERIMENTAL DESIGN AND BASIC TREATMENTS

Generally three types of data were obtained in a manometric experiment in the following pattern:

(1)Eq---(2)L---(3)Eq---(4)1/2L---(5)Eq---(6)D.

In the above sequence, "Eq" denotes an equilibration period with the manometers open to the atmosphere. "L" and "1/2L" denote light periods for the measurement of photosynthetic rates--at full respirometer illumination and at one-half respirometer illumination respectively. "D" denotes a dark period for respiration rate determinations. The first equilibrium phase, "(1)Eq", represents the initial temperature equilibration of the system to 21 C (about 30 to 45 minutes necessary). Data as microliters of oxygen evolved or used were taken from the micrometers at appropriate time intervals during an experiment. These intervals varied from 5 to 10 minutes with L, 1/2L, and D, with the particular treatment, and with the species used in the experiment. The L, 1/2L, and D phases were run and data recorded at each time interval until linearity of gas exchange rate was observed in each of the replicates of a treatment. In some of the experiments, a particular phase (L, 1/2L, and/or D) was repeated for confirmation if time was available.

For the majority of the experiments, water samples were collected from the three major stations on the Madison River (Figure 1). These were utilized unaltered and represented the basic treatments of the study. That is, the photosynthetic and respiratory rates of the various species in these three samples were assumed to closely represent the in situ rates of the macrophytes in the upper, middle, and

lower reaches of the Madison River. Further interpretations of the *in situ* effect of carbon dioxide on macrophyte photosynthesis were based on these data.

Some variations in free carbon dioxide concentrations with date and time of collection were observed at the individual stations, but the water obtained at each site was utilized in the experiments as collected with no attempt to alter pH to any given value.

ESTIMATES OF LIGHT SATURATED PHOTOSYNTHESIS

One major disadvantage of the manometric methods described relates to the discrepancy between natural river illumination and that provided by the Gilson Respirometer. Studies on the Madison River in 1970 indicated a typical illumination value of near 6,000 foot candles, as measured with a Gossen light meter calibrated in foot candles, at midday in August with no overcast. Maximum illumination obtained from the instrument was about 780 foot candles. Thus there is the distinct probability that the photosynthetic data obtained in the experiments would not be directly equatable to the case *in situ* due simply to such a disparity in illumination.

This difficulty was circumvented to some extent by also measuring photosynthetic rates at one-half full instrument illumination by using screens which transmitted about 390 foot candles between the light source and samples. Photosynthetic rates (PS) at light saturation for

any treatment were estimated by using the ratio of photosynthetic rates at full illumination (L) to those at one-half light intensity (1/2L). The major assumption in this manipulation is that light saturation curves approach that of a rectangular hyperbola:

$$Y = Y_{max}X/(k + X),$$

where k represents the value of X corresponding to $Y_{max}/2$ and Y_{max} represents the asymptote. This is probably the best mathematically workable model of photosynthetic rates as a function of light intensity currently available (Pickett 1969, personal communication). This rectangular hyperbolic model can be expressed in terms of photosynthetic rates and light intensity as follows:

$$PS_I = PS_{sat}I/(k + I),$$

where PS_I is the photosynthetic rate observed at some light intensity, I, and PS_{sat} is a constant representing a maximum photosynthetic rate in a sample at light saturation.

This model was utilized to calculate from experimentally measured values a photosynthetic rate at light saturation for each treatment. The various algebraic manipulations necessary in the derivation of usable expressions are not presented; however, three basic and critical working equations are summarized in Appendix A. The graphs in Figure 2 were derived from these working equations. With (PS at L)/(PS at 1/2L) = r, Figure 2 can be utilized to obtain a correction

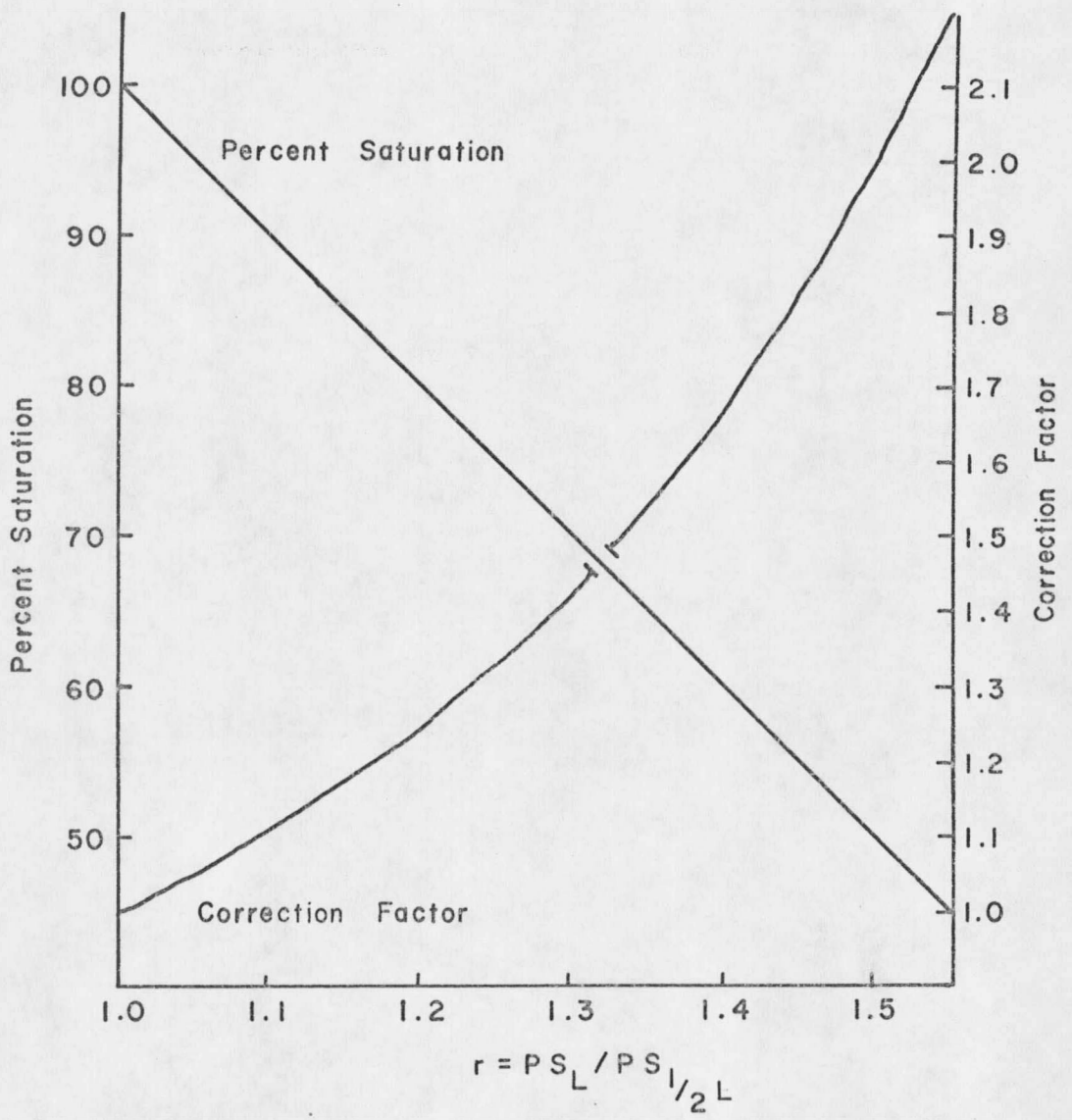


Figure 2. Relationship between percent light saturation of photosynthesis (PS) at respirometer illumination (L = 780 foot candles) and the ratio (r) of net photosynthetic rates at full respirometer illumination to rates at 50% illumination ($\frac{1}{2}$L); also plotted are the corresponding correction factors to adjust PS_L to PS at saturation.

factor (F) to adjust PS at L to PS_{sat} as follows:

$$PS_{sat} = F(PS \text{ at } L).$$

In turn, with r known, the percent light saturation for a tissue at L (respirometer illumination) in a particular water sample can also be obtained from Figure 2.

These manipulations provide only estimates of in situ saturated photosynthetic rates. Such data become most reliable in those cases where saturation intensities in the river approach that available from the respirometer, that is where an "extravagant" illumination impinges upon a particular macrophyte or where PS at 1/2L ($PS_{1/2L}$) approaches PS at L (PS_L). In turn, as the difference between PS_L and $PS_{1/2L}$ for a treatment and species increases (as r increases) the reliability of the estimate declines. However, given the wide disparity between natural and experimental illuminations, such derived rates are probably closer to the actual case than the raw PS_L data obtained directly from the experiments.

PRIMARY CALCULATIONS

Photosynthetic and respiratory rates were determined for each sample by using the readings taken directly from the micrometers of the respirometer. The sequential stretch of readings exhibiting an apparent linearity was used for each phase of the L - 1/2L - D sequence. Rates for each replication were calculated by summing the individual

values of each time interval through the linear sequence of a phase and then by dividing this figure by the total time of the sequence and by the dry weight of the tissue. The results from the replicates of a treatment were then averaged. This provided the major respiratory statistic of a treatment. Where 1/2L data were not obtained, these manipulations also provided the main photosynthetic statistic. In most cases, however, a further adjustment of the photosynthetic data was made which led to an estimate of mean photosynthesis at saturating light intensities (PS_{sat}) under the conditions of a treatment as described previously.

In a few cases, $PS_{1/2L}$ was found to be greater than PS_L with r less than one. This relationship may denote a destruction of the photosynthetic apparatus through an application of unfavorable treatments at instrument light intensities. In these instances, the r values were ignored, and PS at 1/2L was considered to be representative of PS_{sat}.

Photosynthetic rates so obtained were representative of a net photosynthesis (PS_N) since respiration also occurs during the light interval. The addition of a species' respiration rate to its net photosynthetic rate affords an estimate of gross photosynthesis (PS_G) in a treatment: $PS_N = PS_G - R$. Photosynthetic:respiratory ratios (PS_N/R or PS_G/R) were also determined for the treatments as were the

percentages of light saturation of photosynthesis at the maximum light intensity of the respirometer.

ACCESSORY TREATMENTS AND EXPERIMENTS

All species collected from the Madison River were tested with the three basic treatments in a main experiment as described previously. In addition some of the macrophytes were exposed to various accessory treatments as a part of the same experiment. In such accessory treatments, carbon dioxide concentrations of the collected water samples were altered through various means and corresponding changes in photosynthetic rates noted. This was done to confirm that carbon dioxide was the major causal factor of any photosynthetic rate differences between treatments.

Three methods of water alteration were employed in the study. Each method had certain advantages and disadvantages as compared to the others.

The first type of accessory experiment (Method I) involved an alteration of carbon dioxide concentrations prior to loading the reaction vessels. The carbon dioxide concentration of a water sample aliquot was increased or decreased by bubbling carbon dioxide gas (or air) or nitrogen gas through the aliquot of water until the desired higher or lower pH was achieved. Reaction vessels were then prepared with this altered water and manometric measurements made as described previously.

Two other methods of carbon dioxide alteration proved to be more applicable in some cases. In these, reaction vessels that had been previously prepared with water and tissue for a basic treatment were reused, and the accessory run was conducted after the completion of the main experiment. Such accessory treatments were not always completed with the entire pattern of L - 1/2L - D phases; typically the D phase was eliminated since respiratory data had already been obtained in the previous manipulations.

In one of these methods (Method II), the prepared reaction vessels, tissue and water, were stored in the dark for several hours after the completion of the main run. As a result, the pH values of the sample water in the replicates of the basic treatments were expected to approach some similar value after this storage. In the lower Madison water sample, pH would be expected to decrease because of an accrual of carbon dioxide through respiration. In the upper Madison sample, with its initially high carbon dioxide content, evasion to the atmosphere should exceed any respiratory accrual with a concomitant increase in pH. In some of the experiments, quantitative estimates were made of the pH values that might be obtained after dark storage of the basic treatments; similar proportions of tissue to sample water were stored in this case as in the basic treatments but in larger amounts so that the pH could be measured.

In the third of these methods (Method III), carbon dioxide concentrations of the previously tested treatments were increased by bubbling air directly into the water of each reaction vessel. Remonitoring of photosynthetic rates was then conducted immediately following this application. A semi-quantitative approach was generally used by passing air for different lengths of time through two of the treatments with the third left untouched as a control. Typically, twenty-second versus forty-second exposures were utilized, and the latter were expected to contain a greater concentration of free carbon dioxide than the former.

Certain of the aquatic macrophytes were also examined through one or two additional experiments with fresh tissue. Typically, an incomplete, preliminary experiment was conducted in which various experimental difficulties unique to a type of macrophyte were pinpointed and these difficulties then circumvented in the subsequent main experiment. In addition, a third experiment was occasionally conducted with certain of the species following the main experimentation. This confirmatory or final experiment was included to substantiate the photosynthetic response of a plant to carbon dioxide changes and to confirm the absolute rate values that were obtained in the major experiment. All physiological comparisons and ecological considerations with any particular species beyond the intra-species level were made with data

obtained from the main and complete experiment.

EXPERIMENTAL LIMITATIONS

General Factors

Three to five hours were required to complete a manometric experiment with the L - 1/2L - D phases. This time depended upon the activity of the particular tissue and upon the rapidity of the equilibrations. With this time added to the one to two hours required for preparation, between four and seven hours were necessary to conduct a complete experiment. Added to this could be the time required for collection and travel to, in, and from the field.

Experience indicated that the most consistent results were obtained if all of the various treatments of an experiment were included in a single respirometer run, or in two runs conducted within no more than twelve to fourteen hours of each other. If the treatments were completed at widespread intervals, complications arose related to the adverse influences of prolonged storage of the tissues under laboratory conditions. Data from two runs so separated in time were found to be considerably different.

In certain of the early experiments, attempts were made to include four treatments (water from four river stations) in an experiment. In many cases time factors restricted the design of an experiment to a single respirometer run with the three phases and equilibrium periods

in order to avoid excessive storage. The availability of only fourteen outlets on the Gilson Respirometer in turn limited the number of adequately replicated treatments that could be simultaneously included. Ultimately, experiments consisting of three treatments with four to five replications of each treatment were found to be the most satisfactory in providing the requisite amount, type, and consistency of data.

In addition to time factors, experimentation was also limited by the abundance of a particular species in the river. Several of the macrophytes listed in Table 2 were not studied simply because of the small number of plants that could be found. Further, the difficulty of sampling species with a scattered distribution often precluded any extensive experimentation with these macrophytes, e.g., Potamogeton strictifolius. This aspect was compounded with the smaller plants that were, in addition, difficult to see from the surface, e.g., the mosses. Most satisfactory were those abundant and relatively large macrophytes aggregated to form relatively homogeneous mounds such as Myriophyllum exalbescens, Chara vulgaris, and Berula erecta.

Accessory Treatments

Of the three methods of carbon dioxide alteration, Method I had the advantage of being quantitative in that carbon dioxide concentrations of the altered sample water could be calculated from pH and

alkalinity data. It also had certain disadvantages. The addition of an accessory treatment reduced the number of replications possible for the basic treatments in a single manometric experiment. Furthermore, if a single experiment required two respirometer setups to complete, the extra time needed for the second one increased the probability of adverse storage effects. Nevertheless, this method was found to be the most satisfactory of the three and therefore was applied in all cases where experimental considerations proved amenable to its use.

The major advantage of Methods I and II was the conservation of preparation time. In addition, plant sampling in these cases was not as extensive when tissues were re-used; this proved to be important for the more rare and scattered macrophytes in the Madison River. Also, the accessory treatments did not reduce the number of replicates possible in the basic treatments. One disadvantage was the fact that carbon dioxide concentrations could not be directly quantified with the equipment available, i.e., the impossibility of pH measurement given the small volume of water plus tissue in the reaction vessels. In contrast to the direct water alteration procedure (Method I) which employed freshly sectioned tissues, the secondary use of prepared tissues in Method II and III increased the possibility of deterioration related to storage and manipulation. This latter aspect was more prevalent in

Method II than in III since the former required an additional storage period.

STATISTICAL ANALYSES

Photosynthetic rate data calculated directly from respirometer readings (PS_L) were used in the statistical analyses. Rate data from the three basic treatments were statistically examined as a single unit. With additional accessory treatments in an experiment, the data from these treatments were in some cases pooled with the basic treatments and the combined sets of data reexamined through more extensive statistical calculations. However, statistical conclusions were drawn primarily from the basic treatments with the results from the accessory treatments and experiments providing confirmation.

Analysis of variance (ANOVA) methods were first applied to the data (Lentner 1969). Typically, the more simple one-way classification (completely random design) proved to be the most efficient technique for the experimental design of the study. The common case of unequal replications also dictated the use of this simple ANOVA. In a few cases, the experiments were so designed as to allow for the application of the more complex two-way classification techniques (randomized, complete block design) with blocking made on some morphological aspect. If the calculated F-value was significant to at least 10%, the differences between the individual treatment means were then tested against

each other using the Newman-Keuls Multiple Range Test (Lentner 1969). The ranking of treatments requisite in this particular test was made on the basis of carbon dioxide concentrations.

Other statistical tests were also applied to various sets of data in addition to the primary statistical analyses described above. These include Student-t tests, rank and parametric correlations, linear regression analyses, and standard deviation calculations.

RESULTS--FIELD OBSERVATIONS

AQUATIC MACROPHYTES IN THE MADISON RIVER

Revisions to the River's Flora

Observations made during the course of sampling in this study have contributed to a more complete and accurate representation of the flora in the Madison River. These are primarily additions of six rare species (Table 2) not encountered by Horpestad (1969) or other investigators. In addition, three minor alterations in Horpestad's tabulation of aquatic macrophytes became evident.

First, Horpestad lists only the family nomenclature for the charophytes (Characeae) which includes both Chara spp. and Nitella spp. He found this necessary due to the lack of an efficient and quick separation and taxonomic identification of these macrophytes in the field (Horpestad 1970, personal communication). Although Nitella spp. could feasibly be present in the Madison River since it is abundant in the lower Firehole (Rasmussen 1968), a number of samples collected for this study failed to reveal any of this genus in the study area. Thus, the dominant charophyte in the Madison River appears to be Chara vulgaris as suggested by Wright and Mills (1967).

Second, Horpestad (1969) listed two species of Gramineae in the Madison River--Glyceria borealis and Deschampsia caespitosa. He described the former as occurring in all reaches of the river but recorded D. caespitosa only in the upper sections and in the Gibbon River.

However, identification based on vegetative characteristics indicated that the major graminoid in recent collections from the river was G. borealis; D. caespitosa was then assumed to be entirely absent or extremely rare in the stream. Wright and Mills (1967) and Todd (1967) also listed G. borealis as the common aquatic grass in the Madison River.

Finally, Horpestad (1969) lists only Sparganium angustifolium as being in the river although two distinct morphological forms of leaves were discovered in this study. Hotchkiss (1967) presents a classification of aquatic macrophytes based solely on morphological characteristics for use in the field. He lists Sparganium (burreeds) and the fully aquatic form of Sagittaria cuneata (arrowhead) under a group characterized by possessing "limp, thread-like to ribbon-like leaves". These species in situ appear quite similar when viewed from the surface. One, lacking a distinct midrib, represented the Sparganium angustifolium noted by Horpestad whereas the other, possessing a distinct midrib and a different pattern of leaf venation, was ultimately identified as the submerged form of Sagittaria cuneata. Due to their morphological similarity and to the relative scarcity of S. cuneata, Horpestad apparently tabulated both of these types as a single species.

With these revisions and the addition of six rare species, the list of species in Table 2 appears to be a fairly accurate representation

of the macrophyte flora in the Madison River of Yellowstone Park. Twenty-six species have been definitely recorded for the river. This would be expanded to twenty-eight with the occurrence of Nitella spp. and D. caespitosa. In addition, Wright and Mills (1967) listed Potamogeton alpinus (G, B.) as occurring in the river, and Todd (1967) recorded Sparganium chlorocarpum (Rydb.) (probably S. angustifolium). Both of these investigators also observed Hygroamblystegium fluviatile, a bryophyte, but this identification was later changed to Oncophorus virens by Horpestad (1969).

Distribution and Abundance

Horpestad (1969) obtained abundance data as percent canopy cover for twenty species of aquatic macrophytes at eight stations along the Madison River. The percent canopy cover for each species at Horpestad's stations which corresponded to an upper, middle, or lower reach in the study area (Figure 1) were averaged to provide estimates of the species' canopy cover in each reach. These data are summarized in Table 3. Horpestad's data for Glyceria borealis and Deschampsia caespitosa were combined and considered to be representative of the canopy cover of G. borealis alone since D. caespitosa is apparently absent from the river.

An adjustment of Horpestad's Sparganium angustifolium data also became necessary since S. angustifolium and the morphologically similar Sagittaria cuneata were both found in the river rather than the former

Table 3. The percent canopy cover (CC) and relative abundances (RA) of aquatic macrophytes in three reaches of the Madison River. An asterisk denotes those species examined in this study.

Taxa	Madison River Reach						Total
	Upper		Middle		Lower		
	CC	RA	CC	RA	CC	RA	RA
Tabulated by Horpestad:							
*Berula erecta	38.0	21.1	23.3	22.6	13.8	34.2	77.9
Carex rostrata	0.2	0.1	0	0	0	0	0.1
*Chara vulgaris	24.0	13.3	26.1	25.3	2.1	5.2	43.8
Nitella sp.	(Not Observed)						
*Eleocharis acicularis	0.3	0.2	1.6	1.6	0	0	1.8
*Fissidens grandifrons	0.2	0.1	2.0	1.9	2.8	6.9	8.9
*Glyceria borealis	21.1	11.7	12.1	11.7	0.9	2.2	25.6
Deschampsia caespitosa	(Not Observed)						
*Juncus ensifolius	1.6	0.9	0.1	0.1	0	0	1.0
Jungermannia	0.9	0.5	0	0	0	0	0.5
*Myriophyllum exalbescens	37.7	20.9	10.9	10.6	14.3	35.4	66.9
*Mimulus guttatus	0.6	0.3	Rare	0	0	0	0.3
*Oncophorus virens	1.4	0.8	2.5	2.4	5.9	14.6	17.8
*Potamogeton filiformis	12.3	6.8	12.2	11.8	0	0	18.6
*Potamogeton gramineus	0.3	0.2	0.8	0.8	0.3	0.7	1.7
*Potamogeton natans	4.8	2.7	2.6	2.5	0	0	5.2
*Potamogeton nodosus	30.8	17.1	3.6	3.5	0	0	20.6
*Potamogeton strictifolius	1.3	0.7	0.8	0.8	0	0	1.5
Ranunculus aquatilis	0	0	0.2	0.2	0	0	0.2
*Sparganium angustifolium	1.1	0.6	1.5	1.5	0	0	2.1
*Sagittaria cuneata	0.5	0.3	0.2	0.2	0	0	0.5
*Utricularia vulgaris	3.3	1.8	2.6	2.5	0.3	0.7	5.0

Table 3. Continued.

Taxa	Madison River Reach						Total
	Upper		Middle		Lower		
	CC	RA	CC	RA	CC	RA	RA
Total Abundance:	180.4	100.1	103.1	100.0	40.4	99.9	300.0
Abundance of Experimental Species:	179.3	99.5	102.9	99.8	40.4	99.9	299.2
Not Tabulated by Horpestad:							
Calamogrostis canadensis	Rare		Absent		Absent		
*Ceratophyllum demersum	Absent		Common		Absent		
*Eleocharis thermalis	Rare		Absent		Absent		
*Fontinalis neo-mexicanus	Absent		Rare		Rare		
*Hippuris vulgaris	Absent		Common		Rare		
*Potamogeton pectinatus	Rare		Common		Absent		

alone. This adjustment was accomplished by surveying two locations in each of the three reaches and by counting the individual plants of each species at each location. The percentages of S. angustifolium and S. cuneata individuals in each reach were found to be:

Species	Upper Reach	Middle Reach	Lower Reach
S. angustifolium	70%	90%	0%
S. cuneata	30%	10%	0%

These percentages were then used to adjust Horpestad's canopy cover for S. angustifolium to an estimate of the canopy cover of both species; these adjustments are included in Table 3.

The percent canopy cover in the river varied both between species and between reaches (Table 3). The differences in abundance of macrophyte vegetation between segments of the Madison River are aptly illustrated through comparisons of the total canopy cover of each reach. To eliminate this inter-reach variation for comparative purposes, the relative abundance of each species in a reach was determined as its canopy cover divided by the total canopy cover of the segment. This variable allows for the comparison of vegetation between reaches in terms of floristic composition and species dominance. That is, a comparatively uncommon taxon on a river-wide basis could prove to be the major floristic constituent of a particular reach if the total canopy cover of that reach happened to be relatively low. These aspects are more readily evident in the relative abundances of the species in each

reach than in their canopy cover data. In turn, a summation of the relative abundances of each species through the three reaches of the river should provide an indication of the taxon's conspicuousness through the entire study area (Table 3).

Distribution and abundance data are also available for the Madison River from Todd (1967). He obtained standing crop data (g/m^2) for only the more common macrophytes listed by Horpestad (1969), and he considered the genus Potamogeton and the three species of Musci as two broad categories. A modification of Todd's harvest data for the various taxa are presented in Table 4 and expressed for each reach as a mean of the standing crop of each species harvested at those stations that were included within a particular reach of the river as defined in Figure 1. The relative abundance variable is also of value for comparing the standing crop data obtained by Todd (1967) to abundance data obtained by Horpestad (1969) using a different method. As a result, the relative abundances of the species calculated from the data of both studies are also included in Table 4.

Qualitative estimates of the relative abundances of the six rare species encountered in this study were made from observations taken during the course of sampling. These are illustrated in Table 3 by the "common", "rare", and "absent" designations. Species listed as "common" for a reach were those regularly seen during the various

Table 4. Standing crop (grams dry weight per square meter) of the major taxa of aquatic macrophytes found in the three reaches of the Madison River (data modified from Todd 1967). Also included are relative abundance (RA) values (percentages) calculated from both standing crop (SC) and canopy cover (CC) data (Horpestad 1969).

Taxa	Madison River Reach											
	Upper			Middle			Lower			Totals		
	SC	RA-SC	RA-CC	SC	RA-SC	RA-CC	SC	RA-SC	RA-CC	SC	RA-SC	RA-CC
Berula erecta	29.5	10.8	21.1	11.4	9.7	22.6	5.9	10.8	34.2	46.8	31.3	77.9
Chara vulgaris	129.4	47.2	13.3	40.3	34.3	25.3	1.5	2.7	5.2	171.2	84.2	43.8
Glyceria borealis	10.5	3.8	11.7	20.8	17.7	11.7	Trace	0	2.2	31.3	21.5	25.6
Musci	0.3	0.1	0.9	17.3	14.7	4.3	39.2	72.3	21.5	56.8	87.1	26.7
Myriophyllum exalbescens	11.6	4.2	20.9	8.8	7.5	10.6	7.3	13.5	35.4	27.7	25.2	66.9
Potamogeton sp.	66.3	24.3	27.5	8.6	7.3	19.4	0	0	0.7	74.9	31.5	47.6
Sparganium-Sagittaria	26.7	9.7	0.9	10.3	8.8	1.7	0.3	0.6	0	37.3	19.1	2.6
Totals	274.3	100.0	96.3	117.5	100.0	95.6	54.2	99.9	99.2	446.0	299.9	291.1

sampling dates. A canopy cover near 2.0% might represent a reasonable intuitive estimate of their abundance. "Rare" species are considered to be those that were observed infrequently in a particular reach with only a few individuals noted. This would most likely relate to a canopy cover of about 0.1%. Those listed as "absent" were never observed in a particular segment of the river.

ALKALINITY, pH, AND FREE CARBON DIOXIDE

Analyses of alkalinity on water samples collected in this study revealed a slight but consistent increase in this parameter from the upper to the lower stations on the Madison River. This increase was quite small, however, and averaged only 0.063 me/1. Mean values of alkalinity for the upper, middle, and lower stations through the sampling period were found to be 1.98, 2.02, and 2.05 me/1 respectively. A similar increase of alkalinity was observed by Todd (1967) in weekly tests through the same sampling period (mid-July to the end of October). However, such changes were not evident in the data obtained by Roeder (1966), Wright and Mills (1967), or Horpestad (1969).

Alkalinity was also found to vary seasonally. Lower values were obtained during the early part of the sampling period with the higher river flows than were observed for the late October-early November period.

Values of pH at any particular time also increased downstream

through the three stations and reaches of the river. The diurnal and seasonal fluctuations in pH at any station made it impossible to sample for a specific pH that would characterize a station or reach. Thus ranges of pH were found to be more useful in characterizing the three sections of the river. Water samples were collected from the river on a weekly to bimonthly basis between 1000 and 1600 hours from mid-July to the first of November. Values for pH were determined on all samples. From these data, the reaches and stations of the Madison River were classified as follows:

upper--93% of the pH readings were greater than 7.25 but less than 7.65;

middle--95% of the pH values were at 7.65 or greater but less than 7.90;

lower--93% of the values were greater than 8.10 but less than 8.35.

In turn, free carbon dioxide concentrations were found to decline downstream. Water samples from the upper Madison site consistently had higher concentrations of this chemical component than water samples from the lower Madison, and the free carbon dioxide concentrations of water samples from the middle reach were consistently intermediate to those in samples from the other two locations.

TEMPERATURE

Temperature determinations on the Madison River were made at the same time that water samples were collected from the three reaches of the stream. From mid-July to the end of August, the mean temperature of weekly to bimonthly readings from the river between 1000 and 1600 hours was 20.6 C with the three station averages as follows: upper site--20.7 C, middle site--19.9 C, and lower site--21.1 C. Such mid-summer, midday temperatures for the Madison are closely equivalent to the 21 C temperature chosen for the respirometer experiments.

Toward the end of October, midday temperatures in the Madison River declined to near 10 C. Average temperatures in the river for the entire sampling period were as follows: upper site--17.4 C, middle site--16.5 C, lower site--17.1 C, and the entire river--17.0 C. An average temperature difference of 1.0 C at approximately the same times (one-half to one hour differences between stations due to travel) was observed between the three stations on the Madison River. Since this difference was relatively small, and since the direction of the temperature decline varied seasonally (Todd 1967), a constancy of temperature between the reaches of the Madison River was assumed in this study.

RESULTS--MANOMETRIC EXPERIMENTS

CARBON DIOXIDE ALTERATIONS

Three methods of altering carbon dioxide concentrations in water samples were employed. For quantification in Method II, beakers containing proportions of sample water to tissue similar to those in the reaction vessels were simultaneously stored in the dark with the prepared vessels. Readings of pH were then made on the water, and these values were assumed to be closely equivalent to those in the reaction vessels. Table 5 summarizes the results of pH change in several such experiments with different plant tissues. In most cases, the pH established after the storage period approached a value between that common to the lower reach of the Madison River and a pH characteristic of the upper segment of the stream.

Accessory treatments using the three methods of carbon dioxide alteration were included in the experiments to provide evidence that photosynthetic rate differences between the basic treatments for a given species resulted from the differences in carbon dioxide concentrations between sampling sites rather than the differences in some other variable. If carbon dioxide was a causal factor, the photosynthetic processes of the aquatic plants would be expected to respond to its alteration via these methods in the accessory treatments.

Data in Table 5 indicate that the pH of the different tissue-water samples, and consequently the media's carbon dioxide

Table 5. Values of pH obtained from the dark-storage method of carbon dioxide alteration in tissue-water experimental systems; blanks are post-storage pH values of water without the macrophyte tissues.

	Source of Water Sampling Site		
(1) *Berula erecta* (19 hours storage)			
	Upper	Middle	Lower
Pre-storage	7.22	----	8.00
Post-storage	7.63	----	7.65
Blanks	8.43	----	8.52
(2, 3) *Potamogeton nodosus*, *Juncus ensifolius* (13.5 hours storage)			
	Upper	Middle	Lower
Pre-storage	7.62	7.86	8.37
Post-storage (*Potamogeton*)	7.82	7.82	7.85
Post-storage (*Juncus*)	7.60	7.70	7.77
Blanks	8.32	8.51	8.53
(4) *Hippuris vulgaris* (11.5 hours storage, replicated)			
	Upper	Middle	Lower
Pre-storage	7.53	7.87	8.29
Post-storage (1)	7.55	----	7.53
Post-storage (2)	7.66	----	7.60
Blanks	8.36	----	8.48
(5) *Hippuris vulgaris* (18.5 hours storage, replicated)			
	Upper	Middle	Lower
Pre-storage	7.57	7.81	8.20
Post-storage (1)	7.66	----	7.58
Post-storage (2)	7.58	----	7.62
Blanks	8.40	----	8.53
(6) *Mimulus guttatus* (15 hours storage)			
	Upper	Middle	Lower
Pre-storage	7.33	7.73	8.29
Post-storage	7.63	7.70	7.69
Blanks	8.35	8.42	8.42

concentrations, tended to approach the same value after storage. This tendency for the different preparations to acquire similar pH values after storage is due to an evasion of carbon dioxide from the upper Madison sample with its initially high carbon dioxide concentrations coincidental with the respiratory accrual of carbon dioxide in the lower river sample with its initially low concentrations of this constituent. Thus carbon dioxide levels would tend to decrease in the former case and increase in the latter and approach similar concentrations. In turn, if the photosynthetic processes of the macrophytes were responsive to changes in free carbon dioxide concentrations, several expectations are evident in relation to Table 5 in terms of the relative magnitudes of photosynthetic rates that should be obtained from the Method II accessory treatments.

First, photosynthetic rates in these accessory treatments after storage would be expected to show smaller differences between these treatments than the rate differences between the previously run, basic treatments. Second, photosynthetic rates in the accessory treatments would be expected to approach a similar value given the development of similar carbon dioxide concentrations after storage. Third, photosynthetic rates from the accessory treatments would be expected to lie between the rates obtained in waters from the upper and lower reaches of the river since pH, and consequently carbon dioxide, tended,

after storage, to approach a medium value between those of the upper river and lower river water samples.

An observance of these photosynthetic rate changes in the Method II accessory treatments would confirm that differences in carbon dioxide concentrations among the basic treatments were causing the photosynthetic rate differences between these samples. An absence of these photosynthetic correlations would indicate that some variable other than free carbon dioxide was influencing photosynthesis in the unaltered, basic treatments.

PRELIMINARY EXPERIMENTS

Preliminary experiments were conducted with some of the species to determine tissue metabolic activity and the amounts of experimental tissue necessary to give adequate photosynthetic and respiratory rates and to establish appropriate tissue sectioning and preparatory procedures for a particular type of plant morphology. These experiments were generally incomplete with missing treatments and with missing elements in the L-1/2L-D series. In most cases, PS_L rates for the species were quite similar in both the preliminary and the main experiments. Where rate differences between experiments did become evident, these were generally related to modifications and refinements in methodology subsequent to the preliminary experiment so that a more complete representation of the macrophyte shoot system was

possible in the later work with the species.

For the most part, data from the incomplete preliminary experiments will not be considered in the discussion of the results. But, in a few instances, photosynthetic rate data from the preliminary experiment with a macrophyte were utilized in order to increase sample size for certain statistical analyses (e.g., in the calculation of correlation coefficients between photosynthetic rates and carbon dioxide concentrations). For these species, the results of the preliminary experiment are summarized in Appendix B.

TABULAR CONSIDERATIONS

Results of the manometric experiments on the aquatic macrophytes in the Madison River are considered in the following sections. Each species and the data obtained are considered separately in individual sections. All carbon dioxide concentrations are presented as milligrams per liter (mg/l); alkalinity ("alk") is given as mg/l HCO_3^-. Carbonate concentrations are assumed to be negligible within the range of pH values in the experiments. All photosynthetic and respiratory rates are given as microliters of oxygen evolved or consumed per milligram dry weight per minute. The metabolic rates reported for a treatment or water sample are the means of three to five replicates.

Values designated as PS_L in the tables are mean photosynthetic rates (net photosynthesis) for plant tissues and a treatment at a

light intensity of 780 foot candles. The numbered subscripts in PS_{L1} or PS_{L2} distinguish cases where a repetition of the light phase (L) in the L-1/2L-D pattern was employed in a single experimental run. Values designated as R are mean respiratory rates obtained during the dark phase (D) of an experiment, and subscripts again denote a repetition of this phase. In a few cases, PS_{LA} and PS_{LB} and R_A and R_B subscript designations are used; these subscripts again denote repeats of a phase in a single experiment, but in this case, separate respirometer runs were made using fresh tissue. Other subscripts in the tables, e.g., I, II, and III, denote rates for particular tissue types.

Photosynthetic rate data obtained for the "1/2L" intensity of the respirometer (390 foot candles) are not included in the tables. PS_N data are net photosynthetic rates after the averaged PS_L values have been adjusted to estimate PS_{sat} as described previously; here $PS_N = PS_{sat}$. A percent saturation or "%sat" value is the estimated percent light saturation of photosynthesis for a tissue-treatment under L illumination as determined from Figure 2. Photosynthetic/respiratory ratios are also included in the tables and calculated as PS_N/R.

The "composite" heading included in some of the tables denote estimates of the photosynthetic and respiratory rates for the entire

shoot system of the large aquatic macrophytes.

ANGIOSPERMAE (DICOTYLEDONAE)

Berula erecta (Huds.) Cov.

This species is present throughout the Madison River in Yellowstone National Park, and, on the basis of canopy cover, it is the most abundant plant in the study area (Table 3).

B. erecta is a relatively small plant in the Madison and approaches a rosette morphology. Petioles and leaf-blades form the major portion of the shoot system, and only these plant parts were used in the experiments. Only one minor excision was necessary in sectioning the plant for inclusion into reaction vessels; this was needed to sever the leaf (petiole) from the diminutive stem. In essence, a major portion of the vegetative shoot system was examined in its entirety in each treatment replication. Three to five petiole-blade sections could be included in each reaction vessel.

The results from the experiment with B. erecta are presented in Table 6. This experiment includes four basic treatments, one a duplication of the upper Madison treatment, and three accessory treatments. Both net and gross photosynthetic rates in the basic treatments increased from the lower to the upper river samples, and the sample from the middle site had a photosynthetic rate intermediate between the two. These results directly correspond to concentrations

Table 6. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Berula erecta in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments						
	Basic				Accessory		
Stations	Upper-I	Upper-II	Middle	Lower	Upper	Lower	Lower
Alterations	Unaltered	Unaltered	Unaltered	Unaltered	Method I	Method I	Method II
Alkalinity	117.1	115.9	117.7	118.3	117.1	118.3	118.3
pH	7.38	7.54	7.85	8.19	8.32	7.48	----
Free CO_2	7.76	5.30	2.64	1.21	0.89	6.22	----
PS_L	0.0293	0.0286	0.0249	0.0224	0.0213	0.0278	0.0238
% Saturation	87%	88%	89%	96%	97%	87%	93%
R	0.0096	0.0093	0.0097	0.0096	0.0097	0.0097	0.0095
PS_N	0.0337	0.0326	0.0279	0.0233	0.0219	0.0320	0.0257
PS_N/R	3.51	3.51	2.88	2.43	2.26	3.30	2.71
PS_G	0.0433	0.0419	0.0376	0.0329	0.0316	0.0417	0.0352

of free carbon dioxide. PS_N/R ratios demonstrated a similar relationship to carbon dioxide levels. Photosynthetic rates from the duplicate upper river treatments (Upper-I and Upper-II) were quite similar as were their PS_N/R ratios. Respiratory rates through all seven treatments were closely equivalent. The percent light saturation of photosynthesis at L intensity showed an inverse relationship to photosynthetic rates with a decline in this variable accompanying the higher carbon dioxide concentrations.

The F-ratio from a one-way ANOVA of photosynthetic rates (PS_L) from the four basic treatments was significant at 5% ($F_{16}^{3} = 3.60$). Multiple comparisons indicated that the photosynthetic rate mean from the upper-I sample was significantly different at the 5% level from the mean of the lower sample. However, the upper-II and lower river treatment comparison with fewer replications was not significant at this level nor were the upper-middle and middle-lower combinations.

Photosynthetic rates and PS_N/R ratios from the three accessory treatments also suggest a carbon dioxide-induced photosynthetic response in B. erecta. When carbon dioxide was added to the lower site sample by Method I, photosynthetic rates and PS_N/R ratios were increased to near equivalence with that in the unaltered, upper river sample. In turn, the removal of carbon dioxide from the upper river sample decreased photosynthesis and PS_N/R to a level close to that

obtained in unaltered water from station 3. In the accessory treatment where carbon dioxide was increased in the lower river sample through a brief storage period (Method II), photosynthetic rates also increased slightly.

The F-ratio from a total ANOVA of all seven treatments was significant at 2.5% ($F_{27}^{6} = 3.36$). In all cases, photosynthetic rates and PS_N/R ratios were greater in the relatively high carbon dioxide samples than in samples with lower carbon dioxide concentrations. Inversely, percent light saturation figures were consistently lower in the high carbon dioxide treatments. Photosynthetic rates, PS/R ratios, and percent saturation values were similar through those treatments with similar carbon dioxide contents.

Multiple comparisons of the seven treatments indicated that all mean PS_L differences involving distinct differences in carbon dioxide concentrations between treatments were significant at 5%. No statistically significant differences in photosynthetic rates were obtained in those cases of comparatively small carbon dioxide differences, e.g., the unaltered upper-altered lower and the altered upper-unaltered lower comparisons. These results tend to indicate that B. erecta does respond significantly to changes in carbon dioxide. A rank correlation coefficient between the magnitude of PS_N in the samples and their carbon dioxide concentrations was significant at 1% ($r_s = 0.94$ with four degrees of freedom) (Hoel 1966).

Ceratophyllum demersum L.

C. demersum is an excurrent plant with small, finely dissected leaves, distinct apical buds, and a form that is highly branched. In each individual shoot of stem and whorled leaves, only a short distance separates the terminal bud from the point of branching with the main stem. As a result, three morphological classes of tissue could be sectioned and separately tested in each of the basic treatments. These were: the apical section including the terminal bud (I), an intermediate tissue section below the apical section (II), and the basal section of a shoot just above the point of branching with the main stem (III). In all cases, two identical tissue sections were included in each reaction vessel. The only portions of the shoot system of C. demersum not represented in the experiment were the main branches of the plant which lacked leaves and were non-photosynthetic. Photosynthetic and respiratory rate estimates for the entire shoot in a treatment were calculated as an average of rates obtained from the three tissue-types (Table 7).

Respiratory rates for a given tissue-type were similar through all treatments. However, definite differences in metabolic activity were obtained between the different tissues in each treatment. Both photosynthetic and respiratory rates were highest in the apical sections, but progressively decreased toward the base. In contrast to

Table 7. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Ceratophyllum demersum (I--apical tissue section, II--intermediate tissue section, III--basal tissue section) in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments			Accessory Treatments		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method III	Method II	Method II
Alkalinity	122.0	123.8	125.1	122.0	123.8	125.1
pH	7.37	7.81	8.30	----	----	----
Free CO_2	8.28	3.05	1.00	----	----	----
PS_{LI}	0.1260	0.0972	0.0772	0.1560	0.1131	0.0869
PS_{LII}	0.0704	0.0506	0.0424	0.0816	0.0556	0.0544
PS_{LIII}	0.0339	0.0371	0.0241	0.0580	0.0498	0.0480
Mean PS_L	0.0768	0.0616	0.0479	0.0985	0.0728	0.0631
% saturation	79%	82%	83%	78%	81%	82%
R_I	0.0529	0.0517	0.0466	------	------	------
R_{II}	0.0371	0.0397	0.0378	------	------	------
R_{III}	0.0164	0.0196	0.0163	------	------	------
Mean R	0.0355	0.0370	0.0336	0.0355	0.0370	0.0336
PS_N	0.0968	0.0752	0.0575	0.1261	0.0895	0.0770
PS_N/R	2.73	2.03	1.71	3.55	2.42	2.29
PS_G	0.1323	0.1122	0.0911	0.1616	0.1265	0.1106

respiration, each tissue-type demonstrated differences in photosynthetic rates between samples corresponding to the differences in the carbon dioxide concentrations of the waters. This response was most pronounced in the apical and intermediate segments which had 1.63- and 1.66-fold increases, respectively, in PS_N from the lower to the upper river water sample in contrast to the 1.41-fold increase in the basal segment. The PS_N/R ratios of the tissues were also greater in samples with the higher carbon dioxide concentrations and higher photosynthetic rates. In contrast, the percent saturation of photosynthesis at L intensity varied inversely with carbon dioxide levels and photosynthetic rates.

Due to the lack of abundance of this species, carbon dioxide alterations in the accessory treatments were made by Methods II and III. Results of the accessory treatments with C. demersum also suggest a photosynthetic response to carbon dioxide. Where additional carbon dioxide was added by Method III to the upper station water sample, PS_N and PS_G rates increased markedly over those in the unaltered water. In samples from the middle and lower stations altered by Method II, PS_N and PS_G rates became more similar to each other and were higher than those in each of the unaltered samples. However, photosynthetic rates in these altered samples did not approach those obtained in the unaltered, station 1 water. These accessory

experimental results conform to expectations described previously for the Method II alterations. PS_N/R ratios differed between accessory treatments in accordance with the differences in photosynthetic rates.

The statistical analysis of the main experiment was completed by blocking on tissue-types (two-way ANOVA) to eliminate rate variations within a treatment due to the different activities of the tissue segments. Significance at 10% was obtained for the basic treatments ($F_4^2 = 5.45$) and significance at 0.5% was obtained for the blocks (tissue types) ($F_4^2 = 31.76$) from this analysis. With a total ANOVA for all treatments and blocking, the F-ratio for the treatments was significant at 1% ($F_{10}^5 = 49.61$). Multiple comparisons of the six treatments demonstrated the following: (1) the photosynthetic rate of C. demersum in the altered sample with carbon dioxide added by Method III was significantly greater than those in the other treatments; (2) the photosynthetic rates in the unaltered upper and lower river water samples were significantly different at 5%; (3) the insignificant photosynthetic rate difference between the unaltered lower and middle treatments became significant at 5% after the storage of the latter with its associated carbon dioxide accrual; and (4) none of the photosynthetic rate differences through the remaining treatment combinations were statistically significant since all of these involved small carbon dioxide changes.

Hippuris vulgaris L.

Hippuris vulgaris is an excurrent plant with a distinct terminal bud and whorled, relatively small, but compact leaves basally. Unlike Ceratophyllum demersum however, H. vulgaris is sparsely branched spically. Each individual shoot of stem and leaves is about 30 cm long and therefore too large to be totally included in a treatment. As a result, photosynthetic and respiratory rates for the entire shoot system had to be estimated by indirect means. An abbreviated preliminary experiment indicated that tissues from the apical and basal regions of the plant could be used to provide photosynthetic and respiratory rate data which, when averaged or composited, would approximate the rates of photosynthesis and respiration for the entire shoots. This information is summarized in Appendix B. Accordingly, tissues from these regions of the plant were used to provide such estimates.

Photosynthetic and respiratory rates for younger, apical tissues and for older, basal tissues were separately obtained for each treatment in the main experiment. The apical bud section about 3.0 cm long and the subsequent 1.5 cm to 2.5 cm segment of stem and leaf tissue from an individual shoot were included in a single reaction vessel and replicated for a treatment. In addition, two older tissue sections (2.5 to 3.5 cm long) from the basal part of the plant were included in other replicated reaction vessels. An average of the photosynthetic

and respiratory rates obtained from each of the tissue-types in a particular water sample was assumed to represent that for the entire shoot. For the final experiment, tissues from the apex and from the basal region were sectioned and included in the same sample. This was done to increase the opportunity for sample replication within any treatment.

The gas exchange physiology of H. vulgaris (Table 8) was found to be quite similar to that of Ceratophyllum demersum, another excurrent plant. The photosynthetic rates, respiratory rates, and PS_N/R ratios of both species were quite high in the basic treatments in comparison to those of other species examined in the study. In both species PS_N/R ratios and photosynthetic rates were lower in the basal than in the apical tissues of the plant, and the magnitudes of PS_N/R, PS_N and PS_G for both tissue-types in a basic treatment were directly dependent upon the treatment water's carbon dioxide content concentration. The differences of PS_N, PS_G, and PS_N/R ratios between the lower and the upper river samples were greater in the apical than in the basal sections for both macrophytes. The relative differences in PS_N, PS_G, and PS_N/R between these two treatments equalled 1.56, 1.45, and 1.44, respectively, for an entire shoot of H. vulgaris and equalled 1.68, 1.45, and 1.60 respectively for an entire shoot of C. demersum. However, respiratory rates were about equal within each species for each

Table 8. Inoragnic carbon chemistry and photosynthetic and respiratory rates obtained from the main experiment with Hippuris vulgaris; both apical (I) and basal (II) tissues were separately examined in the experiment. Also listed are estimates of the photosynthetic and respiratory rates of the entire shoot system of H. vulgaris. Rates expressed as ul O_2/mg/min.

	Basic Treatments			Accessory Treatments		
Stations	Upper	Middle	Lower	Upper	Upper	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method I	Method II	Method II
Alkalinity	122.0	123.8	125.1	122.0	122.0	125.1
pH	7.53	7.87	8.29	8.58	----	----
Free CO_2	5.72	2.66	1.02	0.51	----	----
PS_L-I	0.1353	0.1031	0.0898	0.0688	0.1226	0.1234
PS_L-II	0.0831	0.0699	0.0670	0.0466	0.0859	0.1033
% sat--I	68%	73%	84%	85%	59%	77%
% sat--II	75%	87%	72%	92%	79%	66%
R_I	0.0298	0.0297	0.0284	0.0298	0.0298	0.0284
R_{II}	0.0327	0.0315	0.0293	0.0327	0.0327	0.0293
PS_N-I	0.1989	0.1412	0.1069	0.0788	0.2084	0.1604
PS_N-II	0.1105	0.0804	0.0931	0.0508	0.1082	0.1580
$(PS_N/R)_I$	6.67	4.75	3.76	2.65	6.99	5.65
$(PS_N/R)_{II}$	3.38	2.55	3.17	1.61	3.31	5.39
PS_G-I	0.2287	0.1709	0.1353	0.1086	0.2382	0.1888
PS_G-II	0.1432	0.1119	0.1224	0.0835	0.1409	0.1873
			Shoot System Estimates			
PS_L	0.1092	0.0850	0.0784	0.0567	0.1043	0.1134
% sat	71%	79%	79%	88%	68%	72%
R	0.0313	0.0306	0.0289	0.0313	0.0313	0.0289
PS_N	0.1540	0.1071	0.0987	0.0646	0.1533	0.1576
PS_N/R	4.92	3.50	3.42	2.06	4.90	5.45
PS_G	0.1853	0.01377	0.1276	0.0959	0.1846	0.1865

of the tissue-types and through all of the different water samples.

A two-way ANOVA (Randomized Complete Block design) with subsampling (Lentner 1969) was applied to the data in Table 9 with blocking on tissue-type. The F-ratio for the blocks was significant at 0.5% ($F_5^1 = 36.47$) which suggested that the photosynthetic rates of the younger, apical tissues were significantly greater than those of the older, basal segments. The treatment F-ratio was also significant ($F_5^5 = 11.76$, $P < 0.025$). Multiple comparisons of the basic treatments indicated that the mean PS_L rate in the upper river sample was significantly greater at the 5% level than those in either the middle or the lower stream water.

Accessory treatments were also applied to H. vulgaris in this experiment. With the removal of carbon dioxide from water of the upper site, PS_L rates became significantly less than those in unaltered water from the upper (at 1%), middle (at 5%), and lower (at 10%) sites on the river. After dark storage, photosynthetic differences between the station 1 and station 3 samples became non-significant as their carbon dioxide concentrations approached a similar value through accrual-evasion equilibrations. The PS_N rate after storage of the upper river samples remained about the same as that before storage; however, PS_N in the station 3 sample increased to close equivalence to that in the post-storage station 1 sample after storage. Data in

Table 5 for H. vulgaris indicate that this should have been the case since pH values through all treatments after dark storage approached those characteristic of water from the upper Madison. PS_L rates in the lower river sample after storage became significantly greater at 5% than rates from the corresponding unaltered sample. In turn, photosynthetic differences between the lower and the upper river samples were no longer significantly different after this manipulation.

In the final experiment with H. vulgaris (Table 9), tissues were tested in unaltered water from the upper and lower sites on the Madison and in two carbon dioxide-altered waters--water from the upper station with carbon dioxide removed and water from the lower site with carbon dioxide added by Method I. The different tissue sampling and preparation procedures described previously probably account for the rate differences that were obtained between the main and final experiments.

The F-ratio from a one-way ANOVA of the PS_L data in Table 9 was significant at 0.5% ($F_{10}^{3} = 15.73$). Similar to the main experiment, multiple comparisons indicated that PS_L rates were significantly greater at 1% in the unaltered upper river sample than in the unaltered lower river sample. With an addition of carbon dioxide to station 3 water, PS_L rates in this sample become significantly greater at 1% than those in the normal station 1 water (at 5%) due to the addition

Table 9. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the final experiment with Hippuris vulgaris in altered and unaltered samples collected from two sites on the Madison River (alterations made by Method I). Rates expressed as ul O_2/mg/min.

	Treatments			
Stations Description	Upper Unaltered	Upper Altered	Lower Unaltered	Lower Altered
Alkalinity	123.2	123.2	126.3	126.3
pH	7.57	8.29	8.20	7.41
Free CO_2	5.27	1.00	1.27	7.81
PS_L	0.1006	0.0874	0.0794	0.1179
% saturation	75%	83%	89%	79%
R	0.0352	0.0350	0.0358	0.0373
PS_N	0.1338	0.1049	0.0889	0.1486
PS_N/R	3.80	3.00	2.48	3.98
PS_G	0.1690	0.1399	0.1247	0.1859

of an excessive amount of carbon dioxide in the alteration. In turn, with the removal of carbon dioxide from the station 1 sample, photosynthetic rate differences between this sample and the normal station 3 sample become insignificant; however, rates in the CO_2-purged, upper river water were significantly less at 10% than those in the normal station 1 water and significantly less at 1% than those in the altered station 3 sample. In all cases, photosynthetic rates were significantly greater in water with the higher carbon dioxide concentrations.

The percent light saturation of H. vulgaris at 780 foot candles was relatively low through the various treatments in comparison to many of the other aquatic plants examined. This was possibly due to the more compact nodes and greater density of foliar tissue in H. vulgaris than in the other macrophytes which would cause a greater internal shading in the reaction vessels. This could act as an added variable, and with foliar differences between individual shoots of H. vulgaris, this would also tend to mask in this species the inverse relationship between percent light saturation and carbon dioxide levels that was fairly obvious in Berula erecta. Although not completely consistent in all of the treatments in the two experiments, percent light saturation in H. vulgaris also appeared to be inversely related to carbon dioxide concentration as was the case for B. erecta.

Mimulus guttatus Fischer

This species was found to be an extremely rare macrophyte in the Madison River. As a result, the difficulty of location and collection of adequate tissue restricted experimentation to one major manometric run. The results of this experiment are summarized in Table 10.

Morphologically, M. guttatus is a somewhat excurrent plant with a large, rosette-like apical region and three to four lower nodes with opposite leaves. Photosynthetic and respiratory rates of the rosetted apex of the plant were determined separately from the basal nodes, and both tissue classes were replicated two or three times in a treatment. Accessory treatments and carbon dioxide alterations were made by Method II due to the lack of tissue.

Photosynthetic data from the two tissue-types are not distinguished in Table 10 because there were no marked or consistent differences between them in photosynthetic rates. PS_L rates of combined treatment replications were found to be slightly greater in the basal section than in the apical section of the plant, 0.0463 versus 0.0443 ul O_2/mg/min respectively. This contrasts to the results obtained for other excurrent plants, e.g., Ceratophyllum demersum and Hippuris vulgaris, in which apical photosynthetic rates were significantly greater than those basally.

M. guttatus illustrates the typical photosynthetic response to

Table 10. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Mimulus guttatus in water from the upper, middle, and lower stations in the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments					
	Basic			Accessory		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II	Method II	Method II
Alkalinity	122.0	123.2	126.3	122.0	123.2	126.3
pH	7.33	7.73	8.29	----	----	----
Free CO_2	9.07	3.64	1.03	----	----	----
PS_L	0.0664	0.0375	0.0311	0.0199	0.0199	0.0218
% saturation	81%	85%	89%	95%	84%	84%
R	0.0211	0.0204	0.0224	------	------	------
PS_N	0.0817	0.0443	0.0348	0.0209	0.0237	0.0259
PS_N/R	3.87	2.17	1.55	----	----	----
PS_G	0.1028	0.0647	0.0572	------	------	------

carbon dioxide that has been obtained from several of the aquatic macrophytes examined in the study. The inverse relationship between the percent light saturation of photosynthesis at 780 foot candles and carbon dioxide concentration is again evident as are the differences in PS_N, PS_G, and PS_N/R that accompany differences in free carbon dioxide in the basic treatments. That is, values from the upper river sample were greater than those in station 2 water, with both of these then greater than the species' photosynthetic rates in water from the lower Madison River. In addition, the absolute values of PS_N were in close accord with the carbon dioxide concentrations of a treatment; i.e., the difference in PS_N between the middle and lower station samples (0.0095 ul O_2/mg/min) was less than the difference between the upper and lower river samples (0.0374 ul O_2/mg/min) which corresponds to the greater difference in carbon dioxide in the latter case (2.61 versus 5.43 mg/l). The relatively high differences of PS_N and PS_N/R obtained for M. guttatus between the upper and lower river water (factors of 2.35 and 2.50 respectively) suggest that the photosynthetic apparatus of this macrophyte is one of the most sensitive among the Madison species to changes in free carbon dioxide.

The one-way ANOVA of the basic treatments produced an F-ratio that was significant at less than 0.5% ($F_{10}^{2} = 19.74$). Multiple comparisons confirmed the significance of the mean PS_L differences between

the lower-upper and middle-upper river samples (at 1%). The F-ratio from a total ANOVA of all treatments was also significant at 0.5% ($F_{20}^{5} = 18.73$). However, after dark storage, photosynthetic rates in the upper and lower reach samples became quite similar and insignificantly different statistically as carbon dioxide concentrations in these treatments tended to approach a similar value. The general decline of all rates in the accessory treatments was probably related to the adverse influence of storage.

Myriophyllum exalbescens Fern.

M. exalbescens is an abundant, excurrent plant with a distinct apical bud and whorled, relatively small and finely dissected leaves toward the base. It is similar to H. vulgaris in being sparsely branched apically. Individual shoots were too large to use in their entirety in a single treatment. Therefore, metabolic rates for the entire shoot system of the macrophyte were estimated in a manner similar to that used for H. vulgaris.

Klarich (1972) obtained photosynthetic and respiratory rates for nine sequential, 3 cm segments of M. exalbescens from the terminal bud to the basal portion of the plant. From these data, photosynthetic and respiratory rates for the entire shoot system of the macrophyte were determined as 0.0164 and 0.0058 ul O_2/mg/min respectively. Simple averages of rates from the two most apical segments and the two

basal sections were calculated as 0.0334 and 0.0127 ul O_2/mg/min. These latter two rates were found to be approximately twice as high as the photosynthetic and respiratory rates calculated for the entire shoot system. This relationship was utilized in this study as an approach for estimating the in toto shoot system metabolic rates of this species.

Experiments with M. exalbescens were designed to be applicable to the results obtained by Klarich (1972). The apical bud and the following section from a shoot were excised and included with sample water in a single reaction vessel and run in several replications. Two basal sections, 6 to 12 cm from the terminal bud, were also put together in other samples and replicated. This provided two sets of replicated PS_L and respiratory data for any treatment. Such rates from the two tissue-types in a treatment were then summed and reduced by 75% to provide for in toto estimates.

Results of the main experiment with M. exalbescens with three basic treatments and the two tissue-types are summarized in Table 11. The inverse relationship observed in other species between carbon dioxide concentrations and percent light saturation of photosynthesis is evident in Table 11. This relationship is most distinct in the apical tissues of M. exalbescens. Net photosynthetic rates in both tissue-types were greater in sample waters with the higher carbon

Table 11. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the main experiment with apical and basal tissues of Myriophyllum exalbescens; also listed are the photosynthetic and respiratory rates of the entire shoot system of M. exalbescens estimated as 25% of the sum of rates in the apical and basal tissues. Rates expressed as ul O_2/mg/min.

	Basic Treatments							
	Apical Tissues				Basal Tissues			
Stations	Upper	Middle	Lower	Means	Upper	Middle	Lower	Means
Alkalinity	123.2	123.8	124.4		123.2	123.8	124.4	
pH	7.50	7.84	8.23		7.50	7.84	8.23	
Free CO_2	6.19	2.85	1.16		6.19	2.85	1.16	
PS_{L1}	0.0364	0.0308	0.0247	0.0306	0.0235	0.0233	0.0191	0.0220
PS_{L2}	0.0345	0.0296	0.0244	0.0295	------	------	------	------
Mean PS_L	0.0355	0.0302	0.0246	0.0301	------	------	------	------
% saturation	78%	88%	92%	85%	90%	90%	91%	90%
R	0.0158	0.0166	0.0162	0.0162	0.0061	0.0064	0.0076	0.0067
PS_N	0.0454	0.0344	0.0268	0.0354	0.0261	0.0259	0.0210	0.0244
PS_N/R	2.87	2.07	1.65	2.19	4.28	4.05	2.76	3.64
PS_G	0.0612	0.0510	0.0430	0.0516	0.0322	0.0323	0.0286	0.0311

	Shoot System Estimates		
Stations	Upper	Middle	Lower
Sum Mean PS_L	0.0590	0.0535	0.0437
(0.25) Sum PS_L	0.0148	0.0134	0.0109
% saturation	83%	88%	92%
Sum R	0.0219	0.0230	0.0238
(0.25) Sum R	0.0055	0.0058	0.0060
PS_N	0.0178	0.0152	0.0119
PS_N/R	3.24	2.26	1.98
PS_G	0.0233	0.0210	0.0179

dioxide concentrations than in those with less carbon dioxide. However, the F-ratio from a one-way ANOVA of PS_L rates was not significant at 10% with the apical tissues of M. exalbescens ($F_{18}^{5} = 1.99$) and neither was the F-ratio for the basal tissues ($F_{9}^{2} = 0.43$). By blocking on tissue-type and with sub-sampling, significant F-ratios were obtained from a two-way ANOVA. Ratios of $F_{4}^{2} = 16.71$ for the blocks and $F_{4}^{2} = 14.47$ for the treatments were obtained with both significant at 2.5%. A significant sampling error was also obtained from the experiment. This error indicated a high degree of variability between tissue sections within any particular water-tissue combination ($F^{27}_{4} = 66.05$). This probably accounts for the lack of significance in the completely random ANOVA's.

Similar to the findings of Klarich (1972), the significant F-ratio for the blocks would indicate that the basal tissues of the macrophyte had significantly lower photosynthetic and respiratory rates than those nearer the apices. This was also observed in Certophyllum demersum and Hippuris vulgaris. Similarly, differences in PS_N and PS_N/R between tissues in the lower and the upper river samples were greater in the apical tissues (factors of 1.69 and 1.74 respectively) than in the basal tissues of the plant (factors of 1.24 and 1.55 respectively).

Multiple comparisons between the three basic treatments of the

main experiment indicated that the mean PS_L differences between the upper and lower, and the middle and lower river samples were significant at 5%. Since the respiratory rates of each tissue-type did not significantly vary between treatments, PS_N/R differences between samples generally corresponded to differences in free carbon dioxide. Relative differences in PS_N and PS_N/R between the high carbon dioxide, station 1 sample and the low carbon dioxide, station 3 sample for the entire shoot system of M. exalbescens amounted to 1.50 and 1.63 respectively, and these are quite similar to the same factors for Berula erecta and Hippuris vulgaris.

The results of the final experiment with M. exalbescens are presented in Table 12. The apical and basal tissues of the macrophyte were also examined in this experiment, and two basic treatments along with three accessory applications were utilized. The latter consisted of an extensive addition of carbon dioxide to a lower site water sample, and carbon dioxide removal from water from the upper Madison River. Photosynthetic rates were again greater in the apical than in the basal tissues for any treatment and distinctively different between treatments within each tissue-type. Individual, one-way ANOVA's for the apical and basal tissues were significant at 0.5% ($F_{10}^{4} = 14.94$ and $F_{8}^{4} = 23.13$ respectively). For both tissues, multiple comparisons indicated that the mean PS_L rates in the unaltered upper and lower

Table 12. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the final experiment with apical and basal tissues of Myriophyllum exalbescens; also included are the photosynthetic and respiratory rates of the entire shoot system of the macrophyte estimated as 25% of the sum of rates in the apical and basal segments. Rates expressed as ul O_2/mg/min.

	Treatments				
Stations	Upper	Upper	Middle	Lower	Lower
Alterations	Unaltered	Method I	Method I	Unaltered	Method I
Alkalinity	126.9	126.9	128.7	132.4	132.4
pH	7.27	8.22	7.36	8.23	6.80
Free CO_2	10.83	1.22	8.94	1.24	33.23
			Apical Tissues		
PS_L	0.0619	0.0353	0.0609	0.0288	0.1269
% saturation	69%	96%	78%	100%	57%
R	0.0202	0.0145	0.0138	0.0188	0.0138
PS_N	0.0897	0.0368	0.0781	0.0323	0.2226
PS_N/R	4.44	2.54	5.66	1.72	16.13
PS_G	0.1099	0.0513	0.0191	0.0511	0.2364
			Basal Tissues		
PS_L	0.0363	0.0159	0.0331	0.0171	0.0585
% saturation	78%	95%	83%	96%	74%
R	0.0191	0.0159	0.0143	0.0149	0.0128
PS_N	0.0465	0.0167	0.0399	0.0178	0.0791
PS_N/R	2.43	1.05	2.79	1.19	6.18
PS_G	0.0656	0.0326	0.0542	0.0327	0.0919
			Shoot System Estimates		
Sum PS_L	0.0982	0.0512	0.0940	0.0459	0.1854
(0.25) Sum PS_L	0.0246	0.0128	0.0235	0.0115	0.0464
% saturation	73%	96%	80%	100%	63%
Sum R	0.0393	0.0312	0.0281	0.0337	0.0266
(0.25) Sum R	0.0098	0.0078	0.0070	0.0084	0.0067
PS_N	0.0337	0.0133	0.0294	0.0122	0.0737
PS_N/R	3.44	1.71	4.20	1.45	11.00
PS_G	0.0435	0.0211	0.0364	0.0206	0.0804

river samples were significantly different at 5%, and rates were much greater in the high carbon dioxide, upper river water. With an extensive addition of carbon dioxide to the station 3 sample, photosynthetic rates in the altered water became significantly greater at 1% than those in the other samples. In turn, with the removal of carbon dioxide from the upper river sample, photosynthetic rates in both tissues significantly declined (at the 5% level) to the point where PS_L differences between the altered upper and unaltered lower samples became insignificant.

The magnitude of PS_N obtained from the treatments of the main and final experiments closely corresponded to the carbon dioxide concentrations of a sample. This can be illustrated by conducting a rank correlation between PS_N and carbon dioxide (eight possible pairs from the two experiments); a significant rank correlation coefficient was obtained from this analysis ($r_s = 0.857$, $P < 0.01$). These results suggest that M. exalbescens, like many other macrophytes in the Madison River, has a photosynthetic response to natural variations in free carbon dioxide. Waters with a relatively high carbon dioxide concentration and a low ranking of this variable provide correspondingly low rankings of PS_N rates (i.e., relatively high photosynthetic rates).

Utricularia vulgaris L.

Utricularia vulgaris (common bladderwort) is an insectivorous

macrophyte that is ubiquitous in the Madison River drainage system of Yellowstone National Park, but it is not particularly abundant in the Madison River. Another form of bladderwort, probably U. minor (McDougall and Baggley 1956), was also located in the Madison; however this species was quite scarce and was not examined in this study. One experiment was completed with U. vulgaris (Table 13) utilizing the three basic treatments and two accessory treatments with carbon dioxide alterations made by Method I. A large portion of the shoot system of this excurrent species could be contained in a single reaction vessel.

As observed for the other excurrent plants, similar respiratory rates were obtained in U. vulgaris in contrast to distinct photosynthetic differences between samples. Higher photosynthetic rates and PS_N/R ratios accompanied the higher carbon dioxide concentrations. In contrast, the percent saturation of photosynthesis at 780 foot candles was found to be consistently lower in treatments with the greater carbon dioxide contents. Rank correlations between treatment differences in carbon dioxide and the corresponding differences in PS_N, PS_G, PS_N/R, percent light saturation, and R provided the following coefficients with eight degrees of freedom:

$$PS_N \text{ -- } r_s = 0.976 \ (P<0.01)$$
$$PS_G \text{ -- } r_s = 0.976 \ (P<0.01)$$
$$PS_N/R \text{ -- } r_s = 0.988 \ (P<0.01)$$
$$\% \text{ sat -- } r_s = -0.979 \ (P<0.01)$$
$$R \text{ -- } r_s = 0.039 \ (P>0.10)$$

Table 13. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Utrichularis vulgaris in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments				
	Basic			Accessory	
Station	Upper	Middle	Lower	Upper	Lower
Description	Unaltered	Unaltered	Unaltered	Method I	Method I
Alkalinity	123.2	126.3	126.3	123.2	126.3
pH	7.57	7.81	8.20	8.29	7.41
Free CO_2	5.27	3.11	1.27	1.00	7.81
PS_L	0.0408	0.0386	0.0302	0.0295	0.0463
% saturation	95%	98%	100%	100%	75%
R	0.0235	0.0245	0.0257	0.0250	0.0260
PS_N	0.0428	0.0394	0.0312	0.0300	0.0616
PS_N/R	1.82	1.61	1.21	1.20	2.37
PS_G	0.0663	0.0639	0.0569	0.0550	0.0876

However, the relative differences in PS_N and PS_N/R between the lower and the upper river samples were found to be relatively low -- factors of 1.37 and 1.50 respectively. Apparently, the photosynthetic response of U. vulgaris to carbon dioxide was less distinct than that in the other excurrent species established in the river.

U. vulgaris also differs from the other macrophytes in the Madison River in showing relatively low PS_N/R ratios in all treatments. With the exception of Sagittaria cuneata, these were markedly lower than the PS_N/R ratios obtained for any other species examined. For example, the lowest PS_N/R ratio in each basic treatment for the remaining twenty species of aquatic plants was 11% to 29% higher than those in U. vulgaris. Such low ratios are possibly related to the insectivorous nature of the macrophyte. However, both the photosynthetic and respiratory rates of this species fall well within the range of rates obtained for the other twenty-one species studied.

The F-ratio from the one-way ANOVA of the basic treatments was found to be significant at 0.5% ($F_6^2 = 15.05$). An ANOVA of all treatments was also highly significant at 0.5% ($F_9^4 = 21.59$). Multiple comparisons of the basic treatments indicated that the mean PS_L rates in the upper and the middle river water samples were significantly greater at 1% than the rate in the lower sample. The mean PS_L rate difference between the station 1 and the station 2 samples, however,

was insignificant at 10%. Photosynthetic rates proportionately increased or decreased with carbon dioxide addition or removal water from a sample. Such induced photosynthetic alterations in the accessory treatments were shown to be statistically significant at the 1% level.

ANGIOSPERMAE (MONOCOTYLEDONAE)

Eleocharis acicularis (L.) R. & S.

This species is relatively abundant on a canopy cover basis in the Madison River (Table 3). E. acicularis (needle spike-sedge) is a diminutive plant, and like most spike-sedges, possesses vestigial, nonfunctional leaves. The stem therefore forms the major portion and photosynthetic organ of the vegetative shoot system. Due to the small size of the macrophyte, numerous individual plants could be sectioned and included in the samples. Both a main experiment and a final or confirmatory experiment were completed with E. acicularis. Carbon dioxide alterations in the final experiment were accomplished primarily by Method II, but the alteration of one accessory treatment was by Method I.

The results in Tables 14 and 15 indicate that the photosynthetic rates of E. acicularis were distinctively different between basic treatments in correspondence to differences in free carbon dioxide. Both photosynthetic rates and carbon dioxide concentrations followed the

Table 14. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the main experiment with Eleocharis acicularis. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered
Alkalinity	122.6	124.4	127.5
pH	7.24	7.81	8.30
Free CO_2	7.40	3.06	1.02
PS_L	0.0202	0.0150	0.0126
% saturation	80%	88%	100%
R	0.0064	0.0065	0.0066
PS_N	0.0253	0.0171	0.0127
PS_N/R	3.95	2.63	1.92
PS_G^N	0.0317	0.0236	0.0193

Table 15. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the final experiment with Eleocharis acicularis (unalt.--unaltered, M.--Method). Rates expressed as ul O_2/mg/min.

	Treatments							
Stations	Upper	Middle	Lower	Lower----Lower		Upper	Middle	Lower
Alterations	Unalt.	Unalt.	Unalt.	M.I------M.II		M.II	M.II	M.II
					--------(6 hours storage)---------			
Alkalinity	126.9	128.7	132.4	132.4	132.4	126.9	128.7	132.4
pH	7.27	7.74	8.23	6.80	----	----	----	----
Free CO_2	10.83	3.72	1.24	33.24	----	----	----	----
PS_L	0.0235	0.0169	0.0118	0.0389	0.0307	0.0250	0.0197	0.0140
% sat	91%	100%	100%	89%	----	----	----	----
R	0.0064	0.0065	0.0070	0.0071	------	------	------	------
PS_N	0.0259	0.0178*	0.0130*	0.0436	------	------	------	------
PS_N/R	4.05	2.74	1.86	6.14	----	----	----	----
PS_G	0.0323	0.0243	0.0200	0.0507	------	------	------	------

An asterisk denotes those cases where $PS_{1/2L} > PS_L$.

same sequence--upper reach > middle reach > lower reach. The relative difference in PS_N was larger by a factor of 1.99 in the upper than in the lower river samples in both the main and confirmatory experiments.

One-way ANOVA's provided significant F-ratios of the basic treatment's PS_L in both the main and confirmatory experiments--$F^2_9 = 16.28$ and $F^2_8 = 21.57$, respectively, with both significant at 0.5%. In the main experiment, multiple comparisons showed the mean PS_L differences between the upper-lower and upper-middle river samples to be significant at 1%; the difference between the middle and lower samples was not significant at 10%. In the final experiment, similar conclusions were drawn from the multiple comparisons, except that in this case, the mean PS_L difference between the middle and lower river samples was also significant (at 5%).

Photosynthetic and PS_N/R values for a given treatment were quite similar in these two experiments and the preliminary experiment (Appendix B). Differences directly corresponded to variations in carbon dioxide concentrations between samples of the same treatment. If the PS_G and PS_N rates of a treatment in the main and confirmatory experiments are compared, a mean difference of only 2.9% was obtained for these parameters between experiments. By combining all of the non-storage treatments of the experiments (including the preliminary experiment) and ranking both PS_N and carbon dioxide concentrations from

low to high values, from one to ten in this case, a highly significant rank correlation coefficient was obtained for E. acicularis between PS_N and carbon dioxide ($r_s = 0.93$, $P<0.01$) (Hoel 1966). This indicates a positive relationship between the magnitude of photosynthetic rates and carbon dioxide concentrations. An insignificant coefficient was obtained between alkalinity and photosynthesis ($r_s = -0.06$).

PS_L differences in the accessory treatments also suggest a photosynthetic response in E. acicularis to carbon dioxide. Of significance was the distinct increase in photosynthetic rates after an alteration of lower sample water by Method I (Table 15). Rates greater than those of the unaltered samples were obtained in response to the addition of carbon dioxide beyond that normally found in the river. In the storage treatments in the final experiment (Method II), PS_L rates, when compared to those of the unaltered samples, were higher in the station 2 and station 3 samples and were also slightly higher in the upper river sample; however, rates were lower after storage in the sample originally altered by a marked addition of carbon dioxide by Method I. Such changes in photosynthesis would be expected based on the chemical results of the storage experiments reported in Table 5.

The F-ratio from the total ANOVA of PS_L in the confirmatory experiment was highly significant at much less than 0.5% ($F_{20}^{7} = 26.79$). Multiple comparisons between the basic treatments of this experiment

and the station 3 sample altered by Method I indicate that the latter sample had significantly great PS_L rates at 1% than all of the basic treatments. In addition, the mean difference between the unaltered lower and upper river samples was also significant at this level. The remaining photosynthetic differences between basic treatments, the lower-middle and middle-upper combinations, were significant at 5%. Numerous additional comparisons are possible, both between individual accessory treatments and between accessory and basic treatments. Most of these mean PS_L differences were found to be significant at least to 5% with the exception of the unaltered lower-altered middle and the unaltered middle-altered upper combinations which were insignificant. This lack of significance in these cases reflects the trend for carbon dioxide values in the accessory samples to approach a similar value which results in a reduction of photosynthetic rate differences after storage. Significant differences in photosynthesis typically were obtained between an individual accessory treatment and its basic counterpart.

Respiratory rates for the macrophyte were quite similar through all treatments and experiments. Therefore, the magnitudes of the PS_N/R ratios paralleled those for photosynthesis, as a result of which greater PS_N/R ratios were observed for the high carbon dioxide waters. The *in situ* PS_N/R ratios of *E. acicularis* in the Madison River

therefore would be highest in the upper reach of the river with a progressive decline downstream to station 3. As for PS_N, the rank correlation coefficient between PS_N/R and carbon dioxide concentrations was significant at 1% (r_s = 0.964 with five degrees of freedom).

The tissues of E. acicularis were apparently near light saturation at respirometer illumination (780 foot candles) in water collected from the lower reach of the river (Tables 14 and 15). However, this was not the case in upstream samples. In contrast to PS_N and PS_N/R, percent light saturation was inversely related to carbon dioxide concentrations. A significant but negative rank correlation coefficient was obtained between these variables with E. acicularis (r_s = -0.564, $P<0.05$ with eight degrees of freedom). In samples from the lower river, E. acicularis appears to be more substrate limited than light limited with 100% light saturation at respirometer illumination; this corresponds to the low carbon dioxide levels in this segment of the river. Upstream, with the greater carbon dioxide concentrations, the macrophyte becomes light limited at 780 foot candles.

Eleocharis thermalis Rydb.

E. thermalis is another species of spike-sedge that has been observed in the Madison River; however, it was found to be extremely rare. Like E. acicularis, E. thermalis lacks functional leaves, and

the stem represents the predominant portion of the shoot system. E. thermalis was also easily sectioned for insertion in the reaction vessels. Its rarity in the river was the main reason for restricting experimentation with the species to one experimental series.

Distinctively different PS_N, PS_G, and PS/R values also were obtained with this species in the three basic treatments (Table 16). A close correspondence was evident between the carbon dioxide concentrations and the photosynthetic rates in a treatment. The one-way ANOVA of PS_L data in Table 16 provided a significant F-ratio at 1% ($F_9^2 = 9.22$). The mean PS_L difference between the upper and lower river samples was found to be significant at 1% by Newman-Kuels Multiple Range Test; mean PS_L differences between the lower-middle and middle-upper treatment combinations were significant at 10%.

The relative differences in PS_N rates between the station 1 and station 3 water samples in E. thermalis (a factor of 1.73) was somewhat less than this factor for E. acicularis (1.99). PS_N/R ratios in E. thermalis also paralleled dioxide concentrations in the treatments; a slightly greater than 50% decline in this parameter would occur from the upper to the lower reaches of the river. Both the differences in PS_N/R between samples and the values of the PS_N/R ratios in the treatments were quite similar in both species of spike-sedge. However, PS_N, PS_G, and respiratory rates were slightly greater in E. thermalis

Table 16. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Eleocharis thermalis. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered
Alkalinity	119.0	120.8	123.2
pH	7.48	7.70	8.17
Free CO_2	6.26	3.48	1.32
PS_L	0.0155	0.0130	0.0106
% saturation	52%	56%	62%
R	0.0075	0.0080	0.0093
PS_N*	0.0298	0.0233	0.0172
PS_N/R	3.97	2.91	1.85
PS_G	0.0373	0.0313	0.0265

*Rough estimates of net photosynthesis at 100% saturation due to the low light saturation at respirometer illumination and the large correction factors; i.e., $r=PS_L/PS_{1/2L}$ was quite high.

than in E. acicularis.

As with the other species, percent saturation figures for E. thermalis were inversely related to carbon dioxide concentrations. This species was unique in the Madison River in its relatively low percent of photosynthetic light saturation at 780 foot candles. All percent light saturation values were less than 62% in E. thermalis in contrast to ones typically greater than 75% for the other macrophytes. This indicates a relatively high light requirement in this macrophyte which, when coupled with the plant's small size, might explain its scarcity in the upper reach of the Madison River. This reach is characterized by a dense, aggregate type of macrophyte growth and distribution wherein E. thermalis might be unable to successfully compete with other species due to shading. The distinct, 50% decrease in PS_N/R downstream shown by E. thermalis which accompanies the decline in carbon dioxide concentrations could account for the absence of this species from the lower Madison. That is, in waters where PS_N/R approaches one, respiration would utilize a large proportion of the photosynthate leaving little matter for the anabolic processes necessary in ecesis. Such considerations might also apply to E. acicularis which demonstrated a similar drop in PS_N/R downstream and was absent from the lower segment of the river.

Glyceria borealis (Nash) Batchelder

G. borealis has distinct grass leaves which form the major portion of the shoot system. Since the vegetative stems of the plant are quite small, tissue samples were primarily composed of several leaves cut into appropriately sized sections. Two distinct types of this macrophyte were recognized growing grouped into separate, homogeneous mounds in the river. In some cases, the mounds consisted of plants with relatively long (25-33 cm) and wide leaves of a dark green color; in other mounds, plants had smaller, shorter (15-23 cm) and finer leaves of a lighter green color. However, using vegetative characteristics, both plant-types were determined to be the same species. Such differences were probably indicative of different stages of clonal growth and development.

G. borealis was a common and abundant aquatic plant in the study area and was extensively examined in this study. A main experiment with the species was conducted in the summer and a final experiment in the late fall. In the main experiment, plants of both leaf-types were examined in the basic treatments. Averages of the photosynthetic and respiratory rates from the two types of plant were taken to represent a composite for the species in each treatment. Only the larger-leaved plants were used in the final experiment.

Distinct differences in photosynthesis and respiration were

evident between the two plant types in the main experiment (Table 17). Plants with the smaller and probably younger leaves had the highest photosynthetic and respiratory rates and the greatest PS_N/R ratios for any given treatment. G. borealis also demonstrated a close parallel between photosynthetic rates, PS_N/R ratios, and carbon dioxide concentrations in the basic treatments, but an inverse relationship between percent saturation and carbon dioxide was evident in both types of plants.

The ANOVA of PS_L data in Table 17 provided a highly significant F-ratio ($F_{18}^{5} = 8.51$, $P<0.005$). Multiple comparisons indicated that all mean PS_L rates between the two plant types in the same treatment were significantly different at 5%. Within both plant forms, the PS_L difference between the upper and lower river samples was significant at 5% while the difference between the upper and middle stream samples was significant at 10%. With the smaller plants, mean PS_L rates in the station 2 and 3 samples were also significantly different at 10%, but this treatment combination was insignificant with the larger plants. For the composite of G. borealis, 2.07- and 2.21-fold differences in PS_N and PS_N/R were obtained between the upper and lower river water samples.

The final experiment with G. borealis included several accessory treatments with carbon dioxide alterations. This experiment was

Table 17. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the main experiment with large and small plants of Glyceria borealis in unaltered samples collected from three sites on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments					
	Small Plants			Large Plants		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	119.0	120.8	123.2	119.0	120.8	123.2
pH	7.40	7.69	8.18	7.40	7.69	8.18
Free CO_2	7.53	3.92	1.29	7.53	3.92	1.29
PS_L	0.0223	0.0189	0.0152	0.0147	0.0098	0.0074
% saturation	65%	76%	82%	66%	83%	84%
R	0.0101	0.0100	0.0112	0.0080	0.0083	0.0082
PS_N	0.0341	0.0248	0.0185	0.0225	0.0118	0.0088
PL_L/R	3.38	2.48	1.65	2.81	1.42	1.07
PS_G	0.0442	0.0348	0.0297	0.0305	0.0201	0.0170

	Plant Means (Composite) by Treatment		
Station	Upper	Middle	Lower
PS_L	0.0185	0.0144	0.0113
% saturation	65%	79%	83%
R	0.0091	0.0092	0.0097
PS_N	0.0283	0.0183	0.0137
PS_N/R	3.11	1.99	1.41
PS_G	0.0374	0.0275	0.0234

performed to confirm the direct relationship between photosynthetic rate differences and the differences in water sample, carbon dioxide concentrations that were observed in the first experiment. Two separate runs using fresh tissues in each were made in this experiment. All carbon dioxide alterations were made by Method I (Table 18).

Respiratory rates in this experiment and those for the larger plant-type in the main experiment were quite similar in all of the treatments. Photosynthetic rates, however, differed to some extent between the two experiments within the same treatments and plant-type; such differences possibly stem from the different seasons of collection. However, as observed in the main experiment, PS_N rates in the three basic treatments of Run I were greater in water from the upstream stations with their higher carbon dioxide concentrations. The F-ratio from an ANOVA of these treatments was significant at 5% ($F_6^2 = 6.36$).

In Run II, photosynthetic rates in water from the upper site were again distinctly higher than those in water from the lower station. This was also true of the PS_N/R ratios for which markedly low values were obtained in the downstream, low carbon dioxide waters. Addition of carbon dioxide to water from the lower site greatly increased photosynthesis and PS_N/R whereas the removal of carbon dioxide from water from the upper site reduced photosynthesis and PS_N/R to levels

Table 18. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the final experiment (two separate runs) with Glyceria borealis (large plants); all sample alterations were made by Method I. Rates expressed as ul O_2/mg/min.

	Basic and Accessory Treatments							
	Run I				Run II			
Stations	Upper	Middle	Lower	Lower	Upper	Upper	Lower	Lower
Description	Unaltered	Unaltered	Unaltered	Altered	Unaltered	Altered	Unaltered	Altered
Alkalinity	126.3	130.5	131.8	131.8	126.3	126.3	131.8	131.8
pH	7.42	7.91	8.35	7.20	7.58	8.59	8.28	7.02
Free CO_2	7.62	2.55	0.94	13.21	5.28	0.52	1.10	19.99
PS_L	0.0136	0.0049	0.0045	0.0178	0.0098	0.0032	0.0034	0.0202
% saturation	100%	100%	100%	79%	100%	100%	100%	84%
R	0.0080	0.0096	0.0085	0.0068	0.0065	0.0094	0.0077	0.0073
PS_N	0.0136	0.0085*	0.0068*	0.0224	0.0111*	0.0058*	0.0075*	0.0240
PS_N/R	1.70	0.89	0.80	3.29	1.71	0.62	0.97	3.29
PS_G	0.0216	0.0181	0.0153	0.0292	0.0176	0.0152	0.0152	0.0313

An asterisk denotes those cases where $PS_{1/2L} > PS_L$.

closely equivalent to those obtained from the unaltered, station 3 sample. In general, photosynthetic rates were similar in all treatments with similar carbon dioxide concentrations. Significant rank correlation coefficients were obtained between treatment carbon dioxide concentrations and PS_N and PS_G rates; a perfect rank correlation was obtained with PS_N ($r_s = 1.00$, significant at 1%), and a coefficient of $r_s = 0.94$ was obtained for PS_G (also significant at 1%).

A one-way ANOVA of mean PS_L in all the treatments of the final experiment provided an F-ratio that was highly significant at much less than 0.5% ($F_{18}^{5} = 61.61$ compared to $F_{15}^{5} = 5.37$ with $P = 0.995$). Multiple comparisons indicated that the mean differences of PS_L between high and low carbon dioxide waters were significant at 5% in most cases, i.e., photosynthetic rates in the high carbon dioxide waters typical of the upper Madison River were significantly greater than rates in waters typical of the lower Madison. In turn, this species would be expected to have distinctively lower in situ PS_N rates and PS_N/R values in the lower reach of the Madison than in the high carbon dioxide, upper segment.

Free carbon dioxide concentrations on the order of 6.7 and 5.2 ppm higher in the final experiment increased the net photosynthetic rates of G. borealis by factors of 2.0 and 1.5 respectively, and gross

photosynthetic activity by factors of 1.4 and 1.2. PS_N/R ratios increased by factors of 2.1 and 1.7. However, such 8.1- to 4.8-fold increases in free carbon dioxide resulted in only very slight increases in the total inorganic carbon (CO_2 plus HCO_3^- as ppm CO_2) available for photosynthesis, less than a 3 ppm increase, or less than a 1.03 factor increase, from the lower to the upper station. On this basis, much smaller increases in photosynthesis would have been expected if carbon dioxide and bicarbonate were equally suitable as carbon sources for photosynthesis in G. borealis.

Juncus ensifolius Wiks.

The monocot J. ensifolius, a member of the rush family, was found to be a relatively unabundant species in the Madison River, and it also had a scattered distribution which made the location and collection of adequate tissue for experimentation somewhat difficult. Vegetative stems were found to be a minor component of the aquatic shoot system of J. ensifolius, so the leaves primarily were used in the experiments. Since leaves of this species of rush are somewhat similar to those of Glyceria borealis, sample preparation procedures with J. ensifolius followed those adopted for G. borealis. Only two to three leaves could be included in each sample.

Due to the general scarcity of J. ensifolius in the river, accessory treatments in the main experiment were made by Method II. Parallels between photosynthetic rates and carbon dioxide were observed in

the basic treatments of the experiment (Table 19) with this species as has been described for other species. This was also the case for PS_N/R ratios and PS_G rates, and greater values of both were obtained in water collected from upstream sites in the Madison River.

The F-ratio from an ANOVA of the basic treatments was significant at 2.5% ($F_{10}^{2} = 6.34$). Through multiple comparisons, PS_L rates in the upper-lower and middle-lower river sample combinations were shown to be significantly different at 1% and 10% respectively. However, with dark storage and the resultant carbon dioxide equilibration in the treatments, photosynthetic rates approached a similar value. All PS_L differences between samples became insignificant statistically after storage as would be predicted by the results in Table 5.

Potamogeton filiformis Pers.

The genus Potamogeton (pondweeds) is one of the most abundant macrophytes in the Madison River, both in terms of standing crop and canopy cover (Tables 3 and 4). Six morphologically diverse species of pondweeds were examined in this study.

P. filiformis (threadleaf or slender pondweed) is one of the most abundant of the pondweeds in the river (Horpestad 1969) and one of the most common of the aquatic macrophytes in the stream. Threadleaf pondweed is a very leafy and bushy, deliquescent plant with extremely thin leaves; all leaves of this species are of the same type. The

Table 19. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Juncus ensifolius in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments					
	Basic			Accessory		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II	Method II	Method II
Alkalinity	122.6	124.4	127.5	122.6	124.4	127.5
pH	7.37	7.64	8.24	----	----	----
Free CO_2	8.32	4.53	1.16	----	----	----
PS_L	0.0252	0.0224	0.0184	0.0228	0.0210	0.0193
% saturation	81%	81%	97%	93%	92%	97%
R	0.0110	0.0123	0.0118	0.0110	0.0123	0.0118
PS_N	0.0310	0.0276	0.0190	0.0246	0.0229	0.0199
PS_N/R	2.82	2.24	1.61	2.24	1.86	1.69
PS_G	0.0420	0.0399	0.0308	0.0356	0.0352	0.0317

shoot system of an individual plant of P. filiformis was much too large to be placed in a single reaction vessel. In a preliminary experiment, an attempt was made to select specific leaf and stem tissue (small branches or "sprigs") from morphologically equivalent points along individual stems. This ultimately proved to be unsatisfactory since a high variance in the photosynthetic data of a treatment was obtained. In the later experiment, the method of "sprig" randomization which provided lower variances and standard errors in the photosynthetic data was utilized in the preparation of tissue samples.

In the main experiment (Table 20), photosynthetic rates were greatest in those basic treatments with the higher carbon dioxide contents. The F-ratio from a one-way ANOVA was significant at 0.5% ($F_{10}^{2} = 12.81$). Mean PS_L differences between the upper and lower and upper and middle treatment combinations were shown to be significant at 1% and 5%, respectively, by the Newman-Keuls Multiple Range test. The mean PS_L difference between the middle and lower river samples was not significant at 10%.

PS_N/R ratios followed a pattern similar to that for PS_L, PS_N, and PS_G. Photosynthesis was not saturated at L light intensities in the main experiment, and percent light saturation was inversely related to the carbon dioxide concentration of a treatment. However, this latter aspect was not totally consistent through the accessory treatments

Table 20. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with Potamogeton filiformis in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments					
	Basic			Accessory		
Station	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method I	Method I	Method I
Alkalinity	122.0	123.2	126.3	123.2	126.3	126.3
pH	7.51	7.75	8.28	8.02	7.25	6.95
Free CO_2	5.99	3.48	1.05	1.87	11.28	22.47
PS_L	0.0382	0.0271	0.0191	0.0200	0.0495	0.0525
% saturation	78%	85%	92%	94%	86%	90%
R	0.0096	0.0096	0.0105	------	------	------
PS_N	0.0489	0.0320	0.0208	0.0213	0.0576	0.0583
PS_N/R	5.09	3.33	1.98	------	------	------
PS_G	0.0585	0.0416	0.0313	------	------	------

(Table 20). As described for Hippuris vulgaris, differences between samples in the amount of internal shading related to the amount and disposition of the tissues in each reaction vessel could account for these inconsistencies. That is, given a similar concentration of carbon dioxide in two samples, the sample with the greater shading factor would tend to have a lower light saturation at 780 foot candles. Such variations then would tend to mask the inverse relationship between percent light saturation and carbon dioxide concentrations.

The total ANOVA F-ratio for the basic and accessory treatments was highly significant at less than 0.5% ($F_{19}^{5} = 18.78$). With the removal of carbon dioxide from the upper reach sample, photosynthetic rates declined relative to the rates for unaltered water. In turn, with the addition of carbon dioxide to the lower and middle water samples, photosynthetic rates significantly increased.

Of the fifteen possible treatment comparisons in the experiment, three were shown to be insignificant at 5% through multiple comparisons of mean PS_L differences. These were altered upper-unaltered lower, the altered upper-unaltered middle, and the unaltered middle-unaltered lower treatment combinations. Carbon dioxide differences between samples of these three insignificant pairs were less than 2.5 mg/1. Carbon dioxide differences in all but three of the treatment combinations were therefore greater than 2.5 mg/1. In all fifteen of the combinations, photosynthetic rates were higher in the samples with the greater

carbon dioxide value (a rank correlation coefficient of $r_s = 1.00$ between PS_N and carbon dioxide concentrations). This would suggest that if a particular water sample had a carbon dioxide content on the order of 2.5 ppm higher than that in any other sample, significantly greater photosynthetic rates might be expected in P. filiformis in that sample. This is the in situ case for the Madison River which has greater than 2.5 mg/l differences in carbon dioxide concentration between the lower and the middle reaches and between these two reaches and the upper segment of the river (Table 20). Thus P. filiformis would be expected to have significantly higher photosynthetic rates in the upper reach of the Madison River than in the downstream segment.

Potamogeton gramineus L.

P. gramineus (variable pondweed) was not a conspicuous or common component of the flora in any reach of the river in Yellowstone National Park. It had a scattered distribution which made the location and collection of adequate tissue difficult. This plus its relatively large size and diverse morphology restricted experimentation with P. gramineus to a single experiment.

Like P. filiformis, variable pondweed is a highly branched, deliquescent species. In addition, P. gramineus has two types of leaves: terminal floating leaves with long petioles, and sessile underwater leaves located near the base of the plant. The latter leaves were

highly variable in size and shape, but they were generally longer and narrower than the terminal leaves.

To facilitate experimentation, the shoot system of P. gramineus was separated into three categories which were separately studied. These three morphological groups can be described as follows: class I--the floating leaves and petioles; class II--the underwater leaves immediately basal to the floating leaves and also including small stem sections above and below the nodes; class III--the smaller underwater leaves near the extreme basal portion of a plant and associated stem sections.

Plants were sectioned and tissues separated as to morphological class and then dried and weighed. The weight proportions of the entire plant represented by each class was then calculated by dividing the weight of an individual class by the total weight of the collected plants. These weight fractions were used to adjust the metabolic rate data of a class leading to a composite (weighted mean) estimate for the shoot system. The following equation was used:

$$M_c = \sum_{i=I}^{III} (F_i)(M_i),$$

where $\sum_{i=I}^{III} F_i = 1$. In these equations, M_c is the composite, weighted estimate of net photosynthetic or respiratory rates for the entire shoot

system of P. gramineus. F_i is the weight fraction of a defined tissue class-I (0.344), II (0.262), or III (0.394), and M_i is the net photosynthetic or respiratory rate of one of the three classes of tissue.

The three basic treatments and several accessory treatments were applied to all morphological classes of P. gramineus. All three methods of carbon dioxide alteration were used. The slight storage period of the Method II and III alterations apparently had an adverse effect since a general decline in photosynthetic rates were obtained in these treatments as compared to the unaltered samples.

Distinct photosynthetic rate differences are evident between the basic treatments of all tissue classes (Table 21). With a few exceptions, photosynthetic rates of the same tissue-type were markedly greater in the upper than in the middle or lower river water samples and slightly greater in the middle river sample than in the lower. These variations in photosynthetic rates, also evident in the PS_L means of Table 21, directly parallel the pattern of carbon dioxide content. A similar parallel is evident in the PS_N/R ratios which were higher in samples collected toward the origin of the Madison River. Although PS_L rates were lower in the Method II and III accessory treatments, their relative magnitudes through the three samples also closely coincided with the pattern of probable carbon dioxide concentrations. A two-way ANOVA of these data with blocking on tissue-type provided

Table 21. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained in the experiment with three tissue classes of Potamogeton gramineus: class I--terminal floating leaves and petioles; class II--large, basal underwater leaves and associated stem; and class III--small, basal underwater leaves and associated stem. Rates expressed as ul O_2/mg/min.

	Basic Treatments			Accessory Treatments			
Stations	Upper	Middle	Lower	Upper	Middle	Lower	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II	Method III 40 sec	Method III 20 sec	Method I
Alkalinity	122.0	123.2	126.3	122.0	123.2	126.3	126.3
pH	7.47	7.83	8.28	----	----	----	6.80
Free CO_2	6.57	2.90	1.05	----	----	----	31.71
PS_L--I	0.0474	0.0416	0.0393	0.0192	0.0314	0.0164	0.0524
PS_L--II	0.0664	0.0556	0.0505	0.0327	0.0444	0.0457	0.0712
PS_L--III	0.0690	0.0500	0.0550	0.0322	0.0578	0.0390	0.0861
Mean	0.0609	0.0491	0.0483	0.0280	0.0445	0.0337	0.0699
% sat--I	91%	100%	100%				87%
% sat--II	80%	80%	90%				85%
% sat--III	97%	89%	91%				88%
R--I	0.0152	0.0149	0.0141				0.0151
R--II	0.0146	0.0142	0.0143				0.0161
R--III	0.0123	0.0117	0.0129				0.0115
Mean R	0.0140	0.0136	0.0138				0.0142
PS_N--I	0.0521	0.0416	0.0404				0.0603
PS_N--II	0.0830	0.0695	0.0561				0.0840
PS_N--III	0.0711	0.0560	0.0605				0.0982
Mean PS_N	0.0687	0.0557	0.0523				0.0808
(PS_N/R)--I	3.43	2.79	2.87				3.99
(PS_N/R)--II	5.68	4.89	3.92				5.22
(PS_N/R)--III	5.78	4.79	4.69				8.54
PS_G--I	0.0673	0.0565	0.0545				0.0754
PS_G--II	0.0976	0.0837	0.0704				0.1001
PS_G--III	0.0834	0.0677	0.0734				0.1097

significant F-ratios as follows: blocks--F^2_4 = 15.24 ($P<0.025$) and treatments--F^2_4 = 9.54 ($P<0.05$). A two-way ANOVA of data from the basic treatments also provided significant F-ratios as follows: F^3_6 = 9.63 for the treatments ($P<0.025$) and F^2_6 = 13.18 for the blocks ($P<0.01$). The significant block F-ratio would indicate that the mean PS_L differences between tissue-types were significant statistically.

Multiple comparisons indicated that photosynthetic rates in the terminal floating leaves and petioles were significantly less than those in the basal underwater leaves and stems. In addition, although respiratory rates were similar between treatments for any particular tissue-type, distinct differences between morphological classes did become evident. The underwater-leaves and stems were apparently the most efficient in accruing a net photosynthate since these tissues had greater PS_N/R ratios than the floating leaves.

Multiple comparisons statistically confirmed the significance of the PS_L differences between treatments. The mean PS_L rate in the upper reach sample with its high carbon dioxide content was significantly greater at 5% for all tissue-types than rates in the middle or lower treatments. As a result, the shoot system of P. gramineus would have significantly greater PS_N and PS_G rates and higher PS_N/R ratios in sample water collected from the upper reach of the Madison River than would be the case downstream. This is illustrated in

Table 22. After the addition of carbon dioxide to the lower reach sample by Method I, photosynthetic rates markedly increased and became significantly greater than rates in the unaltered lower or middle reach samples. In turn, the mean PS_L differences between the upper and lower samples from the river became insignificant after the addition of carbon dioxide to the latter.

Potamogeton natans L.

P. natans (floatingleaf-pondweed) was the largest species of aquatic macrophyte collected from the Madison River. Floating-leaf-pondweed is a deliquescent plant characterized by large and distinct, terminal floating leaves with long petioles. The terminal leaves of this pondweed (petioles and blade) represent a major portion of the macrophyte's shoot system. Sessile, underwater leaves are also present, and these leaves are longer, much narrower, and thinner than the floating leaves.

To estimate shoot system metabolism for this species, tissue classifications and separations were made as with Potamogeton gramineus. Three major tissue classes with six sub-categories were defined for P. natans. The first class included the terminal, floating leaves. The blades were generally quite thick and leathery; however, in a few instances, smaller, thinner and greener floating-leaf blades were collected. This class was then subdivided into three further groups: one

Table 22. Inorganic carbon chemistry and photosynthetic and respiratory rates estimated for the entire shoot system of Potamogeton gramineus; I, II, and III designate three tissue-types defined for the macrophyte. Rates expressed as ul O_2/mg/min.

	Treatments			
	Basic			Accessory
Stations	Upper	Middle	Lower	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method I
Alkalinity	122.0	123.2	126.3	126.3
pH	7.47	7.83	8.28	6.80
Free CO_2	6.57	2.90	1.05	31.71
Adjusted:				
PS_L--I	0.0163	0.0143	0.0135	0.0180
PS_L--II	0.0174	0.0146	0.0132	0.0187
PS_L--III	0.0272	0.0197	0.0217	0.0339
Sum PS_L	0.0609	0.0486	0.0484	0.0706
% saturation*	91%	90%	95%	87%
R--I	0.0052	0.0051	0.0049	0.0051
R--II	0.0038	0.0037	0.0037	0.0038
R--III	0.0048	0.0046	0.0051	0.0048
Sum R	0.0138	0.0134	0.0137	0.00137
PS_N	0.0670	0.0540	0.0509	0.0811
PS_N/R	4.86	4.03	3.72	5.92
PS_G	0.0808	0.0674	0.0646	0.0948

*--Percent saturation calculated as the Sum PS_L divided by the Sum $PS_{1/2L}$ after PS_L and $PS_{1/2L}$ had been adjusted by the weight fraction of each tissue-type.

consisting of the relatively small blades, a second of the larger blades, and a third subclass of floating leaf tissue consisting of the long petioles. The second tissue class consisted of the basal underwater leaves. Stem tissues were placed in the final category, and this category was further subdivided into tissues obtained from the apical portion and those from the more basal portion of the shoot. All of these tissues were cut into smaller pieces before they could be randomly put into the reaction vessels.

Each class of tissue was examined with the three basic treatments. Proportional weight fractions were also determined for each morphological class, and the metabolic data was then adjusted on this basis to provide estimates of the _in toto_ shoot system metabolic rates of _P. natans_. The weight fractions of each tissue class can be summarized as follows:

1.	Floating leaves		0.35
	a. Large	0.83	
	b. Small	0.17	
	c. Petioles		0.23
2.	Underwater leaves		0.12
3.	Stems		0.30
	a. Apical	0.50	
	b. Basal	0.50	
			1.00

Experimental results from the three tissue classes of floating leaves from _P. natans_ are presented in Table 23. The smaller leaf blades had lower photosynthetic rates than the larger leaf blades but

Table 23. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained in the experiment with floating leaves of Potamogeton natans in unaltered water samples from the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments (Floating leaf Blades)					
Tissue Class	Large Blades			Small Blades		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	122.0	123.8	126.3	122.0	123.8	126.3
pH	7.45	7.72	8.26	7.45	7.72	8.26
Free CO_2	6.88	3.76	1.10	6.88	3.76	1.10
PS_L	0.0608	0.0412	0.0361	0.0501	0.0299	0.0230
% saturation	78%	90%	100%	92%	100%	100%
R	0.0131	0.0122	0.0134	0.0165	0.0169	0.0157
PS_N	0.0778	0.0457	0.0364	0.0546	0.0299	0.0240
PS_N/R	5.94	3.75	2.72	3.31	1.77	1.53
PS_G	0.0909	0.0579	0.0498	0.0711	0.0468	0.0397

	Composite (Leaf Blades)		
Stations	Upper	Middle	Lower
PS_L	0.0590	0.0393	0.0339
% saturation	81%	91%	100%
R	0.0137	0.0130	0.0138
PS_N	0.0726	0.0432	0.0343
PS_N/R	5.30	3.32	2.49
PS_G	0.0863	0.0562	0.0481

	Basic Treatments (Petioles)			Composite (Floating leaves)		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	122.0	123.8	126.3	122.0	123.8	126.3
pH	7.45	7.72	8.26	7.45	7.72	8.36
Free CO_2	6.88	3.76	1.10	6.88	3.76	1.10
PS_L	0.0277	0.0208	0.0165	0.0465	0.0319	0.0269
% saturation	84%	89%	100%	81%	91%	100%
R	0.0125	0.0115	0.0118	0.0132	0.0124	0.0130
PS_N	0.0330	0.0233	0.0167	0.0568	0.0352	0.0273
PS_N/R	2.64	2.03	1.42	4.30	2.84	2.10
PS_G	0.0455	0.0348	0.0285	0.0700	0.0476	0.0403

also had higher respiratory rates. As a result, PS_N/R ratios were lower in the small blades than in the larger ones. In addition, the thinner, smaller blades were found to be more nearly light saturated at the light intensity of the respirometer. PS_G rates for any given treatment were greater in the larger floating-leaf blades which, along with their higher PS_N/R ratios, would indicate a greater ability to accumulate a net photosynthate in the older and more mature leaf tissue.

The petioles of the terminal floating leaves were also found to be quite active, but photosynthetic and respiratory rates for the long petioles were lower than those of both types of leaf blades. In addition, both PS_G rates and PS_N/R ratios were lower in the petioles than in the blades. By combining the photosynthetic and respiratory rates of the petioles with that from the terminal leaf blades of P. natans, a composite analysis for the entire floating-leaf was determined (Table 23).

Photosynthetic and respiratory data from the under water leaves of P. natans are presented in Table 24. PS_N and PS_G rates of the basal leaves were similar to those calculated as the composite of the terminal leaves, and respiratory rates were also closely equivalent in the two types of leaves. However, due to the difference in the weight proportions of the two tissues, the terminal leaves would be much more significant to the total photosynthate accrual of P. natans than the

Table 24. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained with underwater leaves from Potamogeton natans in unaltered samples from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alkalinity	122.0	123.8	126.3
pH	7.45	7.72	8.26
Free CO_2	6.88	3.76	1.10
PS_L	0.0438	0.0329	0.0230
% saturation	68%	87%	100%
R	0.0156	0.0147	0.0159
PS_N	0.0644	0.0378	0.0235
PS_N/R	4.13	2.57	1.48
PS_G	0.0800	0.0525	0.0394

underwater-leaves.

Although respiratory rates within each type of the leaf tissue were about the same in all treatments, distinct photosynthetic differences between treatments were evident in each class of tissue. In all cases, photosynthetic rates were greater in the upper river sample than in water from the lower reach of the Madison River. Photosynthetic rates intermediate to these were obtained in water from the middle sampling site. These photosynthetic relationships closely coincided with the carbon dioxide concentrations of the three treatments.

Such photosynthetic rate differences between samples were also

obtained with stem tissue from P. natans (Table 25). The decline in PS_L rates in the upper river water sample after dark storage plus the increase in PS_L rates in the lower sample are in accord with the predicted changes in the carbon dioxide concentrations of the waters as a result of the carbon dioxide evasion-respiratory accrual that occurs during the storage period.

Individual ANOVA's and multiple comparisons of the PS_L data of each tissue-type statistically confirmed the significance of these photosynthetic differences between treatments. F-ratios from one-way ANOVA's and the results of the multiple comparisons are summarized in Table 26. These results indicate that the shoot system of P. natans (Table 27) had significantly greater photosynthetic rates in water samples collected from the upper, high carbon dioxide reach of the river than in water obtained from the lower and middle reaches of the stream.

Percent light saturation in P. natans was inversely related to the carbon dioxide concentrations of the water samples. In water from the lower reach, P. natans was light saturated at 780 foot candles under low carbon dioxide tensions. However, with the higher concentrations of this constituent in samples from the two upstream reaches, the macrophyte become progressively more light limited at this intensity. Potamogeton gramineus, in contrast, was light limited at respirometer illumination in water from all reaches of the river including the lower

Table 25. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained in the experiment with apical (I) and basal (II) stem tissues from Potamogeton natans. Rates expressed as ul O_2/mg/min.

	Basic Treatments			Accessory Treatments		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II	Method II	Method II
Alkalinity	122.0	123.8	126.3	122.0	123.8	126.3
pH	7.40	7.75	8.22	----	----	----
Free CO_2	7.72	3.50	1.21	----	----	----
PS_{L1}--I	0.0112	0.0072	0.0046	0.0084	0.0062	0.0079
PS_{L2}--I	----	----	----	0.0076	0.0063	0.0087
PS_{L1}--II	0.0133	0.0083	0.0054	0.0100	0.0079	0.0083
PS_{L2}--II	----	----	----	0.0084	0.0058	0.0076
Mean PS_L*	0.0123	0.0078	0.0050	0.0086	0.0066	0.0081
% saturation**	86%	90%	100%			
R--I	0.0104	0.0097	0.0113			
R--II	0.0080	0.0075	0.0074			
Mean F*	0.0092	0.0086	0.0094			
PS_N	0.0143	0.0087	0.0055			
PS_N/R	1.55	1.01	0.59			
PS_G	0.0235	0.0173	0.0149			

* Composite photosynthetic and respiratory rates for the item calculated as a mean of rates from the apical and basal tissues since their weight fractions were equal.

**Percent saturation calculated as mean PS_L divided by mean $PS_{1/2L}$.

Table 26. Analyses of variance and multiple comparisons of PS_L rates from Potamogeton natans in unaltered water from the upper, middle, and lower reaches of the Madison River.

Tissue	F-ratios and Significance levels	Multiple comparisons of mean PS_L and significance levels		
		Lower < Middle	Lower < Upper	Middle < Upper
Floating Leaves				
Blades	F_7^5=13.06 (P<0.005)			
Large Blades		P>0.10	P<0.01	P<0.01
Small Blades		P>0.10	P<0.01	P<0.05
Petioles	F_9^2-8.90 (P<0.01)	P>0.10	P<0.01	P<0.05
Underwater Leaves	F_{10}^2=41.56 (P<0.005)	P>0.01	P<0.01	P<0.01
Stems (Apical and Basal)	F_{10}^2=63.10 (P<0.005)	P>0.05	P<0.01	P<0.01

Table 27. Estimated photosynthetic and respiratory rates for the shoot system of Potamogeton natans (composite analysis) in three unaltered samples collected from the Madison River; also listed are the inorganic carbon chemistries of the samples. Rates expressed as ul O_2/mg/min.

	Treatments		
Station	Upper	Middle	Lower
Alkalinity	122.0	123.8	126.3
Free CO_2	7.14	3.68	1.13
PS_L	0.0360	0.0248	0.0199
% saturation	80%	90%	100%
R	0.0124	0.0116	0.0122
PS_N	0.0450	0.0276	0.0203
PS_N/R	3.63	2.38	1.66
PS_G	0.0574	0.0392	0.0325

segment (Table 22). Thus, the photosynthetic apparatus of P. natans would appear to be more limited by the quantity and quality of carbon sources that are available in water from the lower reach than is the case for P. gramineus.

The concentrations of bicarbonate were essentially constant through all three reaches of the Madison and nearly identical in the experiments with the two species, and equally low levels of carbon dioxide were available for photosynthesis in the lower reach in both cases. The fact that P. gramineus was not light saturated in lower river water suggests the greater availability of a suitable carbon source for this species in the station 3 water sample than is the case

for P. natans which was light saturated in this sample. Given the comparatively low ability of P. natans to utilize bicarbonate, this species would not be light limited in the low carbon dioxide, station 3 water since the availability of carbon useable by this species would be relatively low compared to its requirements. However, in the upstream water samples with greater carbon dioxide concentrations, photosynthesis in P. natans does become light limited as a result of the greater amount of useable carbon substrate.

Differences between species are also evident in their direct photosynthetic responses to the differences in carbon dioxide between the upper and lower river water samples at constant bicarbonate. Greater than two-fold differences in PS_N and PS_N/R were obtained for P. natans between these two samples whereas factors less than 1.4 were obtained for P. gramineus. In turn, both species had relatively high PS_N rates and PS_N/R ratios, greater than 3.00, in samples with high concentrations of both bicarbonate and carbon dioxide from the upper Madison reach. In the lower reach, however, the PS_N/R ratio of P. natans approaches 1.6 whereas that of P. gramineus remains at levels greater than 3.0. The lower PS_N/R of P. natans in this reach points to a reduced ability to accumulate photosynthate for anabolic reactions in this species compared to P. gramineus. This in turn corresponds to, and possibly accounts for, the absence of P. natans from the lower,

low carbon dioxide reach of the Madison River (Table 4). In contrast, P. gramineus is present in the lower reach, and this could be because of its potentially greater ability to utilize bicarbonate which results in higher PS_N/R ratios in the station 3 samples and a greater facility for photosynthate accumulation in the lower river.

Potamogeton nodosus Poiret.

P. nodosus (American or longleaf pondweed) is the most abundant species of pondweed in the Madison River (Horpestad 1969). This species is commonly described as having floating-leaves (Winterringer and Lopinot 1966, Hotchkiss 1967), but such leaves were not found in any of the plants of American pondweed collected from the Madison. Therefore, relatively long and expanded, submerged leaves formed the most distinctive portion of the shoot system of this macrophyte. These submerged leaves had relatively long petioles. P. natans also had pronounced and abundant fruiting-flowering stalks during the period of tissue collection.

Due to the large size of P. nodosus, a tissue classification and separation had to be made with this species similar to that with P. natans. Several classes and subclasses of tissue were ultimately defined and separately examined in the three basic treatments. These categories and their respective weight fractions are summarized as follows:

Submerged leaf tissue			0.677
1. Leaf blades		0.523	
a. Apical	0.216		
b. Basal	0.307		
2. Petioles		0.154	
a. Large	0.133		
b. Small	0.021		
Stem tissues			0.324
3. Vegatative		0.253	
a. Apical	0.144		
b. Basal	0.109		
4. Fruit stalks	0.071	0.071	
	1.001	1.001	1.001

The leaves, petioles and blades of P. nodosus comprised approximately two-thirds of the dry weight of the shoot system. Basal leaf blades made the greatest contribution to the foliar weight. Leaf blades taken from the apical portion of the shoot were generally larger than those located basally and were attached to the main axis of a plant. Typically only one apical blade, cut into four sections, could be placed in a single reaction vessel. The smaller, basal leaf blades were commonly associated with lateral branches arising from the main axis near the substrate level. Two of the basal leaves cut in four sections were used in each vessel.

Petioles from the submerged leaves of P. nodosus were separated into two classes. The petioles smaller in both length and girth were most commonly associated with the basal leaves. Eight of the smaller petioles could be put in a reaction vessel whereas only six of the larger petioles associated with apical blades could fit in a vessel.

The apical and basal stem tissues accounted for one-third of the shoot system biomass in P. nodosus, although the leafless fruiting stalks did not make a significant contribution to the total biomass of the plant. All portions of the shoot system of P. nodosus were green except the fruit and flowers, and all tissues were photosynthetically active.

Apical stem sections from which the larger petioles and leaf blades were obtained came from the main axis of the plant. Basal stem sections were obtained primarily from the lateral branches and were somewhat thinner than the apical sections, but in both cases, only four to five, 2-3 cm segments could be contained in each reaction vessel. The apical stem-leaf tissues and the basal tissues were about equally represented on a weight basis in the species' shoot system (49.3% and 43.7% respectively).

Results of the experiments with each tissue class of P. nodosus are presented in Tables 28 and 29. Distinct photosynthetic and respiratory rate differences between tissues were evident within any treatment as were distinct photosynthetic differences between treatments within a given tissue-type. Respiratory rates were quite similar in all treatments for each class of tissue. In general, photosynthetic rates were greater in basal tissues than in those from the apical portions of the shoot system. Photosynthetic and respiratory rates were

Table 28. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained in the experiment with underwater leaves of Potamogeton nodosus in unaltered samples collected from the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments (Leaf Blades)					
	Apical, Larger Blades			Basal, Smaller Blades		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	123.2	123.8	124.4	123.2	123.8	124.4
pH	7.45	7.69	8.23	7.50	7.84	8.23
Free CO_2	6.95	4.01	1.16	6.19	2.85	1.16
PS_L	0.0367	0.0334	0.0292	0.0681	0.0485	0.0465
% saturation	80%	89%	85%	93%	95%	100%
R	0.0141	0.0137	0.0155	0.0192	0.0188	0.0194
PS_N	0.0459	0.0374	0.0345	0.0735	0.0509	0.0465
PS_N/R	3.26	2.73	2.23	3.83	2.71	2.40
PS_G	0.0600	0.0511	0.0500	0.0927	0.0697	0.0659

	Basic Treatments (Petioles)					
	Apical, Larger Petioles			Basal, Smaller Petioles		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	123.2	126.3	126.3	123.2	126.3	126.3
pH	7.35	7.80	8.32	7.35	7.80	8.32
Free CO_2	8.75	3.17	0.96	8.75	3.17	0.96
PS_L	0.0431	0.0252	0.0236	0.0456	0.0309	0.0253
% saturation	71%	88%	92%	72%	81%	84%
R	0.0138	0.0116	0.0139	0.0178	0.0193	0.0185
PS_N	0.0608	0.0287	0.0257	0.0634	0.0380	0.0301
PS_N/R	4.41	2.47	1.85	3.56	1.97	1.63
PS_G	0.0746	0.0403	0.0396	0.0812	0.0573	0.0486

Table 29. Inorganic carbon chemistry and photosynthetic and respiratory data obtained in experiments with three classes of stem tissue from Potamogeton nodosus in unaltered water collected from the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments								
Tissue	Apical Stem Tissue			Basal Stem Tissue			Fruit Stalk Tissue		
Station	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower
Alkalinity	123.2	126.3	126.3	123.2	126.3	126.3	123.2	126.3	126.3
pH	7.35	7.80	8.32	7.35	7.80	8.32	7.44	7.65	8.11
Free CO_2	8.75	3.17	0.96	8.75	3.17	0.96	7.11	4.50	1.56
PS_L	0.0209	0.0127	0.0104	0.0263	0.0193	0.0136	0.0152	0.0131	0.0110
% saturation	73%	90%	100%	74%	93%	100%	76%	85%	96%
R	0.0067	0.0074	0.0068	0.0106	0.0101	0.0102	0.0109	0.0107	0.0114
PS_N	0.0286	0.0141	0.0107	0.0355	0.0208	0.0141	0.0199	0.0155	0.0114
PS_N/R	4.27	1.91	1.57	3.35	2.06	1.38	1.83	1.45	1.00
PS_G	0.0353	0.0215	0.0175	0.0461	0.0309	0.0243	0.0308	0.0262	0.0228

greater in leaf than in stem tissue, and photosynthetic rates were generally greater in leaf blades than in petioles. Respiratory rates were higher in small petioles and basal blades than in the larger petioles and apical leaf blades.

Distinct photosynthetic rate differences between the three basic treatments were shown with both the apical and basal leaf blades (Table 28). Greater photosynthetic rates were obtained in both kinds of leaves in water with the higher carbon dioxide contents. PS_N and PS_N/R differences between the lower and upper river water samples were slightly greater in the basal than in the apical leaf blades with upper sample/lower sample ratios of these variables as follows: apical, PS_N--1.33 and PS_N/R--1.46; basal, PS_N--1.58 and PS_N/R--1.60. This indicates that the basal leaf blades were more responsive photosynthetically to differences in carbon dioxide than were the apical blades, and that P. nodosus was less responsive than Potamogeton natans. The greater photosynthetic and respiratory rates in the basal leaf blades, coupled with their greater proportion of total shoot weight, indicate that the smaller blades actually contributed more to the total metabolism of P. nodosus than the larger and more conspicuous apical leaf blades. As with the leaf blades, photosynthetic differences were also obtained between treatments in each of the five classes of petiole and stem tissue. These classes of tissue, however, contribute less to the

total shoot system metabolism than the leaf blades due to their lower weight proportions and to their generally lower photosynthetic and respiratory rates.

The combined ANOVA of PS_L data from the two types of leaf blade was significant at ($F_{18}^{5} = 8.04$). Mean PS_L differences between the apical and basal leaf blades within any treatment were shown to be significant at less than 5% by multiple comparisons. The F-ratios from one-way ANOVA's of PS_L data for the two classes of petioles and three classes of stem tissue were also significant at 0.5%--$F_{6}^{5} = 19.44$ and $F_{12}^{8} = 16.18$ respectively. However, multiple comparisons of mean PS_L rates between the large and small petioles within each treatment failed to demonstrate significant photosynthetic rate differences between these tissues. In contrast, similar analyses of the stem tissues indicated that, in most treatments, the basal segments had significantly greater photosynthetic rates than the apical segments ($P<0.05$) whereas the apical segments had significantly greater rates than the flowering stalks.

Multiple comparisons of mean PS_L differences between samples were conducted for each tissue class. These results are summared in Table 30. These analyses suggest that the photosynthetic rates of the entire shoot system of P. nodosus would be significantly greater in water samples possessing free carbon dioxide concentrations on the order of 4.0

Table 30. Multiple comparisons of mean net photosynthetic rates at 780 foot candles from Potamogeton nodosus in unaltered water from the upper, middle, and lower reaches of the Madison River.

Tissue	Sample comparison and significance levels		
	Lower < Middle	Lower < Upper	Middle < Upper
Leaves			
Apical Blades	P>0.10	P<0.10	P>0.10
Basal Blades	P>0.10	P<0.05	P<0.05
Large Petioles	P>0.10	P<0.01	P<0.01
Small Petioles	P>0.10	P<0.01	P<0.01
Stems			
Flowering Stalks	P>0.10	P<0.10	P>0.10
Apical Segments	P>0.10	P<0.01	P<0.01
Basal Segments	P<0.05	P<0.01	P<0.05

mg/l higher than the carbon dioxide levels in some other samples with a similar alkalinity near 2.0 me/l. This is illustrated in Table 31. A two-way ANOVA of PS_N data from all tissue classes and treatments also indicate the significance of photosynthetic rate differences in Madison water samples. The treatment F-ratio was significant at 0.5% ($F_{12}^{2} = 27.80$), and multiple comparisons of mean PS_N differences between treatments for the shoot system indicate that the composite photosynthetic rate of the macrophyte in water from the upper reach (Table 31) was significantly greater at 1% than composite rates in samples from the middle and lower reaches of the stream. The significant, block F-ratio from this analysis confirms the significance of photosynthetic

Table 31. Composite photosynthetic and respiratory rates obtained from the leaf blades, petioles, leaves, and stems of Potamogeton nodosus; also included are estimates of composite photosynthetic and respiratory rates for the plant's shoot system in samples collected from three reaches of the Madison River. Rates expressed as ul O_2/mg/min.

	Leaf Blades			Leaf Petioles		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
R	0.0171	0.0167	0.0178	0.0143	0.0126	0.0145
PS_N	0.0618	0.0457	0.0414	0.0613	0.0299	0.0262
PS_N/R	3.61	2.74	2.33	4.29	2.37	1.81
PS_G	0.0789	0.0624	0.0592	0.0756	0.0425	0.0407

	Leaves		
Stations	Upper	Middle	Lower
R	0.0164	0.0158	0.0171
PS_N	0.0617	0.0421	0.0379
PS_N/R	3.76	2.66	2.22
PS_G	0.0781	0.0579	0.0550

	Stems		
Stations	Upper	Middle	Lower
R	0.0090	0.0090	0.0089
PS_N	0.0290	0.0165	0.0119
PS_N/R	3.22	1.83	1.34
PS_G	0.0380	0.0255	0.0208

	Shoot System		
Stations	Upper	Middle	Lower
Alkalinity	123.2	125.7	125.7
Free CO_2	7.47	3.35	1.11
% saturation	83%	92%	95%
R	0.0140	0.0135	0.0144
PS_N	0.0511	0.0338	0.0296
PS_N/R	3.65	2.50	2.06
PS_G	0.0651	0.0473	0.0440

rate differences among the various tissues of this macrophyte. For example, Least Significant Difference multiple comparisons (Letner 1969) demonstrate that all leaf tissues had significantly greater PS_N rates at 1% than the three classes of stem tissue (Table 31).

PS_N/R ratios in all tissue classes of P. nodosus and, as indicated in Table 31, in the composite estimate of the shoot system's PS_N/R also differed between samples in direct correspondence to the differences in free carbon dioxide. Conversely, percent light saturation of photosynthesis at respirometer illumination was generally lower in the high carbon dioxide treatments. However, the relative differences in PS_N and PS_N/R between the upper reach and lower reach river samples for the entire shoot system of P. nodosus (factors of 1.73 and 1.77) were intermediate to the high values obtained for P. filiformis and P. natans and the relatively low values obtained for P. gramineus. Apparently considerable variability in response to carbon dioxide exists among species within the pondweed genus.

Potamogeton pectinatus L.

This species of pondweed has only submerged leaves and is similar to Potamogeton filiformis in having a bushy, deliquescent life-form. As a result, preparatory procedures of P. pectinatus were the same as those used for P. filiformis. Due to the scarcity of plants, only one experiment consisting of basic treatments was performed with

P. pectinatus.

Respiratory rates in all treatments were about the same, but the pattern of photosynthetic differences among samples typical for the other species of pondweed was also obtained with this macrophyte (Table 32). An ANOVA F-ratio of PS_L data for P. pectinatus was highly significant ($F_{10}^{2} = 80.82$, $P<0.005$). Multiple comparisons indicated that all mean PS_L differences between treatments were significant at 1% in a sequence that paralleled the free carbon dioxide concentrations of the treatments--upper>middle>lower for both the PS_N and the carbon dioxide levels of the samples. Relative differences in PS_N and PS_N/R between the station 1 and station 3 samples were quite high for P. pectinatus with factors of 2.38 and 2.16 respectively which were closely similar to these factors in P. filiformis.

Potamogeton strictifolius Benn.

P. strictifolius (narrowleaf-pondweed) is morphologically similar to P. filiformis and P. pectinatus and is characterized by possessing rather short, stiff leaves tapering to a bristle tip (Winterringer and Lopinot 1966). Tissue preparation for this macrophyte was the same as that used for P. filiformis. This species is relatively rare in the Madison River. In addition, and in contrast to many species which were found growing aggregated in relatively homogeneous mounds, P. strictifolius typically had a scattered distribution with a few

Table 32. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from Potamogeton pectinatus in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered
Alkalinity	122.0	123.2	126.3
pH	7.33	7.73	8.29
Free CO_2	9.07	3.64	1.03
PS_L	0.0391	0.0264	0.0160
% saturation	83%	100%	100%
R	0.0111	0.0108	0.0101
PS_N	0.0469	0.0283*	0.0197*
PS_N/R	4.22	2.62	1.95
PS_G	0.0580	0.0391	0.0298

An asterisk denotes those cases where $PS_{1/2_L} > PS_L$.

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individual plants intermixed with the dominant species of a particular mound. These factors made the location and collection of adequate tissue for experimentation difficult and time-consuming. As a result, only one experiment of basic treatments was completed with this species.

Respiratory rate constancy in P. strictifolius in the three basic treatments markedly contrasted to the distinct photosynthetic differences that were obtained (Table 33). PS_L differences were found to be statistically significant ($F^2_9 = 8.16$, $P<0.01$) with significantly

Table 33. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from Potamogeton strictifolius in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered
Alkalinity	120.8	122.0	125.1
pH	7.59	7.86	8.30
Free CO_2	4.93	2.68	1.00
PS_L	0.0280	0.0211	0.0172
% saturation	82%	83%	100%
R	0.0127	0.0133	0.0131
PS_N	0.0342	0.0253	0.0177*
PS_N/R	2.69	1.90	1.35
PS_G	0.0469	0.0386	0.0308

An asterisk denotes the case where $PS_{1/2L} > PS_L$.

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greater (at 5%) mean rates obtained in the upper river, high carbon dioxide sample than in water from the lower or middle stations with lower concentrations of free carbon dioxide. Mean PS_L differences between the lower and middle treatments were insignificant at 10%. Photosynthetic rates of P. strictifolius for a given treatment were generally similar in magnitude to those for P. pectinatus and P. filiformis. In addition, relative differences in PS_N and PS_N/R between the lower and the upper site samples were high in

P. strictifolius (1.93 and 1.99 respectively), as they were in P. pectinatus. However, respiratory rates were somewhat greater in the narrowleaf pondweed, and as a result, PS_N/R ratios were lower in this species than in the other morphologically similar species of pondweed.

With the absence of P. strictifolius from the lower reach of the Madison River, the distributions of all six species of Potamogeton directly corresponded to their photosynthetic responsiveness to free carbon dioxide. P. gramineus, the one species of pondweed that does occur in the lower, low carbon dioxide reach of the river, had the smallest relative differences of all the pondweeds in PS_N and PS_N/R between waters from the lower reach and the upper, high carbon dioxide reach (1.29 and 1.28 respectively). In contrast, the remaining five species of Potamogeton which were absent from the lower section of the river but established in the upper reaches had comparatively high differences in PS_N and PS_N/R (factors greater than 1.70). Such relationships might be a reflection of an effect of free carbon dioxide in influencing distributional patterns in the river.

Sagittaria cuneata Sheldon

S. cuneata (northern arrowhead) is a heterophyllous macrophyte which can be either amphibious or totally submerged. Young plants of S. cuneata are submerged clumps of small stems and ribbon-like leaves

dissimilar to the arrowhead-shaped leaf that characterizes this genus. Sometimes the plants remain submerged and retain the form of reduced rosettes of narrow, ribbon-like leaves (Fassett 1969). In this form, the leaves constitute the major portion of the shoot system, and only this form of the macrophyte was observed in the Madison River study area.

In the preparation of experimental tissues, the leaves of S. cuneata were cut into several two-to three-centimeter segments. Since these leaves were neither extremely long nor thick, all sections from a single leaf could be contained in a single reaction vessel. The experiment was completed with three basic treatments and one accessory treatment in which carbon dioxide alterations were made by Method I. The results of this experiment are summarized in Table 34.

Respiratory rates in S. cuneata were quite similar among the four treatments. Tissues were light saturated at respirometer illumination in water from the lower, low carbon dioxide station but not in that from the upstream, higher carbon dioxide stations. Photosynthetic rates were consistently higher in waters with greater carbon dioxide contents. PS_N/R ratios were also greater in samples with the higher carbon dioxide concentrations, and an exceptionally low PS_N/R ratio was obtained in the low carbon dioxide water collected from the lower reach. S. cuneata demonstrated the lowest PS_N/R ratio for this

Table 34. Inorganic carbon chemistry and photosynthetic and respiratory data obtained from the main experiment with Sagittaria cuneata in water from the upper, middle, and lower stations on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments			
	Basic			Accessory
Stations	Upper	Middle	Lower	Upper
Alterations	Unaltered	Unaltered	Unaltered	Method I
Alkalinity	122.6	124.4	127.5	122.6
pH	7.53	7.69	8.29	7.34
Free CO_2	5.75	4.03	1.04	8.90
PS_L	0.0501	0.0442	0.0225	0.0565
% saturation	95%	98%	100%	87%
R	0.0236	0.0255	0.0229	0.0246
PS_N	0.0526	0.0451	0.0237	0.0638
PS_N/R	2.23	1.77	1.03	2.59
PS_G	0.0762	0.0706	0.0466	0.0884

treatment of any of the species examined during the study.

By combining the results of the preliminary experiment (Table 60, Appendix B) with those from the main experiment and by ranking carbon dioxide differences between various treatment combinations versus corresponding PS_N and PS_N/R differences (21 total pairs), highly significant rank correlation coefficients were obtained between the three variables: CO_2 and PS_N--$r_s = 0.926$ and CO_2 and PS_N/R--$r_s = 0.903$, both significant at less than 1%. Similar manipulations between carbon dioxide concentrations and percent light saturation produced a significant negative rank correlation coefficient ($r_s = -0.906$, $P<0.01$).

The ANOVA of the PS_L data from the main experiment provided an F-ratio ($F_{20}^{3} = 18.37$) that was significant at much less than 0.5%. Mean PS_L differences between the lower-upper and lower-middle river samples were shown to be significant at 1% by multiple comparisons. Data from the accessory treatments in the main experiment followed expectations of such a carbon dioxide-photosynthetic response in the macrophyte. The addition of excess carbon dioxide to the upper reach sample increased photosynthesis over that of the unaltered upper sample. The low PS_N/R ratio in water from the lower Madison River suggests that northern arrowhead is probably dependent upon availability of carbon dioxide considerably in excess of one ppm in order to succeed as a fully submerged aquatic plant.

Sparganium angustifolium Michx.

The stems of S. angustifolium, another ribbon-leaf plant, are quite small and represent only a minor proportion of the shoot system. Emergent structures such as flowering stalks were never observed during the sampling period. The leaves of this species were much longer than the submerged leaves of Sagittaria cuneata, and, as a result, a single leaf of S. angustifolium could not be totally included in a reaction vessel. Several plants were collected and leaves sectioned into six tissue classes; each class was then individually randomized. Four of the tissue classes were studied with the respirometer. Metabolic rates for two other classes were estimated as an average of the measured rates from the segments above and below the untested tissue.

Five to seven, three- to five-centimeter sections from a particular tissue class could be contained in a reaction vessel along with 10 ml of sample water. Weight fraction determinations for each tissue class were made following the respirometer run. Photosynthetic and respiratory rates for the shoot system of S. angustifolium in toto were estimated from the rates and weight fractions of each group as described previously for Potamogeton gramineus. The tissue classes of S. angustifolium leaves and related weight proportions can be summarized as follows:

Segment I --apical section	0.121
Segment II --next apical section	0.167
Segment S_1 --next section, omitted	0.217
Segment III--intermediate section	0.210
Segment S_2 --next section, omitted	0.151
Segment IV --basal section	0.135

The results obtained for each tissue class in the basic treatments are summarized in Table 35. The magnitude of PS_L in the three basic treatments for all tissue classes paralleled the carbon dioxide concentrations of the sample water: upper>middle>lower. By blocking on tissue-type, a significant F-ratio was obtained for the treatments--$F_6^2 = 27.88$, significant at less than 0.5%. A significant F-ratio was obtained for the blocks in this analysis--$F_6^3 = 20.04$, also significant at less than 0.5%. Such F-values indicated that there were statistically significant photosynthetic rate differences in the main experiment both between the I-IV, leaf tissue classes within each treatment and between treatments within any given tissue group.

Photosynthetic rates tended to be significantly greater (at 1-5%) in the more basal segments than in the two apical segments of leaf tissue. In contrast to photosynthesis, respiratory rates were closely equivalent among treatments within each tissue-type but were variable between tissue classes. PS_N/R ratios tended to increase from the apical to the basal portions of the leaf. In contrast, the relative differences in PS_N and PS_N/R between the station 1 and station 3

Table 35. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the experiment with four tissue classes of Sparganium angustifolium leaves in unaltered water from three stations on the Madison River (I--apical leaf segment, II and III--intermediate leaf segments, and IV--basal leaf segment). Rates expressed as ul O_2/mg/min.

Tissue Stations	Basic Treatments: Basal III and IV Segments			Tissue Stations	Basic Treatments: Apical I and II Segments		
	Upper	Middle	Lower		Upper	Middle	Lower
Alk.	123.2	126.3	126.3	Alk.	123.2	126.3	126.3
pH	7.44	7.65	8.11	pH	7.44	7.65	8.11
Free CO_2	7.11	4.50	1.56	Free CO_2	7.11	4.50	1.56
PS_L--I	0.0429	0.0339	0.0272	PS_L--III	0.0386	0.0326	0.0309
% sat--I	85%	100%	100%	% sat--III	61%	72%	84%
R--I	0.0202	0.0211	0.0213	R--III	0.0214	0.0197	0.0199
PS_N--I	0.0506	0.0346	0.0272	PS_N--III	0.0633	0.0453	0.0368
(PS_N/R)--I	2.50	1.64	1.28	(PS_N/R)--III	2.96	2.30	1.85
PS_G--I	0.0708	0.0557	0.0485	PS_G--III	0.0847	0.0650	0.0567
PS_L--II	0.0329	0.0218	0.0212	PS_L--IV	0.0449	0.0402	0.0329
% sat--II	87%	80%	93%	% sat--IV	91%	96%	100%
R--II	0.0159	0.0141	0.0154	R--IV	0.0193	0.0185	0.0171
PS_N--II	0.0378	0.0273	0.0229	PS_N--IV	0.0494	0.0418	0.0335
(PS_N/R)--II	2.38	1.94	1.49	(PS_N/R)--IV	2.56	2.26	1.96
PS_G--II	0.0537	0.0414	0.0383	PS_G--IV	0.0687	0.0603	0.0506

samples generally declined from the apical to the basal segments as follows: I--1.86 and 1.95, II--1.65 and 1.60, III--1.72 and 1.60, and IV--1.47 and 1.31. As a result, the basal segments appeared to be more efficient in accruing a net photosynthate but less responsive photosynthetically to differences in free carbon dioxide than were the apical tissues.

With a few exceptions, the percent light saturation of photosynthesis at respirometer illumination of a tissue class was inversely related to the carbon dioxide concentration of a treatment. The magnitude of this variable in each water sample, in turn, was inversely related to the weight/length characteristics of a particular leaf section. This probably is because of a greater shading effect on the chlorenchyma cells of the thicker, intermediately located class II and III leaf segments. In all tissue classes, photosynthetic rates were higher in the station 1, high carbon dioxide sample than in the lower station water sample and were intermediate between these extremes in the station 2 sample with its intermediate carbon dioxide content.

Multiple comparisons from the two-way ANOVA of the results demonstrated that the mean PS_L differences for the tissue classes between the basic treatments were statistically significant as follows--lower versus upper stream water samples at 1%, lower versus middle stream samples at 5%, and the station 1 versus station 2 water samples at 1%.

This indicates that the photosynthetic activity of the shoot system of S. angustifolium (Table 36) significantly declined in water from the downstream station on the Madison River in response to the downstream decline in free carbon dioxide. PS_N/R ratios also declined, whereas light saturation at 780 foot candles increased downstream.

BRYOPHYTA (MUSCI)

Fissidens grandifrons Bird.

F. grandifrons is a relatively abundant plant in the Madison River and is found primarily in the lower reaches of the stream (Table 3). This moss is an extremely small plant compared to many of the other macrophytes in the river, and its small size and scattered distribution made the location and collection of individual plant difficult. Since each plant was only about one to two centimeters in length, several gametophytes from a number of plants could be placed in a single reaction vessel with no sectioning.

Due to the absence of a distinct photosynthetic response in F. grandifrons to carbon dioxide differences between water samples, two experiments were conducted with this species, the last one for confirmation. The three basic treatments were applied in both experiments, and three accessory treatments were tested in the first run. Accessory carbon dioxide alterations were made by Method II and by Method III (two treatments) due to the difficulty of locating specimens. The

Table 36. Estimates of the composite photosynthetic and respiratory rates for the shoot system of Sparganium angustifolium in unaltered water collected from three stations on the Madison River; inorganic carbon chemistries of the water samples are also listed. Rates expressed as ul O_2/mg/min.

	Basic Treatment		
Station	Upper	Middle	Lower
Alkalinity	123.2	126.3	126.3
pH	7.44	7.65	8.11
Free CO_2	7.11	4.50	1.56
PS_L	0.0390	0.0313	0.0282
% saturation	83%	90%	95%
R	0.0192	0.0182	0.0183
PS_N	0.0491	0.0369	0.0305
PS_N/R	2.56	2.03	1.67
PS_G	0.0683	0.0551	0.0488

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results of the two experiments with F. grandifrons are presented in Table 37.

Respiratory rates were similar in all treatments of both experiments, but slight differences in PS_N and PS_G rates were evident between the basic treatments. A rank correlation coefficient between PS_N rates and carbon dioxide concentrations proved to be significant at 5% (r_s = 0.843 with the six paired observations of the basic treatments). The photosynthetic rate differences between samples were relatively small, however, compared to those obtained for most of the macrophytes considered previously. Only about 1.17-fold differences in PS_N and PS_N/R were obtained for F. grandifrons between waters from

Table 37. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from two experiments with Fissidens grandifrons (E--estimated pH). Rates expressed as ul O_2/mg/min.

	First Experiment					
	Basic Treatments			Accessory Treatments		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II	Method III (20 sec)	Method III (40 sec)
Alkalinity	122.0	123.2	126.3	122.0	123.2	126.3
pH	7.44	7.76	8.13	----	7.27E	6.97E
Free CO_2	7.04	3.41	1.49	----	----	----
PS_L	0.0143	0.0140	0.0134	0.0102	0.0138	0.0151
% saturation	86%	85%	91%	84%	84%	82%
R	0.0042	0.0045	0.0048	0.0042	0.0045	0.0048
PS_N	0.0166	0.0165	0.0147	0.0121	0.0164	0.0184
PS_N/R	3.95	3.67	3.06	2.88	3.64	3.83
PS_G	0.0208	0.0210	0.0195	0.0163	0.0209	0.0232

	Second Experiment		
	Basic Treatments		
Stations	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered
Alkalinity	124.4	125.7	129.3
pH	7.32	7.81	8.16
Free CO_2	9.47	3.10	1.42
PS_L	0.0149	0.0138	0.0130
% saturation	81%	83%	85%
R	0.0052	0.0047	0.0045
PS_N	0.0183	0.0166	0.0153
PS_N/R	3.52	3.53	3.40
PS_G	0.0235	0.0213	0.0198

the upper and lower stations on the river. Nevertheless, the magnitude of photosynthesis in the various treatments generally corresponded to the carbon dioxide concentrations of the water.

Data from the accessory treatments with F. grandifrons indicated that the slight photosynthetic differences between basic treatments were related to differences in carbon dioxide. After dark storage of the upper river sample and its accompanying carbon dioxide evasion, mean PS_N was markedly lower than those of the first run under high carbon dioxide levels. In turn, with the addition of carbon dioxide by bubbling the lower reach sample, photosynthetic rates increased to a level greater than that in the unaltered, upper reach water. With the smaller addition of carbon dioxide by a 20 second aeration of station 2 water, mean PS_N remained similar to that of the unaltered, middle river sample since carbon dioxide loss from evasion was replaced by that from the aeration procedure. The PS_N/R ratios of the accessory treatments responded to changes in carbon dioxide in a fashion similar to that of PS_N or PS_G.

However, the small photosynthetic differences between tissues in various water samples, paralleling the water's carbon dioxide concentrations, could not be shown to be statistically significant. F-ratios from individual ANOVA's of the basic treatments in the two experiments were insignificant at 10% ($F^2_6 < 3.46$). Insignificant F-ratios

were also obtained from ANOVA's after combining all the treatments of the first experiment ($F_{10}^{5} = 2.40$, $P = 0.12$) and after combining the basic treatments of both experiments ($F_{14}^{2} = 2.24$, insignificant at 10%). Therefore, the mean PS_L rate of a basic treatment calculated from both experiments (Table 38) was also insignificantly different from the inter-experimental means of other treatments regardless of the added degrees of freedom in this analysis.

Fontinalis neo-mexicanus S. & L.

F. Neo-mexicanus (water moss) also has a small and relatively compact gametophyte, and no sectioning was required for sample preparation. Water moss was extremely rare in the Madison River. Therefore, only two individually incomplete experiments with low numbers of replications could be completed with this macrophyte. The two experiments were combined and averaged for the final analysis. Both basic and accessory treatments were applied to the macrophyte in each experiment, and all three methods of carbon dioxide alteration were used.

Little variation in the respiratory rates of F. neo-mexicanus (Table 39) was found between treatments. Photosynthetic rates were also quite similar in successive experiments with water from the upper and middle stations on the river. However, distinct differences in photosynthetic rates were evident between the three water samples in each experiment. The F-ratio from an ANOVA of the basic treatments

Table 38. Mean carbon dioxide concentrations and alkalinites and mean photosynthetic and respiratory rates obtained from the two experiments with Fissidens grandifrons in unaltered water from three stations on the Madison River. Rates expressed as ul O_2/mg/min.

Stations	Basic Treatments		
	Upper	Middle	Lower
Alkalinity	123.2	124.5	127.8
Free CO_2	8.26	3.26	1.46
PS_L	0.0146	0.0139	0.0132
% saturation	83%	84%	88%
R	0.0047	0.0046	0.0047
PS_N	0.0175	0.0165	0.0150
PS_N/R	3.72	3.59	3.19
PS_G	0.0222	0.0211	0.0197

with F. neo-mexicanus was significant at 1% ($F_6^2 = 12.69$). Multiple comparison analyses indicated that the mean PS_L rate in the upper river sample was significantly greater at 1% than the mean PS_L rate in the lower water sample. In addition, mean PS_L rates from the upper and middle samples were found to be significantly different at 5%. However, the relative difference in PS_N between the lower and the upper river samples, a factor of 1.37, was not particularly large in comparison to those obtained for several other species.

The magnitude of PS_N/R in the basic treatments followed a pattern similar to PS_N and declined in the low carbon dioxide waters collected

Table 39. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from two experimental runs (A and B) with Fontinalis neo-mexicanus (unalt.--unaltered, M.--Method). Rates expressed as ul O_2/mg/min.

	Basic Treatments			Accessory Treatments				
Stations	Upper	Middle	Lower	Lower----	Lower	Upper	Middle	Lower
Alterations	Unalt.	Unalt.	Unalt.	M.I	M.II	M.II	M.III (20 sec)	M.III (40 sec)
Alkalinity--A	122.0	123.2	126.3	-----	-----	122.0	123.2	126.3
Alkalinity--B	123.8	125.7	-----	127.5	127.5	123.8	125.7	-----
Mean	122.9	124.5	126.3	127.5	127.5	122.9	124.5	12.63
pH--A	7.44	7.83	8.28	----	----	----	----	----
pH--B	7.46	7.76	----	6.95	----	----	----	----
Free CO_2--A	7.04	2.90	1.05	----	----	----	----	----
Free CO_2--B	6.83	3.48	----	22.69	----	----	----	----
Mean	6.94	3.19	1.05	22.69				
PS_{LA}	0.0202	0.0176	0.0151	------	------	0.0127	0.0195	0.0222
PS_{LB}	0.0215	0.0172	------	0.0225	0.0182	0.0161	0.0150	------
Mean-PS_L	0.0209	0.0174	0.0151	0.0225	0.0182	0.0144	0.0173	0.0222
% saturation	93%	95%	92%	92%				
R_A	0.0058	0.0065	0.0054	------				
R_B	0.0058	0.0051	------	0.0066				
Mean-R	0.0058	0.0058	0.0054	0.0066				
PS_N	0.0226	0.0183	0.0165	0.0245				
PS_N/R	3.90	3.16	3.06	3.71				
PS_G	0.0284	0.0241	0.0219	0.0311				

downstream. However, in contrast to most of the macrophytes in the river, the percent saturation of photosynthesis for F. neo-mexicanus was not markedly different between treatments nor were they observed to follow any consistent pattern or trend as had been observed previously.

Accessory treatments also show a photosynthetic response in F. neo-mexicanus to alterations in free carbon dioxide. Such differences were demonstrated to be statistically significant. The total ANOVA for all treatments was highly significant at less than 0.5% ($F_{14}^{7} = 24.47$). In both cases where high carbon dioxide waters were stored to allow for evasion, PS_L rates distinctly dropped from their original level. This decline in photosynthesis through the same storage period was less pronounced, however, in water with the initially higher carbon dioxide content. With additions of carbon dioxide by Method I and III to the lower river sample, photosynthetic rates in both cases became significantly greater at 5% than the rate of the unaltered sample while now showing no significant differences in photosynthesis from the unaltered upper sample. After dark storage of the upper treatment and the associated carbon dioxide loss, its photosynthetic rate decreased significantly from the initial level and was also significantly different at 1% from that in the carbon dioxide-added, lower reach sample; in addition, this rate was no longer significantly

different from that in the unaltered lower sample. Photosynthesis also demonstrated a significant decline after the storage of the carbon dioxide-added, lower sample.

Oncophorus virens Horp.

O. virens is a third species of Bryophyta examined in this study. O. virens forms an important part of the flora in the lower section of the stream. Due to its small gametophyte tissue and vessel preparation procedures followed those adopted for Fissidens grandifrons and Fontinalis neo-mexicanus.

Two experiments were conducted with this species. PS_L rates from the two light phases of the main experiment were quite similar within each treatment (Table 40). Only slight photosynthetic differences were evident between them, and an insignificant F-ratio was obtained from an ANOVA of PS_L data ($F_{18}^{5} = 0.22$). Nevertheless, the magnitudes of PS_N, PS_G, and PS_N/R in the treatments generally corresponded to a treatment's carbon dioxide concentration. Such possible photosynthetic responses in O. virens to free carbon dioxide, however, were quite small in comparison to those obtained from several other species. For example, the relative differences in PS_N and PS_N/R between the lower and the upper river samples in this macrophyte were relatively low (factors of 1.28 and 1.27 respectively) in comparison to most of the Potamogetons, with factors greater than 1.70, and were most similar to factors

Table 40. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the main experiment with Oncophorus virens (unaltered samples). Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alkalinity	123.2	125.7	126.3
pH	7.52	7.80	8.23
Free CO_2	5.91	3.16	1.18
PS_{L1}	0.0245	0.0217	0.0209
PS_{L2}	0.0252	0.0227	0.0219
Mean	0.0249	0.0222	0.0214
% saturation	83%	94%	92%
R	0.0127	0.0131	0.0126
PS_N	0.0299	0.0235	0.0233
PS_N/R	2.35	1.79	1.85
PS_G	0.0426	0.0366	0.0359

obtained from the other mosses.

Since photosynthetic data from the main experiment with O. virens were statistically insignificant, the final experiment with this macrophyte was directed towards accessory treatments with extensive carbon dioxide alterations. In this experiment, the carbon dioxide concentration of the upper river sample was increased over in situ values in order to determine if the photosynthetic difference between the lower and upper river samples could be enhanced by this manipulation. In turn, a water sample from the upper site on the Madison River was purged of carbon dioxide to ascertain if the photosynthetic activity of O. virens would respond to this alteration. Unaltered water from the middle and lower stream stations were included in the experiment for comparison. The results of this experiment are presented in Table 41.

Photosynthetic rates in the basic treatments of the final experiment were generally similar to those obtained previously, although respiratory rates were slightly lower. Percent light saturation values were greater in the final experiment (near 100%), which could relate to a smaller amount of tissue in each reaction vessel in this experiment and a reduction of internal shading in each vessel.

Photosynthetic rate differences between the lower and middle river water samples were again quite small in the final experiment, and this corresponded to a relatively small carbon dioxide difference. However,

Table 41. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the final experiment with Oncophorus virens; all sample alterations were completed by Method I. Rates expressed as ul O_2/mg/min.

	Treatments			
Stations	Upper	Upper	Middle	Lower
Description	Altered	Altered	Unaltered	Unaltered
Alkalinity	125.1	125.1	129.3	129.9
pH	7.11	8.47	7.83	8.23
Free CO_2	15.42	0.67	3.04	1.22
PS_L	0.0266	0.0164	0.0212	0.0204
% saturation	100%	99%	100%	98%
R	0.0120	0.0108	0.0093	0.0119
PS_N	0.293*	0.0166	0.0215*	0.0208
PS/R	2.44	1.44	2.31	1.75
PS_G	0.0413	0.0274	0.0308	0.0327

An asterisk denotes those cases where $PS_{1/2L} > PS_L$.

such differences between the lower-middle and upper stream samples were more pronounced than in the previous experiment because of the induced and larger carbon dioxide differences between water samples. In addition, removal of carbon dioxide from the upper sample caused a marked reduction in photosynthesis.

These larger differences in PS_L rates in the final experiment were found to be significant statistically ($F_{10}^{3} = 32.18$, $P<0.005$), and multiple comparisons indicated that the CO_2-enriched, upper station sample had significantly greater photosynthetic rates at 1% than all other treatments. With the extensive removal of carbon dioxide from upper-station water, photosynthetic rates became significantly lower at less than 5% than those in the other samples. Photosynthetic rate differences between the unaltered middle and lower station water, however, remained insignificant. By combining PS_N data from the main and final experiments with that from a preliminary experiment with O. virens (Table 61, Appendix B), and by ranking carbon dioxide and PS_N (ten pairs), a significant Spearman rank correlation coefficient was obtained ($r_s = 0.803$, $P<0.01$) (Hoel 1966).

CHLOROPHYTA (CHAROPHYCEAE)--Chara vulgaris L.

C. vulgaris (muskgrass or stonewort) is a relatively complex and odoriferous green alga that, on the basis of standing crop, is the most abundant macrophyte in the Madison River of Yellowstone National Park

(Table 4). The thin, multicellular and filamentous thallus of this macrophyte was easily sectioned into appropriately-sized tissue sections for placement in the reaction vessels. Several individual plants collected from the river were sectioned and all tissue sections transferred to a common pool. Single sections were then randomly transferred from this pool to the randomly ordered reaction vessels containing sample water until each vessel had the necessary amount of tissue.

Two experiments were completed with C. vulgaris (Table 42). Only small differences in photosynthetic rates were evident between basic treatments with this species within an experiment. However, PS_N and PS_G rates were slightly greater in both experiments with C. vulgaris in water from the high carbon dioxide, upper station. PS_N rates were slightly greater in the middle than in the lower river sample, but PS_G rates were essentially the same. None of the photosynthetic (PS_L) differences between treatments were statistically significant at 10% with one-way ANOVA's--$F^2_7 = 0.12$ in the first experiment, and $F^2_9 = 0.59$ and 0.08 for PS_{L1} and PS_{L2} in the second experiment.

Respiratory rates of C. vulgaris were similar in the two experiments and in all of the treatments, and percent light saturation figures for a particular treatment were also about equal in both experiments. However, photosynthetic rates were greater in the second experiment than in the first for all treatments. This was possibly

Table 42. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from experiments with Chara vulgaris in unaltered samples collected from three sites on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments--First Experiment		
Stations	Upper	Middle	Lower
Alkalinity	123.2	125.7	126.3
pH	7.55	7.82	8.30
Free CO_2	5.52	3.02	1.01
PS_L	0.0296	0.0282	0.0287
% saturation	81%	83%	88%
R	0.0105	0.0108	0.0117
PS_N	0.0364	0.0338	0.0327
PS_N/R	3.47	3.13	2.79
PS_G	0.0469	0.0446	0.0444

	Basic Treatments--Second Experiment		
Stations	Upper	Middle	Lower
Alkalinity	123.2	125.7	126.3
pH	7.55	7.82	8.30
Free CO_2	5.52	3.02	1.01
PS_{L1}	0.0340	0.0313	0.0325
PS_{L2}	0.0322	0.0309	0.0313
Mean PS_L	0.0331	0.0311	0.0319
% saturation	84%	84%	88%
R	0.0106	0.0111	0.0116
PS_N	0.0394	0.0370	0.0364
PS_N/R	3.72	3.33	3.14
PS_G	0.0500	0.0481	0.0480

related to better technique in the latter experiment due to experience gained from the first. As indicated in Table 42, the greater photosynthetic rates in the second experiment coupled with a constancy of respiration resulted in greater PS_N/R ratios in all treatments of the second run. Due to these factors, data from the second experiment with C. vulgaris will be used later for further comparisons and interpretations.

Although statistically insignificant, photosynthetic rate differences were obtained between treatments in C. vulgaris, and these paralleled differences in carbon dioxide concentrations. Two other factors in Table 42 are also suggestive of at least a slight response in this macrophyte to differences in free carbon dioxide concentrations. Along with photosynthetic rates, PS_N/R ratios were greater in treatments with the higher carbon dioxide contents; such differences in PS_N/R were quite consistent (i.e., upper>middle>lower in both experiments) and more distinct than those of photosynthesis. In addition, the percent saturation of photosynthesis at instrument illumination was consistently lowest in treatments with the high carbon dioxide concentrations. This same pattern was observed in numerous other macrophytes which demonstrated statistically significant photosynthetic rate differences between high and low carbon dioxide waters.

DISCUSSION

DISTRIBUTION AND ABUNDANCE OF AQUATIC MACROPHYTES

Distinctive in Table 3 is the downstream decline in total canopy cover observed for the Madison River by Horpestad (1969). A 77.6% decrease was noted from the upper to the lower reach. This is generally paralleled by the standing crop data (Table 4) of Todd (1967) which shows an 80.2% reduction in biomass through the same sections of the river. Klarich and Wright (1974) reported a similar decline, near 81%, in standing crop.

Undoubtedly the downstream change in the physical nature of the stream could in part account for this reduction in macrophytic canopy cover and biomass. The swifter, shallower, and more gravelly nature of the lower, riffle section of the river could be less conducive to the establishment of abundant plant populations than the smoother and deeper upstream segment. Horpestad (1969) suggested that current speed and substrate texture were the primary factors influencing the total canopy cover of the river community and therefore the downstream decline. Horpestad (1969) also considered depth, interspecific competition, and total soluble inorganic carbon as important limiting factors for certain of the species. However, he did not completely eliminate the potentiality of the other major variable, free carbon dioxide, as an important factor. This coupled with the results of Wright and Mills (1967), Todd (1967), and Klarich and Wright (1974) suggest that free

carbon dioxide, by itself or in combination with various physical factors, should not be ignored as an important ecological factor in the Madison River.

Certain discrepancies are evident between the relative abundances calculated from Todd's (1967) biomass data and those calculated from Horpestad's (1969) percent canopy cover figures. However, those species harvested and tabulated by Todd represent the dominant species listed by Horpestad and 95.6% to 99.2% of Horpestad's total canopy cover. Major discrepancies between studies relate to differences in the magnitude of contribution by any given species to standing crop versus its contribution to total canopy cover. This is probably a consequence of the two different methods employed in the studies and a reflection of the distinctiveness of the two variables. That is, a small plant and a large plant could contribute equally to total canopy cover as defined by Horpestad (1969) but unequally to total biomass as determined by Todd (1967).

Regardless of such differences, certain patterns of distribution are common to both studies. Downstream changes in floristic composition apparently accompany the reduction in carbon dioxide and the physical change in the stream and follow the declines in total standing crop and canopy cover. Therefore, as indicated in Tables 3 and 4, the three reaches of the Madison River, previously described on the basis of pH

and carbon dioxide, also appear to be fairly distinct floristically. The delineation of definite distributional patterns in the Madison River is of importance to this study for comparison to metabolic data. The demonstration of parallels between distribution, carbon source factors, photosynthetic responses, and PS_N/R relationships would help to confirm that carbon dioxide is an important ecological variable in the stream.

By using Horpestad's (1969) (Table 3) and Todd's (1967) (Table 4) relative abundance data to calculate a mean relative abundance for each taxon and reach, three plant associations, corresponding to the three reaches of the Madison River, become evident in the stream as indicated in Table 43. Factors most obviously characterizing the lower reach of the Madison are the low total canopy cover and standing crop values and the predominance of the mosses which are most notable on a biomass basis. In addition, Berula erecta and Myriophyllum exalbescens are important constituents of this reach. Also distinctive in the lower section of the stream are the reduced abundances of Chara vulgaris, Potamogeton spp., Glyceria borealis, and Sparganium-Sagittaria spp.

In contrast, the upper reach of the river can be characterized by the general prevalence of these five genera in addition to B. erecta and M. exalbescens. However, the latter two species are less conspicuous on a relative basis in the upper section than in the lower. In

Table 43. Three associations of aquatic macrophytes observed in the Madison River of Yellowstone National Park corresponding to three reaches of the stream. Groupings made on the basis of mean relative frequencies calculated from canopy cover and standing crop data (from Todd 1967 and Horpestad 1969). Spar.-Sag.--Sparganium angustifolium plus Sagittaria cuneata.

	Madison River Reach		
	Upper	Middle	Lower
Total Canopy Cover	180.4%	103.1%	40.4%
Standing Crop (g/m^2)	274.3	117.5	54.2
Dominant Taxa			
>40%	None	None	Musci
28-40%	C. vulgaris	C. vulgaris	None
Abundant Taxa			
20-28%	Potamogeton spp.	None	M. exalbescens B. erecta
Common Taxa			
12-20%	B. erecta M. exalbescens	B. erecta G. borealis Potamogeton spp.	None
3-12%	G. borealis Spar.-Sag.	Musci M. exalbescens Spar.-Sag.	C. vulgaris
Rare Taxa			
0-3%	Musci	None	G. borealis Potamogeton spp. Spar.-Sag.
Miscellaneous Species	9	9	3
Total Species	22	21	11

addition, the upper reach is unique in its number of relatively rare species that were not found downstream (Table 3). Thus, the number of species upstream are much greater, twenty-two versus eleven species in the lower reach, and illustrative of a greater floral diversity in the high carbon dioxide portion of the Madison River study area. The relative inconspicuousness of the mosses further distinguishes the upper sections of the Madison River.

The middle reach of the Madison River might be best described as an ecotone; plant abundances, both standing crop and canopy cover, are intermediate between the extremes of the upper and lower reach. Through this ecotone, the dominant taxa of the upper reach, Potamogeton spp. and possibly Chara vulgaris, become less prevalent whereas the dominant taxa of the lower reach, the mosses, become more common, and M. exalbescens and B. erecta remain conspicuous. The ecotonal middle reach association of intermediate plant abundance but high diversity and the charophyte-pondweed dominated upper association of high plant abundance and diversity, and the ubiquitous B. erecta - M. exalbescens pair through all reaches, are all demonstrated in Table 43. Also illustrated is the low diversity and low plant abundance riffle segment dominated by the mosses through this lower reach.

The physical nature of the lower reach in association with the dominance of the mosses indicates that this segment of the Madison is

best characterized as having a torrential community as described by Butcher (1933). In contrast, the abundance of Potamogeton spp. upstream along with the slower currents, greater depths, and greater proportion of silt substrate indicates that this reach approaches Butcher's description of a littoral community.

PHOTOSYNTHETIC AND RESPIRATORY RATES OF AQUATIC MACROPHYTES

Mean net photosynthetic rates of aquatic macrophytes varied considerably in the study through all species and treatments and ranged from 0.004 ul O_2/mg/min in Juncus ensifolius under unfavorable conditions to 0.208 ul O_2/mg/min in Hippuris vulgaris under favorable conditions. This variation in photosynthesis was due to a number of factors: rate differences within a species in relation to differences between treatments, rate differences between the different tissue-types within a species, photosynthetic rate differences between the twenty-two species of aquatic macrophytes per se, and differences corresponding to differing levels of light saturation under treatment conditions. If only the basic treatments of the main experiments are considered along with estimates of light saturated photosynthetic rates for the entire shoot system of the macrophytes, the range of photosynthetic rates in the study is greatly reduced. Rates than ranged from 0.0119 ul O_2/mg/min in Myriophyllum exalbescens to 0.1540 ul O_2/mg/min in H. vulgaris.

Variation in the respiratory rates of aquatic macrophytes was considerably less than that for photosynthesis since inter-treatment differences in respiration within a species were typically negligible. Variation in respiration rate was due primarily to differences between species and tissue types, and ranged from 0.0042 ul O_2/mg/min in Fissidens grandifrons to 0.0529 ul O_2/mg/min in Ceratophyllum demersum. This range is reduced if only the composite, shoot system estimates of the main experiments are utilized. This range then relates only to respiratory rate differences between species.

Several other studies report photosynthetic and respiratory data for aquatic macrophytes (Wetzel 1965, 1969; Hartman and Brown 1966; Sculthorpe 1967; Westlake 1967; McGahee and Davis 1971; and Klarich 1971). Such rates were obtained under a variety of experimental conditions and environmental gradients, e.g., low velocity currents and variations in ionic concentrations, salinity, light intensity, diurnal time and monthly comparisons, oxygen, and temperature. When the photosynthetic-respiration rates reported by these investigators are converted to the units used in this study by application of the Ideal Gas Law (Sienko and Plane 1957), they are comparable to those found for the Madison macrophytes.

In the basic treatments of the main experiments with the twenty-two Madison species, photosynthetic rates of macrophyte shoot systems

varied by a factor of thirteen due to intra-specific treatment effects and due to intra-treatment species differences. Respiratory rates varied by a factor of eight due primarily to species differences. In contrast, combined data from the other studies demonstrated extreme differences in photosynthesis and respiration by factors of 89 and 39 respectively. Such comparisons would indicate that metabolic rate data obtained in this study correspond to a series of values that might be expected from the occurrence of a wide range of environmental variables.

Highly significant and positive correlations were obtained between the respiratory and the net photosynthetic rates of the twenty-two Madison species. Both the rank correlation and the parametric correlation coefficients for the middle reach water sample were significant at less than 1% ($r = 0.84$ and $r_s = 0.88$). Net photosynthetic and respiratory rates in water from the middle reach were found to be related as $PS_N = 2.12R + 0.00485$ with $t_{20} = 6.97$). This indicates that species which were highly active in one process had the tendency to be highly active in the other. As a result, PS_N/R ratios from the basic treatments were much less variable between species than either the photosynthetic or respiratory rates alone. Shoot system estimates of PS_N/R in the basic treatments ranged from 1.03 in Sagittaria cuneata under unfavorable conditions to 5.09 in Potamogeton filiformis under

favorable conditions.

The above equation indicates that when all species are considered, photosynthesis typically exceeded respiration by a factor over two in the middle-reach, intermediate carbon dioxide water (i.e., PS_N/R = 2.46). This factor was found to be greater in the upper river, high carbon dioxide samples but lower in the downstream, low carbon dioxide treatments. The overall PS_N/R ratios in these cases were found to equal 3.46 and 2.17 respectively.

THEORETICAL CONSIDERATIONS OF PHOTOSYNTHETIC/RESPIRATORY RATIOS

In the study, net photosynthetic rates (PS_N) and respiratory rates (R) of aquatic macrophyte tissue were the major statistics obtained from the basic and accessory treatments. In addition, several other parameters were derived from these data. These include estimates of gross photosynthesis (PS_G) calculated as $PS_G = PS_N + R$. Photosynthetic-respiratory ratios (PS_N/R) were also determined and are of particular value in that data from two basic metabolic processes are united into one statistic. In effect, this variable provides an indication of the effectiveness of a tissue or a plant in accumulating a net photosynthate under treatment conditions.

Given a twelve-hour dark:twelve-hour light diurnal cycle plus the assumption of light saturation with maximum photosynthetic rates throughout the light period, a PS_N/R ratio of one would represent a

diurnal compensation point. That is, 24-hour respiratory expenditures would be closely balanced by a net photosynthetic accumulation during the day. However, this is a theoretical, minimal value. A PS_N/R of less than one would definitely be indicative of adverse conditions for plant survival. Even at $PS_N/R = 1$, the overall photosynthetic accumulation would probably be to the "negative side" in relation to the needs of anabolic processes. Therefore, the critical minimum PS_N/R ratio required for plant survival *in situ* is undoubtedly much greater than one considering the requirement of synthetic, anabolic processes for growth and development, for vegetative and sexual reproduction, and for normal regeneration countering entropy.

Terrestial systems have been observed to have PS_N/R ratios considerably greater than one. As examples, a PS_N/R of 3.3 was obtained for an acre of corn during the growing season, and a PS_N/R of 5.6 was obtained for perennial grass-herb vegetation (Transeau 1926, Golley 1960). Viable aquatic systems were also found to have relatively high values. PS_N/R ratios of 3.0 and 3.1 were obtained for the phytoplankton and bottom flora of Lake Mendota respectively (Juday 1940), and a value of 3.8 was obtained for the flora in Cedar Bog Lake (Lindeman 1942).

A further illustration of PS_N/R relationships in plants is provided by the work of McGahee and Davis (1971) who measured the photosynthetic and respiratory rates of *Myriophyllum spicatum* L. along an

estuarine salinity gradient. They found that photosynthesis and therefore the PS_N/R ratios of plant tips (apical bud region) declined from near 2.7 under favorable conditions to about 1.3 at the higher salinities. The low PS_N/R values corresponded to the elimination and eradication of the plant. They suggest that the depression of photosynthesis by increased salinity and its effect on PS_N/R may play a role in controlling the distribution of the plant. This implies that the closer the PS_N/R ratio of plant is to one in a treatment, the greater the likelihood that the natural case represented by this treatment is unfavorable to ecesis of the plant. In turn, relatively high PS_N/R ratios in a treatment, e.g., approaching and exceeding 2.0, would suggest more favorable conditions for the plant. A similar application can be made in relation to the carbon dioxide gradient of the Madison River using the PS_N/R values obtained in this study.

PS_N/R ratios and the relative magnitude of change in PS_N/R for each species through the carbon dioxide gradient of the Madison River were combined for comparative purposes by deriving an equation that used the ratios obtained in water from the upper and lower stations on the stream. The factor obtained from this equation can be utilized as a means of comparing physiological data to distributional patterns. Three aspects were taken into account in this derivation.

First, the magnitude of the PS_N/R ratio in water from the lower

station was considered. It was assumed that the smaller this ratio, the more critical the low carbon dioxide concentrations in the lower reach would be for a species. To represent this, the PS_N/R value from the lower treatment was reciprocated; with this manipulation, a relatively high number would be illustrative of a more critical case.

Second, the difference in PS_N/R in samples from the upper and the lower stations on the river was also considered since a greater change in PS_N/R from high to low carbon dioxide along the gradient would indicate a larger carbon dioxide effect. However, as a final consideration, this PS_N/R change downstream is related to the magnitude of PS_N/R in the lower sample. A large difference in this ratio relative to a low PS_N/R under low carbon dioxide tensions would be more indicative of a critical carbon dioxide level for a species than a similar difference relative to a high PS_N/R in the lower sample. In turn, a small difference in PS_N/R through the gradient in relation to a high ratio in the lower treatment would represent the extreme case of no response to and noncritical levels of carbon dioxide.

These three aspects were combined to derive a PS_N/R factor ($F_{P/R}$) as follows for each species examined:

$$F_{P/R} = \frac{1}{(PS_N/R)_L} \cdot \frac{(PS_N/R)_U - (PS_N/R)_L}{(PS_N/R)_L} \text{ ; or,}$$

$$F_{P/R} = \frac{(PS_N/R)_U - (PS_N/R_L,}{(PS_N/R)^2_L}$$

where $(PS_N/R)_U$ and (PS_N/R_L) are PS_N/R ratios for a species obtained in water samples from the upper (U) and lower (L) stations on the Madison River.

According to this equation, the more pronounced effects of the carbon dioxide gradient in the river are illustrated by relatively high $F_{P/R}$ values for any given species. Theoretical limits on the expression range from zero (no effect) for the case where there are no PS_N/R differences between upper and lower treatments to an infinitely large value wherein the ratio in the lower sample approaches zero and/or the PS_N/R differences between treatments become increasingly large. However, empirical limits for the equation define a much narrower range of values. This range can be illustrated by four examples as follows:

	$(PS_N/R)_L$	$(PS_N/R)_U$	Difference	$F_{P/R}$
Case 1	1.3	2.7	1.4	0.83
Case 2	1.3	2.3	1.0	0.59
Case 3	2.5	3.9	1.4	0.22
Case 4	2.5	3.5	1.0	0.16

The above data suggest that $F_{P/R}$ values approaching or exceeding one, e.g., Case 1 calculated from the Myriophyllum spicatum,

salinity gradient data of McGahee and Davis 1971, are indicative of pronounced effects whereas values less than 0.5 and approaching zero are illustrative of the less critical cases in relation to an environmental gradient. Cases 2 to 4 represent hypothetical and less severe examples of PS_N/R levels and magnitude of change for comparison to the data of McGahee and Davis (1971). A similar range of $F_{P/R}$ values was obtained from the species examined in this study, e.g., Sagittaria cuneata, 1.13; Eleocharis acicularis, 0.55; Fissidens grandifrons, 0.05. On the basis of $F_{P/R}$, the carbon dioxide gradient in the Madison River apparently has a much greater influence on S. cuneata, somewhat similar to the effect of the salinity gradient on the PS/R status of M. spicatum, than on F. grandifrons, and E. acicularis occupies an intermediate position.

THEORETICAL CONSIDERATIONS OF PERCENT LIGHT SATURATION

Another variable derived from the photosynthetic rate data of the experiments was the percent light saturation of photosynthesis at maximum respirometer illumination under the conditions of a treatment. If the availability of carbon substrates were limiting to photosynthesis at constant temperature and light, an increase in a carbon species such as free carbon dioxide should increase photosynthetic rates but decrease the degree of light saturation at constant light intensity. Thus, if free carbon dioxide were a critical variable for a particular

species, an increase in its concentration should then decrease the percent saturation at the 780 foot candles of the respirometer. With the decline in carbon dioxide from the upper to the lower Madison, a corresponding increase in the percent light saturation of a species, coupled with a decline in photosynthesis, would be expected in samples obtained from subsequent sites along the gradient. This in turn would tend to confirm that free carbon dioxide acts as a critical variable in the stream. Percent saturation, along with PS_N/R and $F_{P/R}$, therefore provides another diagnostic and confirmatory tool by which to assess the importance of carbon dioxide relative to the aquatic macrophytes in the Madison River.

EFFECTS OF CARBON DIOXIDE ON MACROPHYTE PHOTOSYNTHESIS

In contrast to respiration, photosynthetic rates of the aquatic macrophytes distinctly differed between treatments. This is illustrated in Table 44 which summarizes data composited from Tables 6 to 42. Any interpretations and conclusions concerning the possible role of carbon dioxide in influencing macrophyte photosynthesis would be derived through comparisons of the photosynthetic rates that were obtained in water samples from the upper, middle, and lower stations on the river, plus those obtained in carbon dioxide alteration experiments. Since temperature was held constant and since physical factors such as current velocity would have no effect in the experiments, some

Table 44. Mean net photosynthetic rates (PS_N) and mean respiratory rates (R) (ul O_2/mg/min), PS_N/R ratios, and percent saturations (% sat) at 780 foot candles obtained from twenty-two species of aquatic macrophytes in unaltered water samples from the upper (U), middle (M), and lower (L) stations on the Madison River. Mean carbon dioxide, bicarbonate, total inorganic carbon concentrations and ranges for the three stations are also listed.

	Parameter and Treatment											
	PS_N			R			PS_N/R			% Sat		
Species	U	M	L	U	M	L	U	M	L	U	M	L
B. erecta	0.0326	0.0279	0.0233	0.0093	0.0097	0.0096	3.51	2.88	2.43	88	89	96
C. demersum	0.0968	0.0752	0.0575	0.0355	0.0370	0.0336	2.73	2.03	1.71	79	82	83
C. vulgaris	0.0394	0.0370	0.0364	0.0106	0.0111	0.0116	3.72	3.33	3.14	84	84	88
E. acicularis	0.0253	0.0171	0.0127	0.0064	0.0065	0.0066	3.95	2.63	1.92	80	88	100
E. thermalis	0.0298	0.0233	0.0172	0.0075	0.0080	0.0093	3.97	2.91	1.85	52	56	62
F. grandifrons	0.0175	0.0165	0.0150	0.0047	0.0046	0.0047	3.72	3.59	3.19	83	84	88
F. neo-mexicanus	0.0226	0.0183	0.0165	0.0058	0.0058	0.0054	3.90	3.16	3.06	93	95	92
G. borealis	0.0283	0.0183	0.0137	0.0091	0.0092	0.0097	3.11	1.99	1.41	65	79	83
H. vulgaris	0.1540	0.1071	0.0987	0.0313	0.0306	0.0289	4.92	3.50	3.42	71	79	79
J. ensifolius	0.0310	0.0276	0.0190	0.0110	0.0123	0.0118	2.82	2.24	1.61	81	81	97
M. guttatus	0.0817	0.0443	0.0348	0.0211	0.0204	0.0224	3.87	2.17	1.55	81	85	89
M. exalbescens	0.0178	0.0152	0.0119	0.0055	0.0058	0.0060	3.24	2.62	1.98	83	88	92
O. virens	0.0299	0.0235	0.0233	0.0127	0.0131	0.0126	2.35	1.79	1.85	83	94	92
P. filiformis	0.0489	0.0320	0.0208	0.0096	0.0096	0.0105	5.09	3.33	1.98	78	85	92
P. gramineus	0.0670	0.0540	0.0509	0.0138	0.0134	0.0137	4.86	4.03	3.72	91	90	95
P. natans	0.0450	0.0276	0.0208	0.0124	0.0116	0.0122	3.63	2.38	1.66	80	90	100
P. nodosus	0.0511	0.0338	0.0296	0.0140	0.0135	0.0144	3.65	2.50	2.06	81	92	96
P. strictifolius	0.0342	0.0253	0.0177	0.0127	0.0133	0.0131	2.69	1.90	1.35	82	83	100
P. pectinatus	0.0469	0.0283	0.0197	0.0111	0.0108	0.0101	4.22	2.62	1.95	83	100	100
S. cuneata	0.0526	0.0451	0.0237	0.0236	0.0255	0.0229	2.23	1.77	1.03	95	98	100
S. angustifolium	0.0491	0.0369	0.0305	0.0192	0.0182	0.0183	2.56	2.03	1.67	83	90	95
U. vulgaris	0.0428	0.0394	0.0312	0.0235	0.0245	0.0257	1.82	1.61	1.21	95	98	100
Overall Means	0.0475	0.0352	0.0284	0.0141	0.0143	0.0142	3.48	2.59	2.08	81	87	92

Table 44. Continued.

	Carbon Dioxide (mg/l)			Bicarbonate (mg/l)			Total Carbon (mg/l)		
	U	M	L	U	M	L	U	M	L
Mean	6.82	3.36	1.17	121.9	123.7	125.6	25.9	25.3	25.1
Minimum	4.93	2.64	1.00	115.9	117.7	118.3	24.2	23.9	23.6
Maximum	9.07	4.53	1.56	123.2	126.3	127.8	26.8	26.1	25.6

aspect of water chemistry was apparently affecting the photosynthetic activities of the plants. In addition, since the magnitude of the photosynthetic rate differences between basic treatments varied with the species, this chemical factor must have been differentially influential between species. Several lines of evidence indicate that the differing carbon dioxide concentrations in the river samples were in fact the causal factor for photosynthetic rate differences between treatments.

As indicated by chemical data from other investigations, e.g., Roeder (1966) and Wright and Mills (1967), water quality variables other than free carbon dioxide often lacked or had relatively minute changes in concentration downstream, e.g., potassium, magnesium, sulfate, and iron. In other cases, they either had non-directional changes in concentration through the reach in Yellowstone Park, e.g., nitrate, ammonia, and possibly calcium, had differences in concentration in an opposite direction to the photosynthetic differences between samples, e.g., alkalinity, or else had some combination of these features, e.g., possibly phosphate and temperature. In addition, some of the chemical variables had relatively high concentrations in the river and therefore were not expected to be limiting. No chemical factor for which information is available for the Madison, such as fluoride or boron, had concentrations that might be expected to approach toxic

levels for aquatic macrophytes.

Thus most of the chemical constituents in the river could be eliminated from consideration on these bases. With one exception, only carbon dioxide was found to have consistent and marked changes in concentration that directly corresponded to the photosynthetic rate differences between samples. However, the magnitude of photosynthesis was also directly related to the hydrogen ion concentrations of the water samples and thereby inversely related to their pH's.

If carbon dioxide was the major factor regulating photosynthesis, then photosynthetic rates of a species would be greater in the upper Madison, high carbon dioxide water than in water from the lower, low carbon dioxide reach. Photosynthetic rates in water from the middle reach with its intermediate carbon dioxide concentrations would be expected to be between the upper and lower extremes. As Table 44 shows, this was the case for all twenty-two species examined in the study and for the overall mean rates for the twenty-two species. In addition, the overall mean photosynthetic rate of the middle reach treatment was closer to the overall mean of the lower reach treatment than to the mean of the upper treatment. This corresponds to the fact that the mean carbon dioxide concentration of the middle reach samples was closer to the mean concentration of the lower than to the mean of the upper samples. A similar relationship also was found in the overall

means of the PS_N/R and PS_G data.

Additional evidence for the influence of carbon dioxide in producing photosynthetic responses was obtained from the accessory, carbon dioxide alterations. If photosynthetic rates responded inversely to the pH changes of these altered treatments, and if the rates obtained from the altered water were consistent with those from the unaltered treatments, then supporting data would be provided for the operation of carbon dioxide as a photosynthetic factor rather than some other chemical variable in the river.

In the case of the Method I alterations, additions of carbon dioxide to the lower reach samples and/or its removal from the upper reach samples consistently produced the expected increases or decreases in photosynthetic rates. In most cases, photosynthetic differences between the altered and the related unaltered samples were statistically significant. In addition, photosynthetic rates in the altered upper and the unaltered lower reach treatments were generally similar as were the photosynthetic rates in the altered lower or unaltered upper samples. Remaining photosynthetic discrepancies between these altered-unaltered pairs were generally explainable on the basis of the residual carbon dioxide differences between the two samples after alteration. In many instances, photosynthetic differences between the upper and lower reach treatments became statistically insignificant after the

carbon dioxide alteration of one of the pair; that is, by reducing carbon dioxide in the upper reach sample, photosynthetic rates in this sample became similar to those of the unaltered lower sample and vice versa. In turn, photosynthetic differences between the upper and the lower treatments became statistically significant in a reverse direction after the carbon dioxide alterations of both samples. All of these results are in accord with the view that carbon dioxide is a critical photosynthetic factor in the river. Similar conclusions were derived from Method II and Method III alterations.

If some factor other than carbon dioxide were influencing photosynthesis in the basic treatments, then photosynthetic responses from the Method I, II, and III alterations would not be expected since the other chemical constituents in the samples, with the exception of hydrogen ion, probably were not affected to any great extent by these manipulations. Most values of pH in the various experiments were within the range of pH's from natural waters (6.5 to 8.5) and were well within the recommended limits for aquatic organisms (6.0 to 9.0) (National Technical Advisory Committee 1968). Toxic effects from this source would therefore not be expected.

As indicated by the equation of Rainwater and Thatcher (1960), differences in hydrogen ion concentration between the samples of an experiment were highly correlated to corresponding carbon dioxide

differences (e.g., r = 0.9999 in one case). Thus, the concentration of hydrogen ions would be positively correlated with photosynthesis in a manner similar to the correlation with carbon dioxide. As an example, in Berula erecta (Table 6) a significant correlation of $r = 0.958$ ($P<0.01$) was obtained for the photosynthetic rate and the carbon dioxide differences between samples ($n = 15$); a similar coefficient of $r = 0.959$ was obtained with the hydrogen ion differences. The relative effects of hydrogen ion and carbon dioxide on photosynthesis therefore could not be directly separated in the experiments. This fact has plagued researchers for many years and forms a part of the debate concerning the importance of carbon dioxide as a eutrophic factor. The various ramifications of the two poles of this debate have been generally summarized by Shapiro (1973a), by Goldman's (1973) criticism of Shapiro's (1973a) paper, and by Shapiro's (1973b) rebuttal to Goldman's comments.

The general role of increased hydrogen ion concentration or decreased pH in accelerating photosynthesis is generally visualized as an effect on nutrient availability and as an effect on enzymes associated with nutrient transport. With such effects on the photosynthetic process, however, certainly the respiratory activities of plants would be influenced to some degree also. In contrast to photosynthesis, the respiratory rates of aquatic macrophytes in this study were not notably

affected by the Method I alterations nor by the hydrogen ion differences between the basic treatments (Table 44). It is concluded, therefore, that carbon dioxide plays the major role in affecting the limiting macrophyte photosynthesis in the Madison River of Yellowstone National Park.

CARBON CHEMISTRY AND PRIMARY PRODUCTION IN THE MADISON RIVER

The mean carbon dioxide concentration in the lower river was found to be 17.2% of that in the upper river. Due to the low pH values of all samples, alkalinity was primarily in the form of bicarbonate, and this was also available as a carbon source for photosynthesis. With the slight 1.03-times increase in alkalinity-bicarbonate downstream, the decline in total inorganic carbon from upper to the lower station was much less evident than that for carbon dioxide; that is, 96.9% of the upstream concentrations in total inorganic carbon were found in the lower river samples (Table 44). As a result, Wright and Mills (1967) and Todd (1967) suggested that the reduction in carbon dioxide was the major factor in the river causing the downstream decline in primary productivity.

If the carbon dioxide-primary productivity hypothesis developed through the earlier research were correct, then distinct differences in the photosynthetic rates of Madison species dependent on free carbon dioxide would be expected when tested in water samples from various

points along the carbon dioxide gradient of the stream. Results indicate that carbon dioxide does have a major and direct effect upon the photosynthetic rates of species in the Madison River. Extrapolation of these respirometer results to the river suggests that the in situ photosynthetic rates of a large portion of the species would markedly decline downstream in response to the downstream decrease in carbon dioxide. This decline in photosynthesis corresponds to the in situ decrease in primary productivity that was observed in other studies. However, a wide difference was observed between species in the magnitude of their photosynthetic responses to carbon dioxide.

PHOTOSYNTHETIC RATE DIFFERENCES AMONG WATER SAMPLES AND BETWEEN SPECIES

Magnitudes of Difference

The photosynthetic rate data in Table 44 suggest that all species of aquatic macrophytes responded to some extent to differences in carbon dioxide between treatments. However, considerable variability was observed between the species as illustrated in Table 45 by the relative differences in PS_N and in PS_N/R that were obtained between the lower and upper reach water samples. Such differences in PS_N ranged from 1.08 in Chara vulgaris to 2.38 in Potamogeton pectinatus indicating a 2.2-fold difference in response by species.

The estimated percent declines in photosynthetic rates of the macrophytes from the upper to the lower reaches of the Madison River are

Table 45. A ranking of species based on the relative differences in PS_N and PS_N/R between water samples from the upper and lower reaches of the Madison River, and the estimated, *in situ* percent declines in these variables from the upper to the lower reaches of the stream.

Rank	Species	(1) Relative Difference in PS_N	(2) Percent Decline in PS_N	(1) Relative Difference in PS_N/R	(2) Percent Decline in PS_N/R
1.	*Potamogeton pectinatus*	2.38	58.0	2.16	53.8
2.	*Potamogeton filiformis*	2.35	57.5	2.57	61.1
3.	*Mimulus guttatus*	2.35	57.4	2.50	59.9
4.	*Sagittaria cuneata*	2.22	54.9	2.17	53.8
5.	*Potamogeton natans*	2.16	53.8	2.19	54.3
6.	*Glyceria borealis*	2.07	51.6	2.21	54.7
7.	*Eleocharis acicularis*	1.99	49.8	2.06	51.4
8.	*Potamogeton strictifolius*	1.93	48.2	1.99	49.8
9.	*Eleocharis thermalis*	1.73	42.3	2.15	53.4
10.	*Potamogeton nodosus*	1.73	42.1	1.77	43.6
11.	*Ceratophyllum demersum*	1.68	40.6	1.60	37.4
12.	*Juncus ensifolius*	1.63	38.7	1.75	42.9
13.	*Sparganium angustifolium*	1.61	37.9	1.53	34.8
14.	*Hippuris vulgaris*	1.56	35.9	1.44	30.5
15.	*Myriophyllum exalbescens*	1.50	33.1	1.64	38.9
16.	*Berula erecta*	1.40	28.5	1.44	30.8
17.	*Utricularia vulgaris*	1.37	27.1	1.50	33.5
18.	*Fontinalis neo-mexicanus*	1.37	27.0	1.27	21.5
19.	*Potamogeton gramineus*	1.32	24.0	1.31	23.5
20.	*Oncophorus virens*	1.28	22.1	1.27	21.3
21.	*Fissidens grandifrons*	1.17	14.3	1.17	14.2
22.	*Chara vulgaris*	1.08	7.6	1.18	15.6
	Means	1.72	38.7	1.77	40.0

(1) Relative difference calculated as the ratio of PS_N or PS_N/R in the upper sample to that in the lower sample.

(2) Percent decline in PS_N and PS_N/R is defined as follows: $(U-L)/U \times 100$, where U and L are PS_N or PS_N/R values for a species treated in the upper and lower reach water samples respectively.

also included in Table 45. These also varied widely among species and ranged from a relatively small decrease of 7.6% in C. vulgaris to a 58.0% decline in P. pectinatus. The distribution of photosynthetic rate declines within these two extremes is summarized in Figure 3 for the twenty-two species.

A distinctly non-normal and skewed distribution is evident. A large number of species are projected to have relatively large declines in photosynthetic rates from the upper to the lower reaches of the Madison, whereas a comparatively small number of species would have relatively low responses to the carbon dioxide differences between reaches. One-half of the species studied would have greater than 40% decrease in net photosynthesis between the upper and lower segments, but only 9% of the species would have less than 20% declines. Equivalent values calculated from PS_N/R ratios generally parallel the relative differences and percent declines of net photosynthetic rates for each species.

Statistical Analyses--Multiple Comparisons

Statistical analyses on the photosynthetic rate data by F-tests and multiple comparisons demonstrate that a large proportion of the photosynthetic rate differences among the twenty-two species in the different water samples is statistically significant. The results of these statistical applications to the various experiments have been

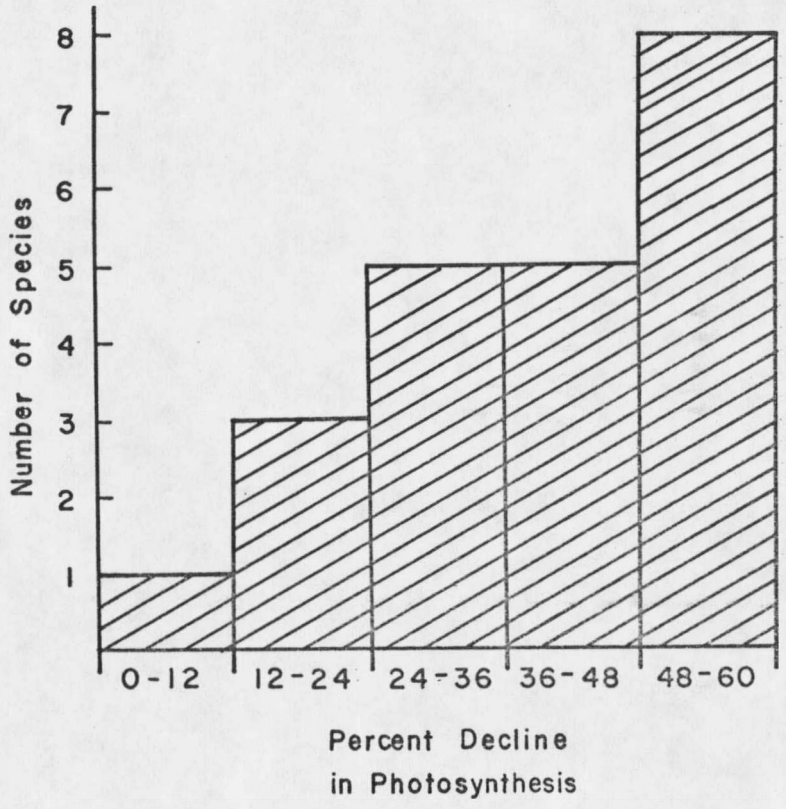


Figure 3. Number of macrophyte species exhibiting percent declines in net photosynthetic rates in water from the upper reach to water from the lower reach of the Madison River in Yellowstone National Park.

extensively discussed previously and are summarized in Table 46. With only three exceptions, statistically significant differences in PS_N rates were obtained between equivalent tissues tested in upper and lower reach water samples. The three exceptional species, Oncophorus virens, Fissidens grandifrons, and Chara vulgaris, are located at the extreme, low-response end of the array in Table 45. All nineteen of the remaining species had statistically significant photosynthetic rate differences between the upper and lower reach samples to the 5% level, and twelve of these species had significant differences between these samples at the 1% level. Of the 66 possible sample pairs for all species in the basic treatments, 64% were found to be significantly different at least to the 10% level, about one-half were significant to the 5% level, and about one-third to the 1% level.

Summary data in Table 44 were also analyzed statistically using a two-way ANOVA and multiple comparisons in which each individual species served as a block. The results of these analyses also illustrate the significant photosynthetic differences that were obtained between water samples and between species. Both the block and treatment F-ratios were significant at less than 0.5% ($F_{42}^{21} = 28.42$ and $F_{42}^{2} = 34.85$ respectively). Multiple comparisons indicate that all of the possible mean net photosynthetic rate differences between the basic treatments were significant statistically at the 1% level.

Table 46. Number of species demonstrating statistically significant photosynthetic rate differences in water samples collected from the upper, middle, or lower reaches of the Madison River in Yellowstone National Park.

Basic Treatment Comparisons		Number of species demonstrating these levels of significance:			
CO_2 Concentrations	Photosynthetic Rates	1%	5%	10%	insignificant
Upper>Lower	Upper>Lower	12	7	0	3
Upper>Middle	Upper>Middle	6	4	4	8
Middle>Lower	Middle>Lower	3	2	4	13
	Total as a Percent	32%	20%	12%	36%
	Cumulative Percent	32%	52%	64%	100%

Multiple comparisons of the blocks show that net photosynthetic differences between species approaching or exceeding 0.0127 or 0.0169 ul O_2/mg/min within any particular treatment would indicate statistical significance at the 1% and the 5% levels respectively.

Correlation and Regression Analyses

In addition to analyses of variance and multiple comparisons, various correlation and simple regression analyses were applied to the photosynthetic data of the various species. Rank correlations for a species were conducted by ranking the net photosynthetic differences between any two samples for all possible treatment pairs and also by ranking the corresponding carbon dioxide differences between these

various pairs. Rank correlation coefficients (r_s) were then computed from these rankings using the equation in Hoel (1966). This approach could be utilized only for those species that were studied in several comparable treatments, basic plus accessory, in an experiment or related series of experiments which allowed for adequate degrees of freedom. With Eleocharis acicularis, an adequate number of comparable treatments was available to allow for the calculation of a direct correlation coefficient between photosynthetic rates and carbon dioxide concentrations.

Rank correlation coefficients were also computed from PS_G, PS_N/R, and the percent saturation data of some of the species. The results of these analyses for each species have been described previously to some extent. In all species, significant and positive rank correlation coefficients were obtained between the carbon dioxide and the net photosynthetic differences between treatments (Table 47). This indicates that the magnitude of the photosynthetic rate differences between tissues in any pair of water samples was closely related to the magnitude of the carbon dioxide differences between these waters. This includes species from the entire spectrum of photosynthetic responses to carbon dioxide arrayed in Table 43. Such correlations suggest that all species responded to some extent to carbon dioxide changes.

In contrast, insignificant rank correlation coefficients were

Table 47. Rank correlation coefficients between water sample to water sample differences in various physiological variables for several species of aquatic macrophytes and the corresponding differences in carbon chemistry (df--degrees of freedom, PS_N--net photosynthetic rates, R--respiratory rates, %sat--percent light saturation at 780 foot candles, alk--total alkalinity, predominantly bicarbonate).

	df	PS_N-CO_2	PS_N/R-CO_2	%sat-CO_2	PS_N-alk	R-CO_2	PS_N-%sat
Berula erecta	13	0.918**	0.911**	-0.868**	-0.046	0.408	-0.955**
Eleocharis acicularis	43	0.980**	0.984**	-0.709**	-0.125	0.122	-0.641**
Eleocharis acicularis (a)	8	0.927**	-----	-0.564*	0.085	-----	-0.370
Fissidens grandifrons	13	0.862**	0.325	-0.727**	-0.656**	0.386	-0.951**
Glyceria borealis	26	0.987**	0.955**	-0.630**	0.238	-0.611**	-0.675**
Hippuris vulgaris	26	0.895**	0.825**	-0.824**	0.123	0.589**	-0.913**
Juncus ensifolius	8	0.928**	-----	-----	-0.155	-----	-----
Myriophyllum exalbescens	26	0.990**	0.933**	-0.957**	0.410*	0.199	-0.961**
Oncophorus virens	19	0.897**	0.841**	0.119	-0.256	0.054	-0.191
Potamogeton filiformis	13	0.907**	-----	-0.601*	0.029	-----	-0.790**
Utricularia vulgaris	8	0.964**	0.964**	-0.973**	0.233	-0.267	-0.936**

(a) Direct rank correlation between variables rather than difference.
** significant at 1%
* significant at 5%

generally obtained between carbon dioxide differences and differences in respiratory rates between water samples. This was expected since respiratory activity should not be markedly affected by carbon dioxide in low concentrations. Further, insignificant coefficients were generally obtained between the alkalinity differences and the net photosynthetic rate differences between treatments. Apparently the small downstream increase in alkalinity in the Madison River had little or no effect on the photosynthetic activities of the plants. The notable exception was Myriophyllum exalbescens which demonstrated a significant and positive rank correlation with alkalinity (Table 47). Klarich (1969) also found that M. exalbescens has a photosynthetic sensitivity to bicarbonate.

Parametric correlation and linear regression analyses were also applied to species having an adequate number of treatments and in which treatment-to-treatment differences were again utilized (Table 48). Significant correlations between treatment carbon dioxide and photosynthetic rate differences were obtained in all cases. In addition, the Student-t value from the regression analysis was also significant. Although these analyses indicate that all species demonstrated significant photosynthetic responses to carbon dioxide, the slope values from the linear regression analyses indicate considerable variation between the ten species.

The m-variable or slope from the regression analyses of Table 48

Table 48. Parametric correlation coefficients (r) and slope (m), y-intercept (b), and Student-t statistics from linear regressions between water sample to water sample differences in various physiological variables for several species of aquatic macrophytes and the corresponding differences in carbon chemistry (df--degrees of freedom; PS_N--net photosynthetic rates, R--respiratory rates, % sat--percent light saturation at 780 foot candles).

Species	Symbol	df	PS_N-CO_2-r	PS_N/R-CO_2-r	% sat-CO_2-r
Berula erecta	Be	13	0.955**	0.936**	-0.843**
Eleocharis acicularis	Ea	43	0.957**	0.965**	-0.562**
Eleocharis acicularis(a)	Eaa	8	0.964**	-----	-0.300
Fissidens grandifrons	Fg	13	0.907**	0.497	-0.750**
Glyceria borealis	Gb	26	0.980**	0.960**	-0.840**
Hippuris vulgaris	Hv	26	0.891**	0.801**	-0.830**
Juncus ensifolius	Je	8	0.834**	-----	-----
Myriophyllum exalbescens	Me	26	0.994**	0.990**	-0.930**
Oncophorus virens	Ov	19	0.793**	0.719**	0.314
Potamogeton filiformis	Pf	13	0.847**	-----	-0.552*
Utricularia vulgaris	Uv	8	0.987**	0.994**	-0.971**

	PS_N-CO_2			PS_N/R-CO_2			% sat-CO_2		
	m	b	t	m	b	t	m	b	t
Be	0.00166	0.00052	11.6**	0.174	0.091	9.6**	-1.37	-0.50	5.7**
Ea	0.00097	0.00000	21.5**	0.139	0.456	16.1**	-0.44	-6.80	4.5**
Eaa	0.00097	0.01301	10.2**	---	---	-----	-0.23	93.34	0.9
Fg	0.00036	-.00034	7.8**	0.043	0.121	2.1*	-0.78	2.50	4.1**
Gb	0.00101	0.00062	25.0**	0.140	0.011	17.5**	-1.04	0.65	7.9**
Hv	0.01179	0.02537	10.0**	0.323	1.261	6.8**	-1.84	-5.96	7.6**
Je	0.00100	0.00145	4.3**	---	---	-----	---	---	---
Me	0.00194	0.00021	48.2**	0.277	0.332	35.6**	-1.12	-4.76	12.9**
Ov	0.00062	0.00275	5.7**	0.052	0.038	4.5**	0.27	-6.48	1.4
Pf	0.00217	0.00712	5.7**	---	---	-----	-0.42	-7.35	2.4*
Uv	0.00420	-.00313	17.4**	0.167	-.039	25.5**	-3.13	5.74	11.5**

(a) Direct parametric correlation between variables rather than difference.

** significant at 1%

* significant at 5%

is defined as the change in photosynthetic differences between treatments per unit change in the corresponding differences in carbon dioxide concentration. A slope calculated in this manner is mathematically equivalent, however, to one that would be obtained from the more common and direct regression between photosynthetic rates and carbon dioxide concentrations, i.e., the change in photosynthetic rates per unit change in carbon dioxide. This is illustrated by Eleocharis acicularis (Table 48) where virtually identical slopes were obtained from both the direct (eight degrees of freedom) and the difference analyses (43 degrees of freedom).

The y-intercept from a direct regression for photosynthetic rates and carbon dioxide concentrations would be much greater than zero and would indicate the "base" photosynthetic rates due to bicarbonate at zero carbon dioxide. The y-intercept would equal zero in this case only if the plant could not use bicarbonate as a carbon source in photosynthesis. In contrast, the y-intercept from a regression analysis of treatment-to-treatment differences should equal zero if carbon dioxide were the only affecting factor, and no change in photosynthesis would be expected in such cases without an alteration in carbon dioxide levels. These aspects are again illustrated in the two types of analysis with E. acicularis; the "base" PS_N rates of this species at zero carbon dioxide would equal 0.013 ul O_2/mg/min.

Most of the species listed in Table 48 demonstrated y-intercepts that were relatively close to zero. The ideal case, as exemplified by E. aciculatis (with n = 45), would probably be evident in more of the other regression equations if adequate numbers of observations were available for these species. The major exception is seen in Hippuris vulgaris, and possibly in Potamogeton filiformis, which had y-intercepts that were much greater than zero. Such large y-intercepts, as seen in H. vulgaris and P. filiformis, would be suggestive of factors other than carbon dioxide also operating to influence photosynthesis. For example, the excurrent and leafy morphology of H. vulgaris indicate that morphological differences between plants or tissues which influence light saturation could also affect photosynthetic rates.

The 33-fold difference in regression slope between species (Table 48) is reduced if the slopes are placed on a relative scale by dividing this value by the species' PS_N rates in water samples from the lower station, which is an approximation of a species' "base", PS_N rates. After this adjustment, photosynthetic rate changes per unit change in carbon dioxide ranged from 2.4% of base rates to 16.3%, a seven-fold difference. Y-intercepts, similarly adjusted, were found to be typically less than 10% of the base rate, i.e., relatively close to zero, with the exception of H. vulgaris and P. filiformis.

Coefficients of determination (r^2) from the parametric

correlations indicate that a high percentage of the photosynthetic rate variations evident between treatments for the various species was related to the carbon dioxide differences. For the ten species submitted to this analysis, 63% to 99% of the treatment-to-treatment photosynthetic rate differences were attributable to differences in carbon dioxide concentrations between water samples, and eight of the ten species had r^2 values greater than 79%.

As an added example, partial and multiple parametric correlation coefficients were also determined for E. acicularis as summarized in Table 49. The coefficient of determination from this analysis (R^2) indicates that 98% of the variability in photosynthetic rates between the tissue-water samples was due to carbon source differences; the partial and multiple correlation coefficients indicate that almost all of this photosynthetic variability was related to the carbon dioxide differences between treatments. PS_N was negatively correlated with alkalinity because of the inverse relationship between carbon dioxide and alkalinity in the water samples. Thus, the downstream changes in carbon dioxide in the Madison River would appear to have a distinct effect upon the photosynthetic activities of E. acicularis and most of the other species in the stream.

PERCENT LIGHT SATURATION OF MADISON RIVER MACROPHYTES

The intensity of light available from the Gilson Respirometer,

Table 49. Partial and multiple, parametric correlation coefficients between net photosynthetic rates (P), carbon dioxide concentrations (C), and alkalinity levels (A) from experiments with Eleocharis acicularis.

Direct Correlation (a)

$r_{PC} = 0.964$ ($P<0.01$)

Difference Correlations (b)

$r_{PC}=0.957$ ($P<0.01$)	$r_{PC \cdot A}=0.990$ ($P<0.01$)
$r_{PA}=-.056$ ($P>0.10$)	$r_{PA \cdot C}=-.866$ ($P<0.01$)
$r_{CA}=0.199$ ($P>0.10$)	$R_{P \cdot AC}=0.990$ ($P<0.01$)

$R^2 = 0.980$

(a) Direct correlation between carbon dioxide concentrations and PS_N (8 degrees of freedom)

(b) Correlation of treatment to treatment differences in PS_N, CO_2, and alkalinity (43 degrees of freedom); partial and multiple correlation coefficients and the coefficient of determination (R^2) calculated from these values (42 degrees of freedom).

about 780 foot candles, was only a small fraction of that impinging upon the Madison River at midday during the summer. However, results from the experiments indicate that most of the plants were quite close to light saturation even with the relatively low illumination available from the respirometer. In only a few cases were percent light saturations in any of the basic treatments much lower than 80% at instrument intensity (Table 44); the notable exception was Eleocharis thermalis at less than 65% saturation in all treatments. Of the remaining species and treatments, only 3% had percent light saturations less than 78% at 780 foot candles which appears to be a relatively high saturation value considering that 780 foot candles is only 13% of the maximum river illumination. Apparently most of the species in the river can achieve light saturated photosynthetic rates at much lower intensities than that at maximum stream illumination.

These results along with the species' marked responses to carbon dioxide support Burr's (1941) contention that aquatic plants are adapted to low light intensities but not to low carbon dioxide tensions. This shade-tolerance coincides with the plants' typical habit of growth in densely aggregated mounds where shading would be a common feature. Due to this low light requirement, only small corrections, typically less than 1.25 times PS_L (Figure 2), were generally needed to provide for an estimate of light saturated rates. Four species--

Eleocharis thermalis, Glyceria borealis, Hippuris vulgaris, and Ceratophyllum demersum--seemed to be most limited by experimental light intensity. This was possibly related to morphology since the latter two species are excurrent plants characterized by a rather bushy, thick, and dense foliage which could impede light penetration to the inner leaves and stem.

Percent light saturation for most species increased in water samples collected downstream on the Madison River (Table 44), and percent saturations in both basic and accessory treatments were lowest in water samples with the greatest carbon dioxide concentrations. This is illustrated in Tables 47 and 48 where statistically significant and negative rank and parametric correlation coefficients were obtained between percent light saturation and carbon dioxide in most of the species examined by these analyses. Negative and significant rank correlation coefficients were also obtained between net photosynthetic rates and percent saturation. This would be expected if these variables were oppositely affected by carbon dioxide. Only Oncophorus virens demonstrated insignificance in these tests.

The results of the regression analyses between percent light saturation and carbon dioxide also suggest a significant but inverse relationship between these two variables. Slopes from the regression equations indicate decreases in percent light saturation on the order

of 0.23% to 1.84% for most species per mg/l increase in free carbon dioxide. The y-intercepts from the regression equations of percent saturation and carbon dioxide were typically much greater than zero; this points to the influence of carbon dioxide on plant photosynthesis with no change in percent light saturation.

Wright and Mills (1967) observed that the Madison River is more light limited in the upper, high carbon dioxide reach and more carbon substrate limited in the lower, low carbon dioxide reach. Similar conclusions can be drawn from the results of this study as illustrated by the following relationships.

If light in the lower reach treatment is increased from 780 foot candles to saturating intensities, mean PS_N rates of all species would be increased by a factor of about 1.09-times (Table 44 and Figure 2). In contrast, a similar increase in light intensity in the upper, high carbon dioxide treatment would result in a mean increase in PS_N of 1.23-times. However, if carbon dioxide is increased in the lower treatment by only 2.2 mg/l, equivalent to the mean change in carbon dioxide between the lower and middle stations on the Madison River, the increase in mean PS_N of all species would be equal to 1.24-times versus the 1.09 factor for light (Table 44). An increase in mean PS_N of 1.72-times would be obtained with a carbon dioxide addition to lower reach water equivalent to that in the upper Madison (Table 45). In

contrast, if an additional 2.2 mg/l of carbon dioxide is added to the upper treatment, average PS_N rates would be increased only by a factor of 1.12-times as calculated from an average of the slope data in Table 48. These relationships are also indicative of a carbon substrate, carbon dioxide limitation in the lower river.

NET PHOTOSYNTHETIC/RESPIRATORY RATIOS OF MADISON RIVER MACROPHYTES

Mean respiration rates within each of the species were nearly the same for all treatments (Table 44). None of the mean differences in respiration rates were shown to be statistically significant, and rank correlations between respiration and several chemical variables such as carbon dioxide were generally insignificant or inconsistent (Table 47). As a result, variations in the PS_N/R values of the macrophytes were due primarily to variations in the PS_N component. Therefore, as illustrated in Table 45, the magnitudes of PS_N/R differences between treatments were quite similar to the corresponding magnitudes of the PS_N differences. A rank correlation of $r_s = 0.95$ ($P<.01$) was obtained for the twenty-two species between their relative differences in PS_N and their relative differences in PS_N/R in different water samples.

Mean PS_N/R differences between the three basic treatments for all twenty-two species (Table 44) were shown to be statistically significant via a two-way ANOVA. The treatment F-ratio ($F^2_{42} = 72.11$) was

significant at less than 0.5%. Multiple comparisons indicated that all possible mean comparisons of PS_N/R were significantly different at less than 1%. In addition, the block (species) F-ratio ($F_{42}^{21} = 9.76$) was also significant at these levels, which demonstrates the occurrence of significant mean differences in PS_N/R between species. In this case, multiple comparisons indicated that statistical significance at 5% or 1% was obtained for any two species having differences in PS_N/R exceeding 0.65 and 0.86 units respectively. This further illustrates the wide range of photosynthetic variation that was found among the species of aquatic macrophytes in the Madison River.

Net photosynthesis was found to be similarly variable between species. For example, within any treatment, seven- to nine-fold differences in extreme PS_N values were obtained between species. The relative declines in net photosynthetic rates in water from the upper Madison to water from the lower Madison were also variable between species (Table 45). Such decreases in PS_N and PS_N/R were similar in magnitude and ranged from near 10% to highs of 57-61% in the species more sensitive to carbon dioxide. The frequency distribution of the percent declines in PS_N among species was abnormal and distinctly skewed (Figure 3) with a large number of species having a pronounced decrease in PS_N between these treatments. A similar frequency distribution was obtained for PS_N/R in the species.

Like net photosynthesis, the PS_N/R values of most species were found to be significantly correlated with free carbon dioxide (Tables 47 and 48). The only exception, Fissidens grandifrons, demonstrated relatively small photosynthetic rate differences between low and high carbon dioxide treatments (Tables 44 and 45). Regression analyses based on treatment to treatment differences in PS_N/R and carbon dioxide provided significant t-values (Table 48). Similar to the PS_N regressions, the non-zero y-intercepts in this analysis reflect the influence of factors other than carbon dioxide on photosynthesis. The regression equations from eight species examined in this manner indicated a wide variation in slope or change in PS_N/R per change in carbon dioxide. In the less sensitive species such as F. grandifrons and Oncophorus virens (Table 45), an increase in carbon dioxide of 5 mg/l would increase PS_N/R by 0.30 to 0.34 units. In the species demonstrating greater sensitivity to carbon dioxide, such as Berula erecta and Glyceria borealis, a five unit increase in this variable would produce more distinctive increases in PS_N/R exceeding 0.7 units.

An overall regression equation was determined for the eight species by calculating the mean slope and y-intercept:

$$PS_N/R = 0.164\ (\Delta CO_2) + 0.201.$$

From this equation, if carbon dioxide were increased by 5 mg/1, PS_N/R would be increased, on the average, by 1.02 units. Thus, if 5 mg/1 of carbon dioxide were added to water from the lower Madison, PS_N/R would be increased from 1.6 to about 2.6. Such an addition of 5 mg/1 of free carbon dioxide corresponds to the *in situ* change in this constituent from the upper to the lower reaches of the Madison River.

As outlined previously, a PS_N/R ratio of 1.6 is possibly representative of an approach to unfavorable environmental conditions and an unsuccessful ecesis whereas a value of 2.6 is more akin to that obtained from successfully established plants. Only one species, *O. virens*, had a PS_N/R ratio less than 2.0 in water from the upper, high carbon dioxide reach (Table 42). The occurrence of such a pronounced and direct effect on a plant's function, in this case as expressed by PS_N/R, in response to the alteration of an environmental variable would suggest that this variable operates as a critical, limiting factor in the system.

An $F_{P/R}$ factor has been defined to quantitatively describe the PS_N/R status of a species in the lower, low carbon dioxide treatment. Values of this factor approaching 1.0 would indicate severe stress on the species under low carbon dioxide levels, whereas an $F_{P/R}$ less than 0.5 and approaching zero would denote a less critical case. A range of $F_{P/R}$ values from 0.05 to 1.13 were obtained with the

twenty-two species studied. Seven of the species had $F_{P/R}$ values less than 0.3 whereas six of the species had factors greater than 0.7. The remaining nine species had intermediate $F_{P/R}$ values between 0.3 and 0.7. Due to their low $F_{P/R}$ values, seven species appeared to be generally unaffected by the carbon dioxide gradient in the river whereas six species, with relatively high $F_{P/R}$ values, appeared to be definitely influenced by the reduction of free carbon dioxide in the lower reach. The demonstration of a direct relationship between such variations in the species' $F_{P/R}$ status and the species' distributional patterns in the river would provide further confirmation of the significance and operation of carbon dioxide as an important limiting factor in the Madison River.

DISTRIBUTIONAL RELATIONSHIPS

Patterns of Distribution Among Species

Two approaches were used to relate physiological data, such as $F_{P/R}$ and the relative differences in PS_N between treatments, to patterns of macrophyte distribution in the Madison River. Three distinct groups of plants corresponding to the upper, middle, and lower reaches of the stream became evident (Table 43). One of the approaches developed to correlate distribution with physiology involved grouping of species having similar distributional patterns. Using data obtained by Horpestad (1969), Table 3, and Todd (1967), Table 4, the twenty-two

species were split into three well-defined groups on the basis of their distributions. First, these macrophytes were separated on the basis of beling relatively abundant or absent-unabundant in the lower reach. Second, for those species considered to be largely absent from the lower reach of the Madison River, a further split was made as shown in Table 50.

In the second approach, an attempt was made to quantify the tendency of a species to be relatively more abundant or less abundant in one of the three reaches by defining a distribution factor (D). In this way, the magnitude of the physiological variables could be quantitatively related or correlated to the magnitude of the D value. The D factor was defined as follows:

$$D = \frac{M + L}{U + M},$$

where U, M, and L are the relative abundances of a species in the upper, middle, and lower reaches of the Madison River, respectively, as listed in Table 3. Thus, a species that was quite conspicuous in the lower reach but uncommon upstream would have a high D value. In turn, species that were absent or in trace amounts in the lower reach but common in the upper section would tend to have D values approaching zero. Values of D = 1.0, or near 1.0, would indicate either the tendency to be equally common through all three reaches or the tendency to have a high relative abundance in the middle section.

Table 50. Distributional groups of macrophyte species in the Madison River.

Group I. Species absent or unabundant in the lower reach and most abundant in the upper reach.

Group II. Species absent or unabundant in the lower reach and most abundant in the middle reach.

Group III. Species present and relatively abundant in the lower reach.

Group I	Group II	Group III
Eleocharis thermalis------Et	Ceratophyllum demersum---Cd	Berula erecta----------Be
Glyceria borealis---------Gb	Eleocharis acicularis----Ea	Chara vulgaris----------Cv
Juncus ensifolius---------Je	Potamogeton pectinatus---Pp	Fissidens grandifrons----Fg
Mimulus guttatus----------Mg	Sparganium angustifolium-Sa	Fontinalis neo-mexicanus-Fn
Potamogeton filiformis----Pf		Hippuris vulgaris--------Hv
Potamogeton natans--------Pna		Myriophyllum exalbescens-Me
Potamogeton nodosus------Pno		Oncophorus virens--------Ov
Potamogeton strictifolius-Ps		Potamogeton gramineus----Pg
Sagittaria cuneata--------Sc		Utrichularia vulgaris----Uv

Table 51 summarizes D values obtained for the macrophytes as organized into the three species groupings described previously. The D values quantitatively describe the distributional characteristics of these three groups of plants in Table 50. Species in Group III generally had D values greater than one while species classified as Group I had D values consistently less than one. The four species of Group II were somewhat intermediate, i.e., D near 1.0. Using a pooled, Student-t test, the mean D values of the three groups were shown to be significantly different as follows: Group I < Group II, Group I < Group III, and Groups I + II < Group III, all at 0.5%; Group I < Groups II + III at 2.5%; and Group II < Group III at 10%.

Parallels Between Distribution and Photosynthetic/Respiratory Ratios

The $F_{P/R}$ factors of the species are also presented in Table 51, and pronounced differences in $F_{P/R}$ are evident between the three distributional groups. Species most generally found in the upper, high carbon dioxide reach of the river (Group I, D < 1.0) had individual $F_{P/R}$ values and a group mean generally greater than $F_{P/R} = 0.70$. In contrast, species most conspicuous in the lower reach (Group III, D > 1.0) had individual values and a group mean typically less than $F_{P/R} = 0.3$. In addition, species most abundant in the middle reach (Group II, D $\longrightarrow$ 1.0) had intermediate $F_{P/R}$ values. This can be further illustrated by partitioning the twenty-two species as follows

Table 51. $F_{P/R}$ factors and D values for three distributional groups of aquatic macrophytes in the Madison River.

	Group I			Group II			Group III	
	D	$F_{P/R}$		D	$F_{P/R}$		D	$F_{P/R}$
Et	0.0	0.62	Cd	1.00	0.35	Be	1.30	0.18
Gb	0.59	0.85	Ea	0.89	0.55	Cv	0.79	0.06
Je	0.10	0.47	Pp	0.91	0.60	Fg	4.40	0.05
Mg	0.0	0.97	Sa	0.71	0.32	Fn	2.00	0.09
Pt	0.63	0.79				Hv	1.10	0.13
Pna	0.48	0.71				Me	1.46	0.32
Pno	0.17	0.38				Ov	5.31	0.15
Ps	0.53	0.74				Pg	1.50	0.08
Sc	0.40	1.13				Uv	0.74	0.42
n	9	9	n	4	4	n	9	9
Means	0.32	0.74	Means	0.88	0.46	Means	2.07	0.16
s	0.26	0.23	s	0.12	0.14	s	1.64	0.13

	Groups I and II		Groups II and III	
	D	$F_{P/R}$	D	$F_{P/R}$
n	13	13	13	13
Means	0.49	0.65	1.70	0.25
s	0.34	0.25	1.46	0.19

(where D ⟶ 1.0 = 0.89 to 1.11, and T = totals):

	$F_{P/R}$				D			
	<.3	.3-.7	>.7	T	<.89	.89-1.11	>1.11	T
I	0	3	6	9	9	0	0	9
II	0	4	0	4	1	3	0	4
III	7	2	0	9	2	1	6	9
T	7	9	6	22	12	4	6	22

The mean $F_{P/R}$ values of the three groups and the two group combinations in Table 51 were shown to be significantly different statistically by using a pooled, Student-t test. These results are summarized in Table 52. As a result, the three groupings of species appear to be statistically distinct entities in terms of their physiological and distributional characteristics since significant differences were obtained in their mean $F_{P/R}$ factors and in their mean D values.

Data in Table 51 indicate that the D and $F_{P/R}$ values of the species are inversely related. This is also illustrated by the significant, negative rank correlation coefficient that was obtained between these variables ($r_s = 0.764$, $P < 0.01$ with twenty degrees of freedom). Apparently those species tending to have a high ranking of the D value tend to have a significantly lower ranking of $F_{P/R}$ that is closer to one. Although there is a considerable scatter of points, this inverse relationship between $F_{P/R}$ and D is illustrated in Figure 4. This graph indicates that a small decline in D at $D < 1$ could potentially produce a relatively large increase in $F_{P/R}$ compared to the increase

Table 52. Pooled, Student-t tests of mean $F_{P/R}$ factors in three distributional groups of macrophyte species (Groups: I, II, and III) in the Madison River of Yellowstone National Park.

Group $F_{P/R}$ Comparison	Degrees of Freedom	Calculated t-value	Test Criteria	Significance Levels
II<I	11	t=2.22	t=2.20	P<0.025
III<I	16	t=6.59	t=2.92	P<0.005
III<II	11	t=3.75	t=3.11	P<0.005
III<(I + II)	20	t=5.38	t=2.85	P<0.005
(II + III)<I	20	t=5.44	t=2.85	P<0.005

---

that would be obtained with a decline in D at D > 1. All species with $F_{P/R}$ values greater than 0.5 had D values less than 1.0 whereas all species having a D factor greater than 1.0 had $F_{P/R}$ values less than 0.5.

A significant parametric correlation coefficient was also obtained between these variables ($r = -0.560$ with $P < 0.05$), but it was smaller in magnitude than r_s. This difference in coefficients is probably a reflection of the nonlinear nature of the relationship between D and $F_{P/R}$ as illustrated in Figure 4.

The $F_{P/R}$ factors of the twenty-two species suggest that conditions in the lower Madison were apparently much more critical and detrimental

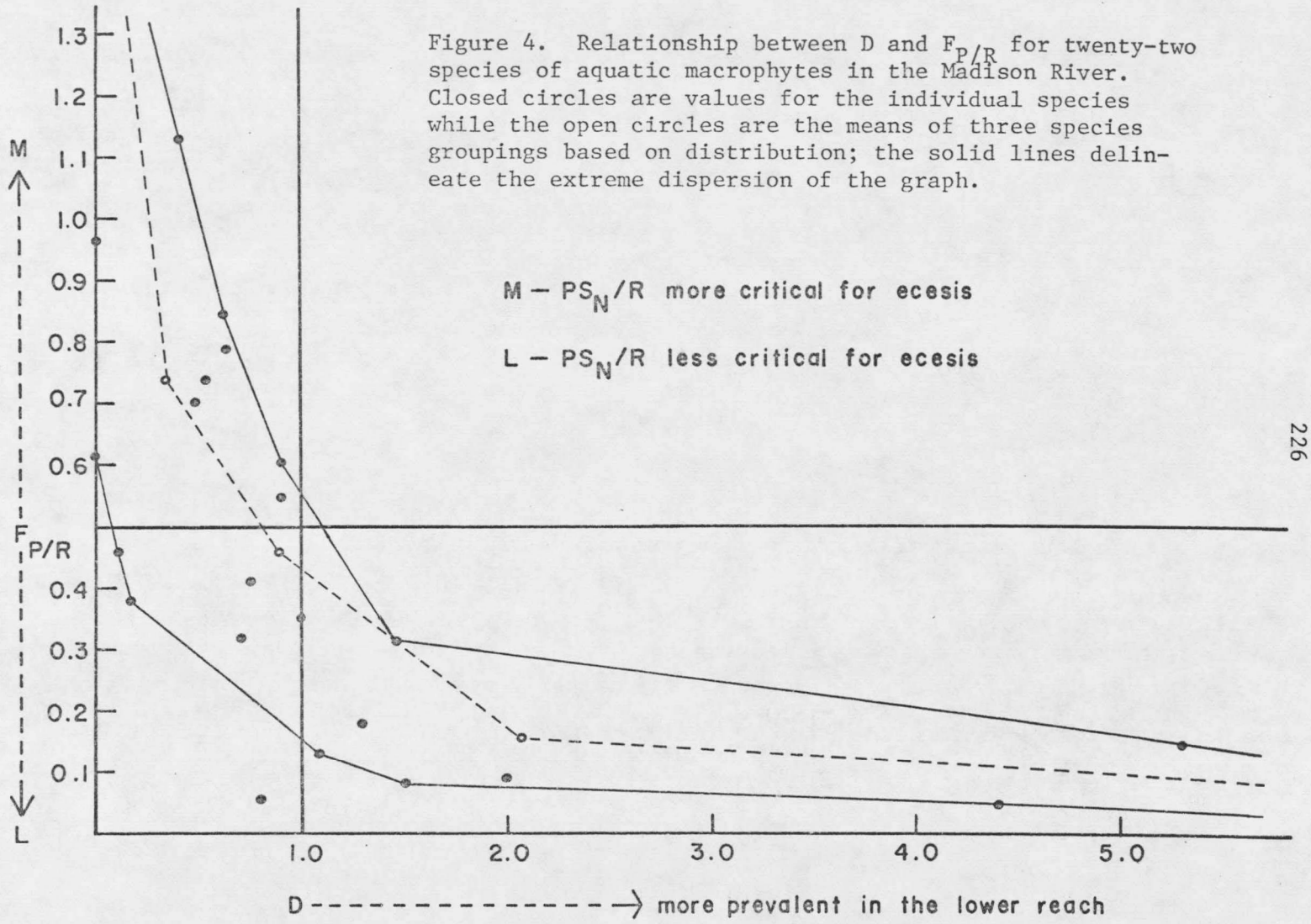


Figure 4. Relationship between D and $F_{P/R}$ for twenty-two species of aquatic macrophytes in the Madison River. Closed circles are values for the individual species while the open circles are the means of three species groupings based on distribution; the solid lines delineate the extreme dispersion of the graph.

to the establishment and ecesis of those species which are largely restricted to the upper reach of the river (Group I, Tables 50 and 51) than for species found throughout the study area (Group III). An intermediate effect was evident in the Group II macrophytes which are most abundant in the middle section. Species largely absent from the lower reach had high $F_{P/R}$ values which would indicate a physiology that was not conducive to success under low carbon dioxide concentrations. These species showed a large photosynthetic response to free carbon dioxide and low PS_N/R ratios in water from the lower reach. A much less detectable photosynthetic response and a lower $F_{P/R}$ were observed in those species present in the lower Madison.

If physical properties were the only limiting factors in the river, such pronounced physiological differences and downstream changes in net photosynthetic rates, PS_N/R, and $F_{P/R}$ between and within species would not be expected. This relationship between physiology and distribution can be construed as evidence supporting a hypothesis that carbon dioxide, by itself or in combination with various physical factors, acts as a factor limiting the establishment of certain species in the lower Madison River and restricts their migration to the lower reach. The remaining species, however, which are photosynthetically less sensitive to carbon dioxide, primarily the Group III macrophytes, have become established and are relatively abundant in this lower, low

carbon dioxide section of the stream.

Distinct differences between the plant groups are also evident in their mean PS_N/R values in the basic treatments (Table 53). In contrast, mean respiratory rates of the three groups are not greatly different, especially the Group I and Group III species, and the differences in mean respiratory rates among all groups are statistically insignificant at 10%. As a result, with inter-group respiratory levels about equal, mean PS_N/R differences between plant groups, and therefore their mean photosynthetic rate differences, do not appear to be the result of non-experimental and random differences in the mean metabolic rates of the distributional groups. Rather, differences in mean PS_N/R are most likely attributable to the differential responses of the grouped species to some influencing environmental factor such as free carbon dioxide.

The mean PS_N/R values of the three groups are quite high and closely similar in the upper river water sample, and they are lower for all groups in the water sample from the lower reach. This decline of PS_N/R is much more distinct for Group I than for Group III, and Group II occupies an intermediate position more like that of Group I than Group III. The relative overall drops in PS_N/R from the upper to the lower reach treatment were: Group I, 53%; Group II, 46%; and Group III, 25%.

Table 53. Mean PS_N, R, PS_N/R, and percent light saturation (%sat) values for the three distributional groups of Madison River species in different treatments (PS_N and R in ul O_2 per mg per minute).

	Treatments		
	Upper Reach Madison Water	Middle Reach Madison Water	Lower Reach Madison Water
Group I--PS_N	0.0447	0.0308	0.0219
Group II--PS_N	0.0545	0.0394	0.0301
Group III--PS_N	0.0471	0.0376	0.0341
Group I--R	0.0134	0.0137	0.0140
Group II--R	0.0181	0.0181	0.0172
Group III--R	0.0130	0.0132	0.0131
Group I--PS_N/R	3.45	2.35	1.61
Group II--PS_N/R	3.36	2.33	1.81
Group III--PS_N/R	3.56	2.95	2.67
Group I--%sat	77%	83%	91%
Group II--%sat	81%	90%	95%
Group III--%sat	86%	89%	91%

Several of the mean differences of PS_N/R within and between groups were shown to be statistically significant by using a one-way ANOVA with LSD multiple comparisons (Lentner 1969) utilized to test the statistical significance of the individual mean differences between treatments and groups. A significant F-ratio of $F^{8}_{57} = 7.32$ ($P<0.005$) was obtained through this analysis. The results of the multiple comparisons indicated that there were no significant mean differences in PS_N/R at the 10% level between groups in the upper treatment ($t_{55}<0.05$); i.e., mean PS_N/R values in the upper reach treatment were essentially the same for the three groups of plants. However, the middle and lower reach treatments provide significant mean differences in PS_N/R between the plant groups as follows: middle treatment--Group I<III, significant at 10% ($t_{55}= 1.74$) with Group II<III and Group I<II insignificant at 10% ($t_{55}=1.41$ and 0.05); lower treatment--Group II<III, significant at 10% ($t_{55}=1.96$) and Group I<III, significant at 1% ($t_{55}=3.08$) with Group I<II insignificant at 10% ($t_{55}= 0.46$). The lack of significance in the mean PS_N/R differences between Groups I and II indicates that the species comprising these two groups were nearly equivalent physiologically.

Many of the intra-group differences in mean PS_N/R between treatments were also shown to be statistically significant. Mean PS_N/R values were significantly greater at 2% in the upper reach water samples

than in the lower reach samples for all three groups of plants. In contrast, mean PS_N/R differences between the middle and the lower station samples were insignificant at 10% in Group II and III plants, although significance at 5% was obtained with Group I species. Mean PS_N/R differences between the upper and middle stream samples were significant at 10% in the Group II and III macrophytes and significant at 1% in the Group I species. Appreciably lower PS_N/R values were obtained from the plant groups when tested in water collected from the downstream, low carbon dioxide stations. Differences in PS_N/R between reach water samples were most distinct in the Group I species.

All groups of species had similar mean PS_N/R values in the upper treatment and therefore a photosynthetic activity in the upper, high carbon dioxide reach that was adequate for providing necessary photosynthate accrual for a successful ecesis ($PS_N/R > 3.35$). The relatively low abundances of the moss species in the upper reach (Tables 3 and 4) were probably related to factors other than carbon dioxide levels, such as competition. However, as carbon dioxide levels decline downstream from the upper reach, photosynthesis of the Group I and III macrophytes drops to levels where PS_N/R relationships might not be conducive to an adequate photosynthate accrual for ecesis ($PS_N/R < 1.9$ in the lower reach). In these cases, carbon dioxide could act as a limiting factor, and its low concentrations could restrict these species

from the lower reach. In contrast, although PS_N/R values declined for all species in the downstream waters, Group III macrophytes maintained generally high PS_N/R levels even in the lower, low carbon dioxide treatment, which indicates adequate photosynthate accrual for ecesis. This apparently was also the case for the Group I and II species in the middle treatment.

Correlations Between Macrophyte Distribution and Photosynthetic Rates

Evidence supporting the hypothesis that carbon dioxide acts as a limiting factor to certain species is also available from the PS_N and the percent light saturation relationships between the three groups of species. As noted, respiratory rates were fairly constant for all species in all treatments. As a result, intra-specific differences in PS_N/R between treatments, and thereby inter-specific differences in $F_{P/R}$, were mainly due to variable PS_N differences between treatments in response to differences in carbon dioxide concentrations. Thus, the high $F_{P/R}$ values of some species were due primarily to the marked lowering of photosynthetic rates in water collected from the lower station. Therefore, species groups in Table 50 should also show differences in PS_N and percent light saturation as well as in $F_{P/R}$ and $PS_{N/R}$. Distinctive inter-group differences in mean PS_N and mean percent light saturation were obtained as indicated in Table 53.

A significant negative rank correlation coefficient was obtained

between the relative differences in PS_N in the upper, station 1, and the lower, station 3, water samples and the species' distributional factor (D)(r_s = -0.647, P<0.01). This indicates that species having relatively high D values (e.g., D>1 with rankings <8) had relatively small differences in PS_N between water from these two sampling locations. This is also evident in the mean differences in PS_N and PS_N/R of the three plant groups in the various water samples (Table 53).

Inter-group variations in mean PS_N and in mean PS_N/R can be further illustrated by the ratio of each of these variables in the upper reach water sample (U) to each groups' mean PS_N or mean PS_N/R values in water from station 3 (L):

	Group I	Group II	Group III
$(PS_N)_U/(PS_N)_L$	2.02	1.92	1.34
$(PS_N/R)_U/(PS_N/R)_L$	2.14	1.84	1.36

These data indicate that the macrophytes' PS_N and PS_N/R differences between the upper and lower Madison water samples were more pronounced for those species confined to the upper two-thirds of the river study area. The decrease in photosynthetic rates from the upper, high carbon dioxide reach to the lower, low carbon dioxide reach was much greater in the Group I and II species than that observed for species abundant in the lower segment of the river.

The significance of the photosynthetic variation between plant

groups (Table 53) was established statistically using pooled, Student-t tests as follows:

PS_N: Group I > Group III -- t_{16} = 3.02, significant at 0.5%
Group II > Group III -- t_{11} = 2.73, significant at 1%
Group I > Group II -- t_{11} = 0.32, insignificant at 10%

PS_N/R: Group I > Group III -- t_{16} = 3.42, significant at 0.5%
Group II > Group III -- t_{11} = 2.22, significant at 2.5%
Group I > Group II -- t_{11} = 1.03, insignificant at 10%

The general similarities of the plants in Groups I and II are described by the lack of statistical significance in the differences between these sets. In contrast, the mean, parametric differences between Groups I-II and Group III are highly significant. These results suggest that those species absent from the lower, low carbon dioxide reach of the Madison River had a significantly greater photosynthetic sensitivity to this carbon source than the Group III species commonly found in this lower section.

Mean photosynthetic rates of all the plant groups were lower in water from the downstream portion of the river (Table 44) with its slightly lower total carbon availability for photosynthesis due to reductions in carbon dioxide concentrations (Table 44). This observation corresponds to the results obtained in other studies (Wright and Mills 1967) wherein distinct decreases in primary production were observed downstream. The differences between the three plant groups in the magnitude of this decline in net photosynthetic rates were

distinct in this study. Group I showed a 51% overall decline in photosynthetic rates from the upper to the lower treatment compared to a decline of 28% in the Group III plants and a decline of 45% in the Group II species.

Mean net photosynthetic rates of the three groups were similar in the upper reach water with its high carbon dioxide levels (Table 53). However, with the drop in carbon dioxide through the middle to the lower reach, photosynthetic rates of Group I species become dintinctly less than those of Group III in the lower reach water samples (Table 53). These results closely parallel the distributional patterns of the groups in the river, and they also implicate a photosynthetically-related sorting effect downstream in the Madison River which could in turn account for the disappearance or reduced abundances of particular species in the lower segment of the stream.

These observations are explainable if the species comprising Group III (Table 43) can utilize bicarbonate in photosynthesis more efficiently than Group I and II plants. Species established in the lower reach of the Madison River therefore would be much less dependent upon the availability of free carbon dioxide as a carbon source in photosynthesis. As a result, Group III plants would not be affected photosynthetically to as great an extent as those of Group I and II by the in situ, downstream decline of carbon dioxide since bicarbonate

concentrations remained essentially constant through the river. The relative ability of some of the species to more efficiently utilize bicarbonate would be then reflected in the $F_{P/R}$ relationships described previously (Table 51) wherein the high $F_{P/R}$ values of the Group III species would indicate successful ecesis in the lower Madison River.

Parallels Between Distribution and Percent Light Saturation

Percent light saturation data are illustrative of and provide confirmation for the differential responses of the three species groups to free carbon dioxide. A significant but positive rank correlation coefficient ($r_s = 0.461$, $P<0.05$) was obtained between the upper treatment/lower treatment, percent light saturation (%sat) ratios (%U/%L) of the species and their distribution variables (D). This suggests that species with high D values and thereby a low ranking of this variable relative to the other species, e.g., $D>1$ and a ranking less than 8, had smaller decreases in light saturation from the lower to the upper reach water samples than the plants with higher ranked D's ($D<1$). Species with high D values and relatively common in the lower reach also had smaller photosynthetic responses to free carbon dioxide. These relationships are further illustrated by the mean percent light saturation data of the three distributional groups of macrophytes (Table 53). The %U/%L ratios of the three plant groups are as follows:

Group I, 0.85; Group II, 0.86; and Group III, 0.94.

The %U/%L ratios of the species were significantly and negatively correlated with their $F_{P/R}$ values ($r_s = -0.534$ with $P = 0.01$). Such significant relationships between percent light saturation and the other chemical, physiological, and distributional variables of the study indicate that species largely restricted to the upper reach of the Madison River had greater photosynthetic responses to the carbon dioxide gradient in the river than did the Group III species, and that this response was apparently of sufficient magnitude to influence their distribution in the stream.

All of the distributional groups of macrophytes had lower light saturations in the higher carbon dioxide treatments (Table 44). In these cases, this is indicative of a greater light limitation at the 780 foot candles of the respirometer in response to a greater amount of available carbon substrate for photosynthesis. Since the total inorganic carbon concentrations were only slightly greater in the upper than in the lower reach water samples (Table 44), this response appears to be primarily related to the greater availability of a more "useable" carbon substrate in the upper Madison River. However, the differences in percent light saturation between the upper and lower samples were greater in the Group I than in the Group III species with the Group II plants occupying an intermediate position. This follows from the

various rank correlation assessments which indicated that species present in the lower portion of the Madison River had a lower photosynthetic sensitivity to free carbon dioxide. In turn, light saturation was relatively high (>90%) for all three groups of macrophytes under the low carbon dioxide levels of the lower river; this implies a greater carbon-substrate limitation which was most severe in the Group I plants.

BICARBONATE-CARBON DIOXIDE RELATIONSHIPS

The differences in mean PS_N between basic treatments (Table 44) for the twenty-two species of plants studied appear to be linearly related to corresponding differences in mean carbon dioxide concentrations rather than to differences in total inorganic carbon or bicarbonate. This approach to linearity between PS_N and carbon dioxide is illustrated by the fact that the change in mean PS_N per unit change in mean CO_2-C concentrations between the upper and the middle river station water samples was similar to that obtained between the middle and the lower station samples, 0.0131 ul O_2/mg/min PS_N per mg CO_2-C and 0.0113 ul O_2/mg/min PS_N per mg CO_2-C respectively. Overall, the change in mean PS_N per unit change in mean CO_2-C between the upper and lower stream water samples equalled 0.0124 ul O_2/mg/min PS_N per mg CO_2-C. Since the mean respiratory rates of the twenty-two species were essentially constant in the treatments (Table 44), the mean gross

photosynthetic rate also appears to be linearly related to carbon dioxide concentrations.

With bicarbonate levels essentially constant between samples, and with the assumption of linearity between PS_G and carbon dioxide, PS_G in water from the upper reach due to CO_2-C and that due to HCO_3^--C can be estimated for each species. Where U and L represent water samples from the upper and the lower stations on the Madison River, requisite calculations can be described in three steps as follows:

1. (PS_{GU} minus PS_{GL}) ÷ (mg CO_2-C_U minus mg CO_2-C_L) = PS_G per mg of CO_2-C;
2. (PS_G per mg of CO_2-C) x mg CO_2-C_U = the PS_{GU} of a species due to carbon dioxide (PS_{GUC});
3. PS_{GU} minus PS_{GUC} = PS_{GUB}, i.e., the PS_{GU} of a species due to bicarbonate.

Such calculations also can be applied to data from the station 2 and station 3 treatments using steps 2 and 3, e.g., PS_{GLC} and PS_{GLB}. A division of PS_{GUC} by mg CO_2-C_U (C) and a division of PS_{GUB} by mg HCO_3^--C_U (B) provide estimates of the rates of gross photosynthesis in the upper station water sample per mg of each type of carbon source, (PS_{GUC}/C) and (PS_{GUB}/B) respectively. The ratio of (PS_{GUC}/C)/(PS_{GUB}/B) provides a factor that describes the magnitude a species' photosynthetic response to carbon dioxide relative to its response to bicarbonate.

Values of (PS_{GUC}/C) and (PS_{GUB}/B) for the twenty-two species are

summarized in Table 54 along with their $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios. PS_{GUC} and PS_{GUB} for each species as a percentage of PS_{GU}, and PS_{GLC} and PS_{GLB} as a percentage of PS_{GL} are included in the table as are (PS_{GUC}/C) and (PS_{GUB}/B) values, mean $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios, and mean PS_G percentages for the three distributional groups of plants.

With one exception, all species of macrophytes apparently utilized carbon dioxide more efficiently in photosynthesis than bicarbonate. Gross photosynthesis due to carbon dioxide ranged from a low of 0.00135 to a high of 0.04508 ul O_2/mg/min per mg of inorganic carbon as carbon dioxide. The range of PS_G per mg of inorganic carbon as bicarbonate was 50% to 90% lower. The greater ability of macrophytes to utilize carbon dioxide over bicarbonate is also illustrated by their $(PS_{GUC}/C)(PS_{GUB}/B)$ ratios which were typically greater than 1.70. Of the twenty-two species examined, only Chara vulgaris with a $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratio less than one was apparently able to utilize bicarbonate as a carbon source in photosynthesis more efficiently than carbon dioxide (Table 54).

Although more than 81% of the photosynthetic activity of all the macrophytes in the lower reach treatments was due to bicarbonate, which accounted for a large percentage of the total inorganic carbon (TIC) in the samples, more than 4.0% of this photosynthetic activity was due to carbon dioxide in 91% of the species even though its concentration

Table 54. Partitioned gross photosynthetic rates per mg of CO_2-C (PS_{GUC}/C) and per mg of HCO_3^--C (PS_{GUB}/B) obtained from twenty-two species of aquatic macrophytes and from three distributional groups of plants (Groups I, II, and III). The percent of PS_G due to each of these carbon sources in waters from the upper and lower reaches of the Madison River and $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios (RA) for the species and plant groups are also listed.

Species	PS_{GUC}/C	PS_{GUB}/B	Percent PS_G due to: Upper CO_2-C	Upper HCO_3-C	Lower CO_2-C	Lower HCO_3-C	RA
E. thermalis	.00800	.00097	36.7	63.3	10.9	89.1	7.9
G. borealis	.00819	.00084	45.2	54.8	12.4	87.6	9.3
J. ensifolius	.00574	.00116	31.0	69.0	5.8	94.2	4.8
M. guttatus	.02073	.00205	50.0	50.0	10.1	89.9	9.8
P. filiformis	.02015	.00102	56.4	43.6	18.5	81.5	19.0
P. natans	.01518	.00112	51.6	48.4	14.5	85.5	13.1
P. nodosus	.01213	.00163	37.9	62.1	8.2	91.8	7.3
P. strictifolius	.01491	.00109	42.9	57.1	13.0	87.0	13.2
S. cuneata	.02295	.00160	47.2	52.8	13.7	86.3	13.8
Group I	.01422	.00128	44.3	55.7	11.9	88.1	10.9
C. demersum	.02070	.00348	35.4	64.6	6.1	93.9	5.8
E. acicularis	.00713	.00069	45.4	54.6	10.4	89.6	10.0
P. pectinatus	.01282	.00105	54.8	45.2	12.1	87.9	11.8
S. angustifolium	.01291	.00173	36.6	63.4	11.5	88.5	7.3
Group II	.01339	.00177	43.1	57.0	10.0	90.0	8.7
Group I + II	.01396	.00142	43.9	56.1	11.3	88.7	10.2
B. erecta	.00669	.00132	27.9	72.1	6.7	93.3	5.0
C. vulgaris	.00163	.00191	5.0	95.0	1.0	99.0	0.8
F. grandifrons	.00135	.00076	13.5	86.5	2.5	97.5	1.7
F. neo-mexicanus	.00406	.00083	27.1	72.9	5.5	94.5	4.8
H. vulgaris	.04508	.00467	37.9	62.1	9.9	90.1	9.4
M. exalbescens	.00394	.00068	28.8	71.2	7.3	92.7	5.8
O. virens	.00519	.00141	19.7	80.3	4.7	95.3	3.6
P. gramineus	.01080	.00247	23.9	76.1	4.8	95.2	4.2
U. vulgaris	.00862	.00216	18.7	81.3	5.3	94.7	3.9
Group III	.00971	.00180	22.5	77.5	5.3	94.7	4.4
Group I + II + III	.01222	.00157	35.2	64.8	8.9	91.1	7.8

contributed to only about 1.2% of the TIC. Such variations between carbon dioxide and bicarbonate in their relative contributions to photosynthesis versus their contributions to TIC were much more distinct in the upper reach, higher carbon dioxide waters. In this case, bicarbonate, while contributing about 93% of the TIC in the water samples, typically accounted for less than 85% of the macrophytes' photosynthetic activity. In contrast, carbon dioxide accounted for more than 18% of the photosynthetic activity in 91% of the species while providing only about 7.3% of the upper treatment's TIC. Carbon dioxide consistently accounted for a greater proportion of the photosynthetic activity of most species than would be expected on the basis of its actual concentrations in the samples. Thus, an increase in TIC, when due to additions of carbon dioxide, would stimulate photosynthesis much more markedly per unit of carbon than would be the case with an increase in TIC due to additions of bicarbonate.

As noted, $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios of the species indicate that 95% of the plants examined in the study utilized CO_2-C more effectively in photosynthesis than HCO_3^--C. However, these ratios also demonstrate a wide variation among species in their photosynthetic responses to carbon dioxide and bicarbonate (Table 54). About 91% of the macrophytes were found to be able to utilize carbon dioxide greater than two times more effectively than bicarbonate in photosynthesis. The

variation among species in this regard is also evident in the relative contributions of CO_2-C to macrophyte photosynthetic rates in the upper and lower reach treatments (Table 54). These variations ranged from 5.0% to 56.4% in samples from the upper Madison and from 1.0% to 18.5% in samples from the lower reach of the river.

Variations between species in photosynthetic response to carbon dioxide versus bicarbonate were correlated with variations in their distributional patterns in the Madison River. A significant and negative rank correlation coefficient ($r_s = -0.60$, $P<0.01$) was obtained between the species' distributional factors, D (Table 51), and their $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios (Table 54). The negative rank correlation and inverse relationships between D and $(PS_{GUC}/C)/(PS_{GUB}/B)$ indicate that species tending to have higher D values and a greater prevalence downstream in the Madison River also tended to have a lower $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios and smaller photosynthetic responses to CO_2-C relative to their responses to HCO_3^--C. The converse was true for species with lower D values largely confined to the upper reach of the Madison. In the latter case, species were more sensitive to free carbon dioxide as a carbon source than the species prevalent further downstream. A significant but positive rank correlation coefficient was obtained between $(PS_{GUC}/C)/(PS_{GUB}/B)$ and $F_{P/R}$ ($r_s = 0.81$, $P<0.01$).

In addition to the negative correlation between D and

$(PS_{GUC}/C)/(PS_{GUB}/B)$, distinct mean differences were also evident between the three distributional groups of macrophytes in the species' relative photosynthetic responses to the two carbon sources (Table 54). Twelve of the thirteen species of aquatic macrophytes that were confined largely to the upper two reaches of the river (Groups I and II) had $(PS_{GUC}/C(PS_{GUB}/B)$ ratios greater than or equal to 5.8. In turn, eight of the nine species abundant in the lower reach (Group III) had ratios less than or equal to 5.8. Most of the plants restricted to the upper river were able to utilize carbon dioxide about 7.0 or more times more effectively than bicarbonate in photosynthesis. For most of the species found to be abundant in the lower, low carbon dioxide reach of the river, this factor was 5.0 or less.

Such differences in $(PS_{GUC}/C)/(PS_{GUB}/B)$ between plant groups were examined statistically via a pooled Student-t test. Assuming that the variance in this ratio was insignificantly different between groups, the results of this test can be summarized as follows: Group I x Group II--t = 0.93 with 11 degrees of freedom, insignificant at 10%; Group I x Group III--t = 3.74 with 16 degrees of freedom, significant at 1%; Groups I + II x Group III--t = 3.84 with 20 degrees of freedom, significant at 1%; and Group II x Group III--t = 2.87 with 11 degrees of freedom, significant at 2%. Thus, Group I and II species demonstrated a significantly higher $(PS_{GUC}/C)/(PS_{GUB}/B)$ ratios and greater

photosynthetic sensitivities to free carbon dioxide than the Group III plants.

Mean differences are also evident between distributional groups in the percentage contribution of CO_2-C and HCO_3^--C in the water samples to their mean photosynthetic rates and in their (PS_{GUC}/C) and (PS_{GUB}/B) values (Table 54). Group I species had higher (PS_{GUC}/C) but lower (PS_{GUB}/B) values than Group III plants, i.e., a greater gross photosynthetic rate per mg of CO_2-C, but a lower gross photosynthetic rate per mg of HCO_3^--C. These values in the Group II macrophytes were intermediate to those of the Group I and III plants.

Differences between species in their photosynthetic sensitivities to CO_2-C and HCO_3^--C paralleled the downstream changes in their distribution along the carbon dioxide gradient of the Madison River. Such differences between species in their photosynthetic responses to the carbon source variations might be one of the factors that brings about downstream alterations in species composition and abundance in the river. For example, the hypothesis that the Group III plants can utilize HCO_3^--C more effectively in photosynthesis than the Group I or II plants, which thereby accounts for their presence in the lower, low carbon dioxide reach of the Madison River, corresponds to the fact that higher mean (PS_{GUB}/B) values were obtained for the Group III species.

All species examined from the Madison River responded to both

carbon dioxide and bicarbonate but to varying degrees. Such differential responses and carbon source requirements among species coupled with the decline in carbon dioxide downstream could account for the distributional and abundance changes that were observed in the Madison at the alkalinity characteristic of the stream, about 2.0 me/l. The carbon dioxide gradient at 2.0 me/l alkalinity would act as a screen to sort out downstream those species that could less effectively utilize bicarbonate in photosynthesis. Since the species also responded to bicarbonate, such alterations in distribution and abundance downstream might not have been evident in the river, given no other limiting factors, if the stream had had higher alkalinities. Thus, although several Potamogeton spp. were absent from the lower Madison with its low carbon dioxide concentrations, these species could be located in another squatic system under low carbon dioxide tensions if the alkalinity of the system was at an adequate level.

The critical alkalinities required by the various macrophytes in order to undergo metabolic ecesis under low carbon dioxide concentrations, e.g., near 1.0 mg/l, can be estimated by using the mean (PS_{GUC}/C) and (PS_{GUB}/B) values of the distributional plant groups and their mean respiratory rates to calculate an HCO_3^--C concentration that affords a PS_N/R somewhat greater than two. For the Group III species, this requirement is less than the 2.0 me/l alkalinity of the

Madison River since this group had PS_N/R's greater than 2.00 in the low carbon dioxide reach.

For Groups I plus II, alkalinity as bicarbonate greater than 2.35 me/l would provide PS_N/R values in excess of 2.67 under low carbon dioxide. For Sagittaria cuneata, which describes the extreme case of Groups I and II, i.e., the lowest PS_N/R in the lower reach (Table 44), an alkalinity in excess of 3.39 me/l would be required to insure a $PS_N/R > 2.67$ and a high probability of ecesis with no other limiting factors. The Group III species probably would not undergo ecesis under low carbon dioxide levels if alkalinities were less than 1.37 me/l since PS_N/R would be less than two under these conditions. PS_N/R ratios would be less than 1.5 with alkalinities less than 1.1 me/l. Thus aquatic systems with low carbon dioxide concentrations approaching one mg/l and alkalinities approaching one me/l would not be expected to produce productive macrophyte communities. High levels of carbon dioxide, therefore, appear to be most critical for aquatic macrophytes in relatively non-alkaline waters such as the Madison River.

TAXONOMIC ASPECTS

Aquatic plants have been grouped into two broad categories based on their ability to use various inorganic carbon sources for photosynthesis (Ruttner 1953). On the basis of this classification system, all of the aquatic macrophytes examined from the Madison River would be

considered as "Elodea-like" because of their ability to utilize bicarbonate as a carbon source. However, this classification system suggests an "all or nothing" effect in that plants are described either as completely dependent upon free carbon dioxide and unable to use bicarbonate, or else as able to utilize bicarbonate with the implication that carbon dioxide is a non-critical factor. Results from this study indicate that this classification is not totally applicable to the macrophytes in the relatively non-alkaline waters of the Madison River.

Under the conditions of the Madison, all species of macrophytes were able to utilize bicarbonate to some extent, and most of the species in the stream were able to utilize carbon dioxide more effectively than bicarbonate as a carbon source (Table 54). However, these capacities varied considerably between species, and carbon dioxide was apparently an important carbon source for those species that had relatively low efficiencies for utilization of bicarbonate. Therefore, the relative importance of carbon dioxide and bicarbonate as photosynthetic carbon sources for the various macrophytes appears to be a matter of degree rather than one of all or nothing.

All species of macrophytes were apparently affected to some extent by the downstream decline in carbon dioxide. But, for some of the species, the associated decline in photosynthetic rates was not

marked and did not reduce PS_N/R ratios to potentially critical levels. For some species, however, PS_N/R ratios did decline to extremely low levels in the lower reach of the Madison, and carbon dioxide, thereby, apparently represents a critical factor for these plants under the *in situ* bicarbonate concentrations of the stream. If bicarbonate levels in the Madison had been in excess of 3.4 me/l, the two categories of carbon source requirements would have been more directly applicable to the stream, and all species, including *Sagittaria cuneata*, would be definitely categorized as "Elodea-like" with no major, inter-treatment photosynthetic and PS_N/R differences between species.

Evidence from this study and from other recent investigations indicate that the variable photosynthetic sensitivities of the aquatic plants to the two carbon sources do not closely correspond to their taxonomic and systematic relationships as implied in Ruttner's (1953) classification system. Considerable variation became evident within certain closely related taxa. As an example, most of the *Potamogeton* species in this study were found to be sensitive to free carbon dioxide with relatively low efficiencies for the photosynthetic use of bicarbonate. However, in this regard, *Potamogeton gramineus* was observed to have different characteristics than other members of this genus.

In addition, early investigators found angiosperms to be efficient utilizers of bicarbonate whereas mosses apparently were dependent

upon free carbon dioxide. However, the opposite was observed for the macrophytes in the Madison River where the bryophytes were more efficient users of bicarbonate and less dependent upon free carbon dioxide than many angiosperms such as Glyceria borealis, Sagittaria cuneata, several species of Potamogeton, and Eleocharis spp.

As added examples, Bristow (1969) found Myriophyllum brasilense to be totally dependent upon carbon dioxide for photosynthesis whereas Steeman Nielsen (1944, 1946, 1947) found that M. spicatum could utilize bicarbonate. Steeman Nielsen also reported the aquatic moss Fontinalis sp. to be dependent upon free carbon dioxide, and this was later confirmed by Ruttner (1969) for F. antipyretica. In the present study, M. exalbescens was also found to be an efficient user of bicarbonate in photosynthesis, although it also responded to alterations in carbon dioxide. In contrast to the findings of Steeman Nielsen (1944, 1946, 1947) and Ruttner (1969) with Fontinalis, F. neo-mexicanus was found to be an efficient user of bicarbonate as were Fissidens grandifrons and Oncophorus virens, the other aquatic mosses in the stream.

These comparisons indicate that all angiosperms are not totally independent of carbon dioxide and that some angiosperms are unable to utilize bicarbonate as a carbon source. In turn, mosses are not totally dependent upon free carbon dioxide, and many of the bryophytes are able to efficiently use bicarbonate in photosynthesis. Therefore, definite

similarities are evident in the carbon source requirements of taxa that are widely separated systematically.

As a result of these observations, a general classification system of aquatic macrophytes with respect to their carbon source requirements cannot be based entirely on taxonomic features, and, in non-alkaline waters, this system would have to be more complex than a simple separation into two broad categories as proposed by earlier investigators. Based on the results of this and other studies, four major categories can be described, and four subgroups of species can be further delineated on the basis of the ability of these plants to utilize carbon dioxide and bicarbonate in photosynthesis relative to that of an "average" Madison species.

The classification system developed through this study can be summarized as follows:

Category 1. Species unable to utilize free carbon dioxide and totally dependent upon bicarbonate.

Category 2. Species unable to utilize bicarbonate and totally dependent upon carbon dioxide.

Category 3. Species able to utilize both carbon sources but able to use bicarbonate more efficiently than free carbon dioxide.

Category 4. Species able to utilize both carbon sources but able to use carbon dioxide more efficiently than bicarbonate.

(A) Species able to utilize carbon dioxide more efficiently and bicarbonate less efficiently than an "average" plant.

(B) Species able to utilize bicarbonate more efficiently and carbon dioxide less efficiently than an "average" plant.

(C) Species that utilize both carbon sources more effectively than an "average" plant.

(D) Species that utilize both carbon sources less effectively than an "average" plant.

No species encountered in the Madison River would fall into Category 1 or Category 2. The occurrence of macrophytes having the characteristics of Category 1 is probably unlikely. Species in Category 2 require a ready source of carbon dioxide in order to undergo ecesis, and these species would not be present in waters with high pH. Category 2 plants therefore would not be expected to be widespread in aquatic environments. Fontinalis antipyretica and Myriophyllum brasilense serve as examples of the Category 2 macrophytes. In the Madison River, only Chara vulgaris was found to have the characteristics of Category 3, whereas the remaining twenty-one species of macrophytes would fall into the four subgroups of Category 4.

Since the Madison macrophytes differed in sensitivity to both carbon sources by a matter of degree, a classification of Category 4 has to be made on the basis of the species' photosynthetic response to carbon dioxide and to bicarbonate relative to some standard value. The delineation of the four subgroups in Category 4 can be quantified by using the PS_{GUC}/C and PS_{GUB}/B values of the species after an adjustment

which involves a division of these values by a species' respiratory rate in order to normalize the natural, inter-specific metabolic variations. In this manner, the mean photosynthetic responses of the Madison species to carbon dioxide and bicarbonate can be determined, and the responses of the individual species then can be compared to these mean or standard values for separation into the four subgroups defined previously. The $(PS_{GUC}/C)/R$ and $(PS_{GUB}/B)/R$ data of the various macrophytes and the "average" Madison plant are summarized in Table 55.

Category 4A includes eight of the species in the Madison River: G. borealis, Mimulus guttatus, Eleocharis acicularis, and S. cuneata along with four of the Potamogeton species--P. filiformis, P. natans, P. strictifolius, and P. pectinatus. All of these macrophytes were either Group I or Group II plants and were most abundant in the upper, high carbon dioxide reach of the river. Category 4B was found to be the opposite of Category 4A both in relative carbon source preference and in distributional features. All of the Madison River species in Category 4B were Group III plants and most abundant in the lower, low carbon dioxide reach of the river. This category includes two species of mosses, F. neo-mexicanus and F. grandifrons, plus Berula erecta, M. exalbescens, and P. gramineus.

Three species are included in Category 4C. Hippuris vulgaris is

Table 55. PS_{GUC}/C and PS_{GUB}/B values of twenty-two species of aquatic macrophytes in the Madison River adjusted by the species' respiratory rates (R) as follows: $(PS_{GUC}/C)/R$ and $(PS_{GUB}/B)/R$.

Species	$(PS_{GUC}/C)/R$	$(PS_{GUB}/B)/R$
"Average" plant	0.850	0.115
Potamogeton filiformis	2.035	0.103
Potamogeton natans	1.255	0.093
Potamogeton pectinatus	1.198	0.098
Potamogeton strictifolius	1.147	0.084
Eleocharis acicularis	1.097	0.106
Mimulus guttatus	0.973	0.096
Sagittaria cuneata	0.956	0.067
Glyceria borealis	0.881	0.090
Potamogeton gramineus	0.794	0.182
Fissidens grandifrons	0.287	0.162
Fontinalis neo-mexicanus	0.712	0.146
Berula erecta	0.704	0.139
Myriophyllum exalbescens	0.679	0.117
Chara vulgaris	0.147	0.172
Hippuris vulgaris	1.488	0.154
Eleocharis thermalis	0.964	0.117
Potamogeton nodosus	0.866	0.116
Sparganium angustifolium	0.694	0.093
Ceratophyllum demersum	0.585	0.098
Juncus ensifolius	0.491	0.099
Oncophorus virens	0.405	0.110
Utrichularia vulgaris	0.305	0.088

most distinctive in its ability to efficiently utilize both carbon dioxide and bicarbonate as carbon sources. This category also includes E. thermalis and P. nodosus. The Category 4C plants apparently had the better of both worlds in a photosynthetic sense in their ability to effectively utilize both carbon sources.

The five species in Category 4D were found to be comparatively inefficient users of both carbon dioxide and bicarbonate (Table 55). The insectivorous Utrichularia vulgaris was most distinct in this regard. Category 4D also consists of Sparganium angustifolium, Juncus ensifolius, Ceratophyllum demersum and O. virens. Representatives of both Categories 4C and 4D were found among all of the Group I, II, and III classes of species based in their distributions in the Madison River.

Species in Category 4 require abundant carbon dioxide concentrations to become established only if bicarbonate is in low supply. This relationship would be most pronounced in 4A class plants and, with adequate alkalinities, less pronounced in 4B, 4C, and Category 3 plants in order of prominence. Category 4C and Category 3 plants would be expected to become widespread in alkaline equatic environments. The Category 4D species apparently represent an amalagation of unique macrophytes or exceptions that would require high concentrations of either or both carbon dioxide and bicarbonate in order to undergo ecesis.

Category 2 macrophytes along with Category 4A and 4C species probably possess the greatest opportunity for ecesis in extremely non-alkaline waters with adequate carbon dioxide concentrations. Category 3 species such as C. vulgaris would probably not be found in these waters.

Several other broad and general relationships were observed among macrophytes in the Madison River in terms of their relative photosynthetic sensitivities to carbon dioxide and bicarbonate. The angiosperms that commonly have been described as amphibious and as occurring in semi-aquatic environments such as swamps and marshes were found to be very responsive to free carbon dioxide and relatively inefficient users of bicarbonate in comparison to macrophytes that have been described as truly aquatic in nature. This comparison includes species such as G. borealis, S. cuneata, and M. guttatus in the first case and species such as M. exalbescens and B. erecta in the second.

Plants which typically have an aerial habit would be expected to be ill-equipped for a totally aquatic existence without the occurrence of unique conditions that would make the aquatic system amenable to the ecesis of these species. Such a condition was manifested in the Madison by the occurrence of high carbon dioxide concentrations in the upper reach of the stream. This was paralleled by the presence in this segment of several amphibious species that were largely absent

from the low carbon dioxide reaches of the stream, e.g., G. borealis, S. cuneata, and M. guttatus.

The mosses and C. vulgaris were found to be more dfficient in the use of bicarbonate and less sensitive photosynthetically to alterations in carbon dioxide than were many of the tracheophytes. Within the angiosperms, the monocots as a group, including most Potamogeton spp., appeared to be more sensitive to carbon dioxide than many dicots, although there was considerable variation in this regard between members of the latter taxon.

ESTIMATES OF PRIMARY PRODUCTION

Standing crop data as grams dry weight per square meter are available from Todd (1967) for the major taxa of aquatic macrophytes in the three reaches of the Madison River. Since the photosynthetic and respiratory data obtained for the taxa in the present study are expressed as a rate per unit of dry weight (mg), such rates can be placed on an area basis (as ml O_2 per square meter per minute) by multiplying PS per unit weight by weight per square meter for each species.

Photosynthetic and respiratory rates are available for each species in water samples collected from each reach of the river and are assumed to represent the in situ rates that would be obtained for each macrophyte in a particular segment of the stream. As a result, net production and respiration for the shoot system of the macrophyte

community in each of the three reaches can be estimated by appropriately summing the calculated rates per unit area obtained for each species in the stream segment. The relative frequencies of the macrophytes in each reach, as calculated from Horpestad's (1969) percent canopy cover (Table 3), were also incorporated into the calculations in order to estimate the standing crops of some of the species that were combined by Todd (1967) into broader taxonomic categories (Table 4).

The gross primary production of the macrophyte community in each reach of the river can then be calculated by adding net production and respiration. Gross primary production in each reach as ml O_2 per m^2 per minute was converted to units of g C per day per m^2 in order to compare the results of this study to that from other production investigations on the river. Wright and Mills (1967) and Klarich and Wright (1974) made such estimates using the upstream-downstream method developed by Odum (1956). The results of these studies are summarized in Figure 5 along with estimates of primary production obtained in this study.

As indicated in Figure 5, estimates of the primary production of various reaches of the Madison River are somewhat higher in this study than those obtained by Wright and Mills (1967) and by Klarich and Wright (1974). However, the variations among studies do not appear to be unreasonable in view of the differences in methodology, and the

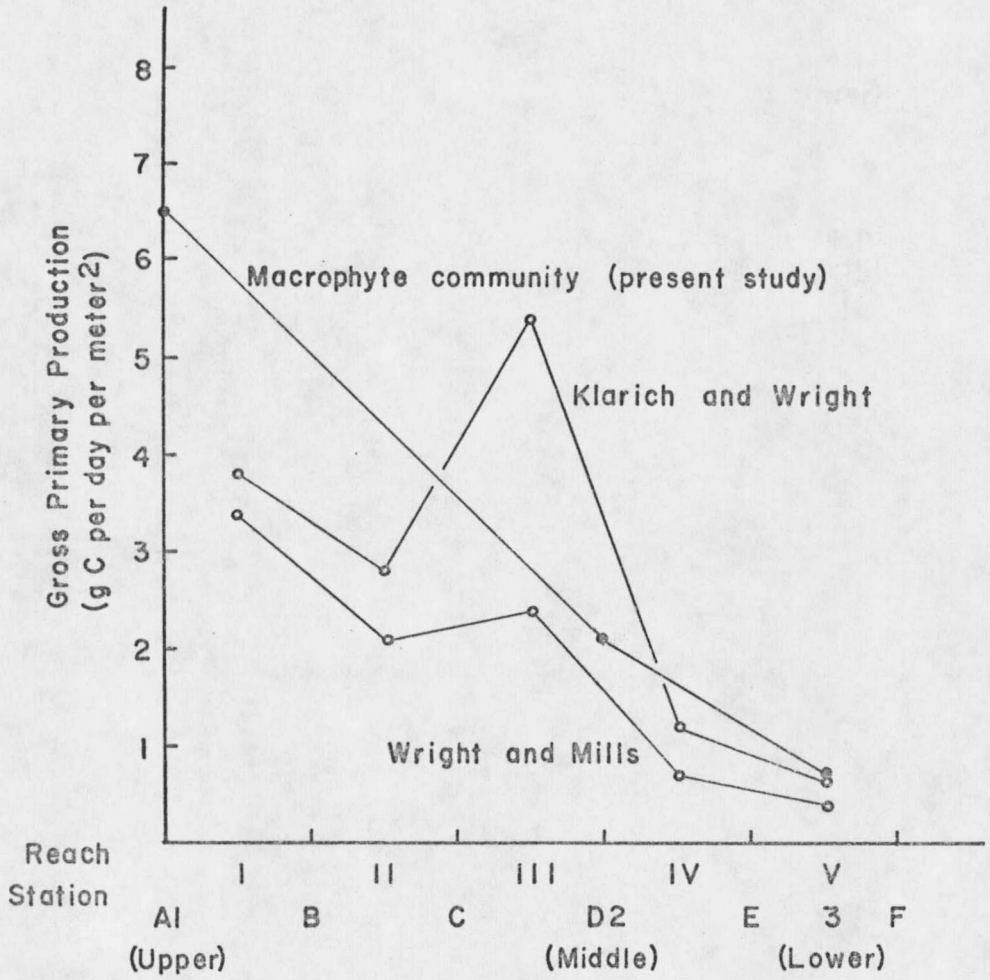


Figure 5. Gross primary production of several reaches of the Madison River in Yellowstone National Park (data taken from Wright and Mills 1967, and Klarich and Wright 1974).

variations between the 1967 and 1974 determinations through the various reaches appear to be about as great as the discrepancies between the estimates from this study and those by the upstream-downstream method.

Estimates of net production and respiration were also generally similar between the various studies. Such similarities are even more pronounced considering the fact that estimates of primary production from photosynthetic and respiratory rates were based on only a part of the total river production that was estimated by the upstream-downstream method. For example, the respiratory activity of the macrophyte root system was not examined in this study nor were the respiratory and/or photosynthetic activities of the bacterial and periphytic components of this rheological system other than organisms attached to the collected macrophytes. This suggests that the macrophyte community was the dominant floral element of the stream and/or that total Madison production was underestimated somewhat by Odum's (1956) method. As an alternative, this could also suggest that the production of the macrophyte community was overestimated through the methods of this study since the primary production calculations were based on saturating light intensities, i.e., $PS_N = PS_{sat}$.

All three studies demonstrated a distinct downstream decline in gross primary production of the Madison River, and these decreases were of a similar magnitude in the studies. Todd (1967) also

determined the primary productivity of the Madison River, but he did not make estimates of respiratory rates in the river segments; as a result, gross production for comparison could not be calculated from Todd's data. However, the net production rates established by Todd (1967) also demonstrated a distinct decline downstream on the same order as that determined by Wright and Mills (1967) and by Klarich and Wright (1974). The similarities between the various studies can be illustrated by the downstream, percent declines in primary production as follows:

This study	88.8%
Wright and Mills (1967)	88.2%
Klarich and Wright (1974)	83.1%
Todd (1967)	85.8%

Wright and Mills (1967), Todd (1967), and Klarich and Wright (1974) have suggested that the progressive downstream decline in free carbon dioxide in the Madison River may have been a major cause for the marked downstream decrease in primary production as depicted in Figure 5. Various studies, e.g., Steeman Nielsen (1944, 1946, 1947), Wright (1960), Bristow (1969), Ruttner (1969), Wright (1969), and Klarich (1971), indicate that the availability of carbon dioxide can act to limit the rates of photosynthesis in certain aquatic species and systems. The distinct and direct photosynthetic responses of the various macrophyte species to alterations in carbon dioxide levels

and to the differences in carbon dioxide concentrations between samples collected along the carbon dioxide gradient of the river support the hypothesis that this chemical variable is an important limiting factor in the river. On this basis, downstream reductions in carbon dioxide as a carbon substrate in macrophyte photosynthesis would reduce the primary production of the lower reaches. However, the standing crop and canopy cover of the macrophyte communities also decrease downstream (Tables 3 and 4) and these factors along with the change in species composition in themselves could account for the decline in primary production regardless of changes in free carbon dioxide.

A general tendency was observed in the Madison River for the downstream macrophyte communities to have lower photosynthetic and respiratory rates at common standing crop and carbon dioxide levels than those located upstream. This is illustrated in Table 56. Such decreases in metabolic rates with the other variables held constant were due to the increase in predominance of species downstream that had lower PS_N and respiratory rates than the macrophytes abundant in the upper reach of the river.

For example, the mosses were prevalent in the lower reach but scarce upstream and had net photosynthetic rates ranging from 0.0165 to 0.0235 ul O_2/mg/min and respiratory rates ranging from 0.0046 to

Table 56. Net and gross primary production (NP and GP) and respiration (R) (ml O_2 per m^2 per minute) and net production/respiratory ratios (NP/R) for three communities of aquatic macrophytes in the Madison River calculated for different carbon dioxide concentrations and for different levels of standing crop per unit area.

	Carbon Dioxide Concentrations of 6.8 mg/l(U)				
	Standing Crop of 274.3 g/m^2(U)			Standing Crop of 54.2 g/m^2(L)	
Parameter	Upper River Community	Middle River Community	Lower River Community	Upper River Community	Lower River Community
NP	11.20	9.47	7.15	2.22	1.41
R	3.20	2.87	2.60	0.63	0.51
GP	14.40	12.34	9.75	2.83	1.92
NP/R	3.50	3.31	2.75	3.50	2.75

	Carbon Dioxide Concentrations of 1.2 mg/l(L)				
	Standing Crop of 274.3 g/m^2(U)			Standing Crop of 54.2 g/m^2(L)	
Parameter	Upper River Community	Middle River Community	Lower River Community	Upper River Community	Lower River Community
NP	8.19	6.93	5.56	1.62	1.10
R	3.36	3.00	2.62	0.67	0.52
GP	11.55	9.93	8.18	2.29	1.62
NP/R	2.44	2.31	2.12	2.44	2.12

(U)--characteristic of the upper river reach.
(L)--characteristic of the lower river reach.

to 0.0131 ul O_2/mg/min in water from the middle segment of the river (Table 44). In contrast, Potamogeton spp. which were abundant upstream but largely absent from the lower segment of the river had somewhat higher net photosynthetic and respiratory rates in water from the middle reach; these ranged from 0.0253 to 0.0540 ul O_2/mg/min and from 0.0096 to 0.0135 ul O_2/mg/min for photosynthesis and respiration respectively. As a result, the downstream reduction in abundance of species having high photosynthetic rates coupled with an increase in the proportion of species having relatively low photosynthetic rates could act to decrease primary production in lower reaches of a stream independent of carbon dioxide alteration as illustrated in Table 56.

Apparently the macrophyte communities of the stream tended to change downstream from one having relatively high metabolic rates and high PS_N/R for ecesis to one having relatively low metabolic rates and undergoing ecesis with relatively low PS_N/R ratios. A large part of such shifts were due to the increased proportion in the lower reach of Oncophorus virens, which has relatively low PS_N/R values (Table 44).

Downstream reductions in biomass of the macrophyte shoot systems per unit area (Table 4) indicate a decrease in the amount of photosynthetic tissue in the lower reaches of the Madison River. As a result, photosynthetic rates per unit area would also be lower in the downstream segments, and this corresponds to the downstream decline in primary

production. Table 56 demonstrates the pronounced reduction in photosynthetic rates of the upper and lower communities in the Madison River as a result of decreases in standing crop at constant carbon dioxide that are equivalent to the in situ situation. As a result, the direct effect of carbon dioxide in causing the downstream decline in primary production by progressively reducing the photosynthetic rates of the macrophytes downstream might not be as great as implied in Figure 5, and it might not be as great as what is suggested by the distinct photosynthetic responses of the individual species to the carbon dioxide differences between water samples collected at different sites along the river. Changes in species composition and reduced standing crops downstream might play a more important role in this regard.

Horpestad (1969), although he did not totally eliminate carbon dioxide as a plausible limiting factor, suggested that a change in the physical characteristics of the stream with the development of a more adverse environment in the lower reach plays the major role in altering species composition and reducing standing crop and canopy cover downstream. A decrease in primary production would thereby follow as illustrated in Table 56. Horpestad's (1969) observations are in accord with the results from other investigations of rheological systems (Pond 1903, Pearsall 1920, Butcher 1933, Curtis 1959) which also implicate

physical features as important factors in regulating the occurrence, distribution, and abundance of aquatic macrophytes.

The potential action of such physical characteristics on the macrophyte communities cannot be quantitatively separated from the potential action of carbon dioxide as indicated by this study. Since Horpestad (1969) did find some relationships between carbon dioxide concentrations and macrophyte diversity, distribution, and abundance in the Madison River, and with the results of this study, possibly both the physical changes in the river and its carbon dioxide gradient were acting in concert to produce a reduction in standing crop and the change in species composition downstream, this then bringing about a decline in primary production. At least some evidence has been provided through this investigation to implicate carbon dioxide as a potential causal factor in this regard.

As noted, three variables directly influence the magnitude of primary production through the various reaches of the river: the decline in carbon dioxide concentrations, the alteration in species composition, and the downstream decrease in standing crop. The direct effect and magnitude of the carbon dioxide variable can be estimated and separated from the effects of the standing crop and species composition changes through several methematical steps. Similar manipulations were utilized to derive the data summarized in Figure 5 and in Table 56.

In the first step, standing crop characteristic of the upper reach (274.3 g/m^2) was utilized and partitioned to correspond to the species composition or proportions of species that characterized each of the three reach communities. In this case, the net photosynthetic and respiration rates separately obtained for each of the species in water samples from the upper reach were used to calculate a rate per unit area under high carbon dioxide conditions, about 6.8 mg/l, but for the three different species compositions corresponding to the three river communities. This then provided an estimate of the *in situ* primary production in the upstream community of the river and an estimate of the component of downstream decline in production due to the changes in species composition through the middle and lower reaches of the stream.

In the subsequent step, a standing crop of 274.3 g/m^2, partitioned for the proportions of species in the middle and lower reaches, was again used but with carbon dioxide concentrations characteristic of the middle and lower segments, about 3.4 and 1.2 mg/l. In this case, the net photosynthetic and respiratory rates of the species in water samples from the middle and lower reaches were used along with the appropriate proportions of species in each reach. The differences in the calculated production rates (ml $O_2/m^2/min$) between step one and step two for the middle and lower reaches then provided an estimate of

the amount of decrease in primary production due directly to the carbon dioxide gradient of the river.

In the third and final step, the standing crop observed by Todd (1967) for each reach was utilized, and metabolic rates were recalculated for each community using the carbon dioxide levels and species composition characteristic of each reach. The difference in photosynthetic rates between step two and step three provided an estimate of the amount of decrease in primary production through the lower two segments due to the downstream decline in biomass per square meter. In addition, this final manipulation afforded an estimate of the *in situ* production in each reach as summarized in Figure 5 but adjusted in this figure to mg C/day/m^2. The differences between gross production calculated for the upper stream community and that calculated for the middle and lower stream communities in step three affords an estimate of the decline in production in each reach due to the change in species composition, to the decrease in standing crop, and to the downstream reduction in free carbon dioxide.

The results of these mathematical manipulations are summarized in Figure 6. Apparently the downstream change in the species structure of the communities to one having a predominance of macrophytes with inherently lower metabolic rates accounted for 21.4% of the decrease in primary production from the upper to the middle reach; this amounted

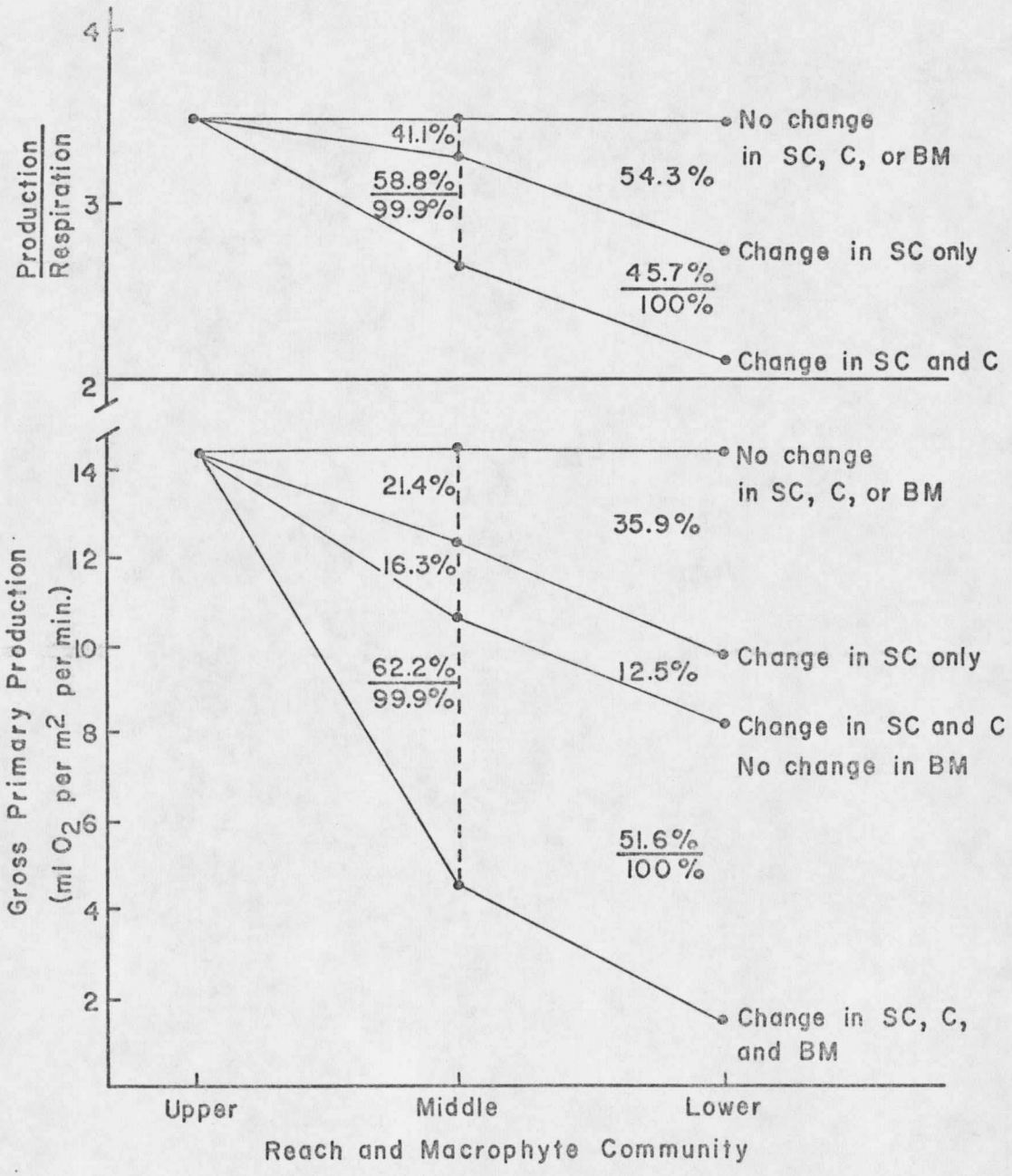


Figure 6. Percentage of the downstream decline in the gross primary production of three Madison River macrophyte communities and their production/respiration ratios due to changes in species composition (SC), in carbon dioxide concentrations (C), and in standing crop or biomass (BM) per square meter.

to 35.9% from station 1 to station 3. An effect of this magnitude might be expected as a result of the distinct differences in floristic makeup between the lower and upper communities. The greatest amount of decrease in production was due to the distinct downstream decline in standing crop (Table 4). This variable accounted for more than 50% of the production decrease through the two downstream segments of the stream.

Carbon dioxide concentration also apparently had a direct effect in reducing community production by depressing the macrophyte's photosynthetic rates at low concentrations. However this effect was comparatively small and accounted for only 13-16% of the decrease in primary production downstream. However, as indicated in Figure 6, carbon dioxide had a much more pronounced effect on the downstream gross production/respiration ratios (GP/R) of the river communities. Changes in standing crop would not affect this particular variable whereas the downstream decrease in carbon dioxide accounted for nearly 50% of the 1.65-fold decline in GP/R to the lower reach. About 54% of this reduction in GP/R was due to the progressive dominance of species in the lower reach with inherently low PS_N/R values under all conditions, particularly Oncophorus virens.

With the influence of carbon dioxide in reducing community GP/R along the natural gradient in the Madison River, carbon dioxide

therefore appears to have, when in low concentrations, the capability of indirectly reducing primary production downstream, in addition to its more direct effect, by lowering the photosynthetic efficiencies of the downstream macrophyte communities. This could then alter the species composition and lower the biomass of the communities, and a decline in production to the lower reaches of the river would thereby follow as illustrated in Figures 5 and 6 and in Table 56. The potential "sorting" of macrophyte species by the carbon dioxide gradient of the Madison River has been described previously. Similar photosynthetic patterns are evident at the community level.

The upper river community as a whole responded significantly to differences in carbon dioxide as demonstrated in Table 56. However, a large proportion of the standing crop in this upper community was represented by Group III plants, primarily Myriophyllum exalbescens and Berula erecta, which were also abundant in the lower river. Thus, the community metabolic rates under different carbon dioxide levels can be partitioned into two sets--that due to these Group III plants and that due to Group I species and Sparganium angustifolium largely confined to the upper reach of the river (Table 57).

Marked differences are evident in Table 57 between these two components of the upper community in their photosynthetic responses to free carbon dioxide. The Group I component responded much more

Table 57. Photosynthetic response of the upper reach macrophyte community of the Madison River to levels of carbon dioxide characteristic of three reaches of the stream: upper reach--6.8 mg/l, middle reach--3.4 mg/l, lower reach--1.2 mg/l; NP, R, and GP are net production, respiration, and gross production respectively as ml $O_2/m^2/min$.

	Total Community		
Parameter	6.8 mg/l CO_2	3.4 mg/l CO_2	1.2 mg/l CO_2
NP	11.20	9.20	8.19
R	3.20	3.25	3.36
GP	14.40	12.45	11.55
NP/R	3.50	2.82	2.44

	Group III Component (170.8 g/m^2) (prevalent in the lower reach of the river)		
Parameter	6.8 mg/l CO_2	3.4 mg/l CO_2	1.2 mg/l CO_2
NP	6.28	5.79	5.54
R	1.71	1.79	1.86
GP	7.99	7.58	7.40
NP/R	3.66	3.23	2.98

	Group I Component (103.4 g/m^2) (most abundant in the upper reach of the river)		
Parameter	6.8 mg/l CO_2	3.4 mg/l CO_2	1.2 mg/l CO_2
NP	4.92	3.41	2.65
R	1.49	1.46	1.50
GP	6.41	4.87	4.15
NP/R	3.31	2.33	1.76

significantly to the downstream differences in this variable than the Group III plants. As a result, a large part of the photosynthetic decline of the total community along the carbon dioxide gradient would be due to the Group I component of the community. The photosynthetic rates of the Group III component demonstrated only a slight decline, and their net production/respiratory ratios (NP/R) in the lower reach were much greater than the proposed critical values which would be suggestive of successful establishment. The decline in NP/R of the total community from the upper to the lower reach would be due primarily to factors affecting the Group I component; the decline in photosynthesis is quite distinct in this case, and NP/R ratios would be decreased in the Group I component to levels that would indicate a relatively low probability of success in the lower river ($PS_N/R < 2.0$). This corresponds to, and could account for, the relative rarity of the Group I component of the upper river community in the lower segment of the Madison River.

SUMMARY

Photosynthetic and respiratory rates of twenty-two species of aquatic macrophytes were manometrically measured in water samples from three sites along the Madison River in Yellowstone National Park. Photosynthetic and respiratory rates were also measured in water samples in which the carbon dioxide concentrations were experimentally manipulated to approximate those in water from upstream or downstream stations. Net photosynthetic rates at light saturation, gross photosynthetic rates, net photosynthetic rate/respiratory rate ratios, and percent light saturation values in the manometer were calculated from these data.

Several investigators have observed an 83% to 88% downstream decline in the primary production of the Madison River. This decline was attributed to carbon dioxide since the concentration of this constituent also decreased downstream in a fashion that paralleled production. A part of the primary production in the Madison is due to a luxurious aquatic macrophyte community which demonstrated downstream changes in plant distributions and species composition and downstream decreases in total standing crop and in percent canopy cover.

If there were no major chemical or physical variables in the twenty-three kilometer reach of the Madison River other than carbon dioxide, then such correlations between primary production and carbon dioxide and between carbon dioxide and abundance and distribution

changes would indicate that the availability of this carbon source acts as a limiting factor. Carbon dioxide appears to be the only major chemical variable in the river with a sufficient magnitude of variation to account for the decline in primary production in the lower reach (Wright 1964). However, distinct downstream changes in various physical factors do occur through a long riffle in the lower one-third to one-half segment of the study area. This riffle brings about increases in the stream's average width and average current speed, shallower depths, and an alteration in substrate from a gravelly-sandy composition upstream to one in the lower section that is rocky in nature. Such physical changes could also restrict and decrease macrophyte establishment in the lower reach which could cause a decline in primary production apart from the carbon dioxide factor. As a result, the relationships between primary production and macrophyte distribution and abundance and carbon dioxide concentrations become clouded with the occurrence of these corresponding physical changes in the Madison River.

The main objective of this study was to determine whether carbon dioxide could act as a limiting factor to aquatic macrophytes in the Madison River, either in causing a downstream reduction in photosynthetic activity and thereby primary production, or in altering distributional patterns and reducing plant abundances which would also affect

production. A physiological approach was used to: (1) determine if photosynthesis responded significantly to the in situ carbon dioxide changes in the stream; (2) establish, through an assessment of net photosynthetic/respiratory ratios, whether the photosynthetic response of a macrophyte to decreased carbon dioxide concentrations would be of sufficient magnitude to indicate a low probability of ecesis under low carbon dioxide tensions; and (3) assess the inter-specific variability of the photosynthetic response to carbon dioxide in relation to the species' distributions in the river.

All species examined in the study were found to respond photosynthetically to some extent to the differences in carbon dioxide levels between water samples from the upper, middle, and lower reaches of the stream. Although similar respiratory rates were obtained for each of the twenty-two species in all water samples, all of the species had greater photosynthetic rates in water from the high carbon dioxide, upper reach than in water from the lower section of the river. Intermediate photosynthetic rates were generally obtained in water from the middle segment where the carbon dioxide concentration was also intermediate. Photosynthetic rates in these water samples could be increased or decreased by the addition or removal of carbon dioxide by various methods. In addition, the percent light saturation of the macrophytes at the 780 foot candles of the respirometer were typically

lower in waters with the higher carbon dioxide concentrations. These observations suggest a photosynthetic response to the downstream decrease in free carbon dioxide, and this corresponds to downstream decline in primary production that has been observed in the Madison River.

Most species demonstrated relatively high light saturation values even at the light intensity of the respirometer, which was relatively low compared to natural river illumination, and this aspect was most pronounced in water from the lower reach with its low carbon dioxide levels. This indicates that the macrophytes were more carbon than light limited. The species appeared to be more substrate limited in the lower reach than in the upper segment since they had higher percent light saturations but relatively low photosynthetic rates in the lower segment. In turn, they appeared to be more light limited in the upper, high carbon dioxide reach than in the lower segment of the river since they had lower percent light saturations upstream along with relatively high photosynthetic rates. However, these relationships were more distinct in some species than in others.

All of the species examined in the study, with the exception of Chara vulgaris, apparently utilized carbon dioxide more effectively than bicarbonate on a per unit carbon basis in photosynthesis. However, the photosynthetic responses of the macrophytes to the in situ carbon dioxide differences at various locations on the river varied

considerably among species. With some species the response was slight, and less than 1.3-fold differences in photosynthetic rates were obtained between the upper and lower reach water samples. In other species, the response was greater, and statistically significant, two-fold or more differences in photosynthetic rates were obtained. In contrast, only a 1.03-fold average difference in total soluble inorganic carbon was evident between the upper and lower reaches of the stream, although the mean carbon dioxide concentration upstream was 5.8-times that downstream.

The decreases in percent light saturations from the lower to upper reach water samples were also greater in the species with the greater photosynthetic responses to free carbon dioxide.

Most macrophyte species had relatively high PS_N/R ratios, generally greater than 2.5, in the upper reach, high carbon dioxide waters. For those species that demonstrated relatively small inter-sample photosynthetic differences, PS_N/R ratios were also relatively high and typically greater than 2.5 in the lower reach waters since respiration rates were fairly constant through all treatments. However, for species with a marked photosynthetic response to carbon dioxide and constant respiration rates, PS_N/R ratios in the lower reach water samples were low enough, typically less than 2.0, to suggest a low

probability of ecesis for these plants in this segment of the river. Thus, the photosynthetic response of some species to the Madison River carbon dioxide gradient appears to be of sufficient magnitude to affect their distributions in the river.

The fact that definite and statistically significant parallels and correlations were obtained between the species' distributions and their photosynthetic responses to free carbon dioxide lends support to the hypothesis that carbon dioxide does act as a limiting and distributional factor in the Madison River. Species that are largely absent from the lower reach of the river, the Group I + II plants, had greater photosynthetic responses to the inter-sample carbon dioxide differences than the macrophytes that were abundant in the lower, low carbon dioxide segment (Group III).

The calculated, total primary production per unit area of the macrophyte communities closely coincides with that obtained by other investigators for the upper, middle, and lower reaches of the Madison River. About 12% to 17% of the downstream decline in production apparently can be attributed to the direct effect of carbon dioxide wherein the decrease of photosynthetic rates along the carbon dioxide gradient produces a corresponding decline in total production. In addition, photosynthetic/respiratory data indicate that carbon dioxide, probably in concert with the physical variables, could affect primary production

indirectly by altering species distributions downstream and by possibly reducing macrophyte abundance. In the first case, this initiated change in macrophyte composition downstream decreases production by 21% to 36% since the Group III species abundant in the lower reach had lower photosynthetic rates at constant carbon and bicarbonate than the Group I + II species most abundant in the upper segment. The downstream decline in macrophyte standing crop accounted for about 51% to 63% of the total decrease in Madison production.

If only physical aspects such as current, depth, and substrate are involved in defining species distribution and abundance and thereby levels of primary production, then a close correspondence and significant correlations between photosynthetic and distribution-abundance data would not be expected; rather, the photosynthetic responses of the species to carbon dioxide would have been more randomly related to their distributions and statistically insignificant. Although the effects of the physical factors cannot be directly and quantitatively separated from those of carbon dioxide, evidence obtained from this study indicates that carbon dioxide concentration does have a strong potential for affecting the distribution, abundance, and primary production of macrophyte communities in relatively non-alkaline waters such as the Madison River. It seems unlikely that all of the parallels observed between the photosynthetic-respiratory variables and the

species' distributions and abundance in this investigation would be totally coincidental in nature. The results of this study therefore tend to confirm the conclusions of other investigators that carbon dioxide acts as an important limiting factor in the Madison River and is instrumental in causing the downstream decline in primary production.

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APPENDICES

APPENDIX A. WORKING EQUATIONS FOR ESTIMATING PHOTOSYNTHETIC RATES AT LIGHT SATURATION

Assuming that photosynthetic rates (PS) as a function of light intensity (I) approach a rectangular hyperbola, experimentally measured photosynthetic rates at L and (1/2)L, light intensity at maximum and 50% maximum respirometer illumination respectively, can be utilized to estimate light saturated photosynthetic rates or PS_{sat} (Pickett 1969, personal communication). The following definitions will be applied:

PS_L = PS(L) = rate of net photosynthesis at full respirometer light intensity;

$PS_{(1/2)L} = PS_{L/2}$ = PS(L/2) = rate of net photosynthesis with 50% screen in the respirometer;

$r = PS_L/PS_{L/2}$;

PS_N = estimated net photosynthetic rate at light saturation = PS_{sat};

and $F = 1/PS_L$ = correction factor used to estimate PS_N from PS_L.

With the assumption that the light saturation curves are rectangular hyperbolae of the form

$$PS(I) = I/(k + I),$$

then $PS(L)/PS(L/2) = r = (2k + L)/(k + L)$,

or $L = k(2 - r)/(r - 1)$,

and $F = 1/PS(L) = 1/(2 - r)$,

and $PS_N = PS_{sat} = F\ (PS_L) = PS_L/(2 - r)$.

If L is far below light saturation, the net rate of photosynthesis

is not carbon substrate limited and is almost proportional to light intensity. In this case r approaches 2.0, and this procedure will provide a poor estimate of $PS_N = PS_{sat}$. However, if L is near light saturation, and if the carbon source is rate-limiting, r approaches 1.0, and this procedure should provide a good estimate of PS_N at light saturation. Since comparisons of carbon limitations of photosynthesis can be made only near light saturation, this procedure will provide useful estimates of the degree to which the light saturated rate of photosynthesis is limited by carbon sources available in various aquatic environments.

APPENDIX B. TABLES OF RESULTS FROM PRELIMINARY EXPERIMENTS WITH VARIOUS MACROPHYTE SPECIES

Table 58. Mean photosynthetic and respiratory rates (ulO_2/mg dry wt/min) of Hippuris vulgaris tissue sections at differing distances from the terminal bud--segments I to IV where segment I is the terminal bud section and segment IV is the basal section. Mathematical methods for estimating the photosynthetic and respiratory rates of the entire shoot system are also presented.

Parameter	Segment I	II	III	IV	Summation
Average distance from the apex (cm)	0.0	6.8	14.2	21.6	---
Segment dry weight (mg)	23.1	26.8	43.9	23.0	116.8
Photosynthetic rate per weight	0.0616	0.0425	0.0266	0.0132	---
Photosynthetic rate per segment	1.423	1.139	1.168	0.304	4.034
Respiratory rate per weight	0.0304	0.0187	0.0190	0.0162	---
Respiratory rate per segment	0.702	0.501	0.834	0.373	2.410

Estimated Shoot System (SS) Photosynthetic Rates (PS):

Method 1. $PS_{SS} = (\sum_{i=I}^{IV} PS/segment)/(\sum_{i=I}^{IV} segment\ weight) = 0.0345\ ulO_2/mg/min$

Method 2. $PS_{SS} = 0.5\ (PS_I + PS_{IV}) = 0.0374\ ulO_2/mg/min$

Estimated Shoot System (SS) Respiratory Rates (R):

Method 1. $R_{SS} = (\sum_{i=I}^{IV} R/segment)/(\sum_{i=I}^{IV} segment\ weight) = 0.0206\ ulO_2/mg/min$

Method 2. $R_{SS} = 0.5\ (R_I + R_{IV}) = 0.0233\ ulO_2/mg/min$

Table 59. Inorganic carbon chemistry and photosynthetic rates obtained from the preliminary experiment with Eleocharis acicularis in water collected from three sites on the Madison River. Rates expressed as ul O_2/mg/min.

	Treatments					
	Basic			Accessory		
Stations	Upper	Middle	Lower	Upper	Middle	Lower
Alterations	Unaltered	Unaltered	Unaltered	Method II (16 hours storage)	Method II (16 hours storage)	Method II (16 hours storage)
Alkalinity	122.6	124.4	127.5	122.6	124.4	127.5
pH	7.60	7.70	8.22	----	----	----
Free CO_2	4.89	3.95	1.22	----	----	----
PS_L	0.0145	0.0142	0.0095	0.0111	0.0124	0.0100
% saturation	84%	86%	99%	----	----	----
PS_N	0.0173	0.0165	0.0096	------	------	------

Table 60. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the preliminary experiment with Sagittaria cuneata in unaltered sample water collected from three sites on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alkalinity	123.2	123.8	124.4
pH	7.45	7.69	8.23
Free CO_2	6.95	4.01	1.16
PS_L	0.0585	0.0372	0.0203
% saturation	93%	98%	100%
R	0.0226	0.0250	0.0230
PS_N	0.0632	0.0379	0.0207
PS_N/R	2.80	1.52	0.90
PS_G	0.0858	0.0629	0.0437

Table 61. Inorganic carbon chemistry and photosynthetic and respiratory rates obtained from the preliminary experiment with Oncophorus virens in unaltered sample water collected from three sites on the Madison River. Rates expressed as ul O_2/mg/min.

	Basic Treatments		
Stations	Upper	Middle	Lower
Alkalinity	119.0	120.8	123.2
pH	7.35	7.73	8.18
Free CO_2	8.45	3.57	1.29
PS_L	0.0180	0.0178	0.0176
% saturation	71%	76%	74%
PS_N	0.0254	0.0233	0.0238

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