

PHOTOSYNTHETIC CHARACTERISTICS OF THE SUBMERSED AQUATIC PLANTS HYDRILLA,  
SOUTHERN NAIAD, AND VALLISNERIA

By

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Chlorophyll content of hydrilla (*Hydrilla verticillata* (L.F.) Royle), southern naiad (*Najas guadalupensis* (Spreng.) Magnus), and vallisneria (*Vallisneria neotropicalis* Marie-Vict.) plant samples varied widely under different environmental conditions. The chlorophyll a to chlorophyll b ratios for hydrilla and vallisneria were approximately 2.0 at the water surface; however, hydrilla samples collected from field populations at a depth of 1.5 m had a greater proportion of chlorophyll b. Hydrilla appeared to be capable of chromatic adaptation.

The leaf area index of hydrilla and vallisneria samples taken from populations grown in earthen ponds 1.5 m deep was 4.8 and 8.7, respectively. In these ponds, the dried standing crop of hydrilla was estimated to be  $161.4 \text{ g m}^{-2}$  and that of vallisneria was  $400.1 \text{ g m}^{-2}$ . The horizontal distribution of hydrilla is such that it forms an extensive canopy which limits light penetration. Tissue analysis of samples of these species collected in September 1973, and January 1974, showed a pronounced seasonal variation in their carbohydrate content.

The pH of culture solutions increased rapidly when these submersed species were grown in sealed vessels under controlled conditions.

Measurements for carbon-14 in plant and culture solutions indicated that free  $\text{CO}_2$  was utilized most readily for photosynthesis; however, carbon for subsequent photosynthesis was derived from  $\text{HCO}_3^-$  which was added to the culture solutions. Uptake of  $^{14}\text{C}$  by southern naiad and vallisneria gradually decreased from maximum at pH 4.0 to nearly zero at pH 8.5.

Hydrilla had the highest rate of  $^{14}\text{C}$  uptake in solutions between pH 5.0 to 8.5 with a maximum absorption in solutions of pH 6.0. No evidence of direct  $\text{HCO}_3^-$  utilization was shown by any species studied. The  $\text{CO}_2$  compensation points of all the species were about  $8.0 \text{ } \mu\text{l l}^{-1}$ . Photosynthetic characteristics of submersed aquatic plants could not be used to categorize these species into either the  $\text{C}_3$  or  $\text{C}_4$  plant groups.

Data indicated that these submersed aquatic plants were most like  $\text{C}_3$  species, but they possess distinct characteristics which may be attributed to the adaptation of aquatic plants to growth under low light intensities and low concentrations of free  $\text{CO}_2$ .

CHAPTER I  
GENERAL INTRODUCTION AND REVIEW

Introduction

One of the less obvious signs of American affluence is the accelerated eutrophication of natural waters and the contributing growth of submersed aquatic plants. Native plants which once helped maintain a well balanced aquatic system now often occur in troublesome and sometimes dangerous quantities. Nutrification of natural waters by improper land management and waste disposal has undoubtedly increased growth of aquatic plants. The introduction and sale of exotic and native species by the pet fish and aquarium industry has resulted in the spread of undesirable plants from one area to another (52).

Florida is the leading source of aquatic plants for sale to aquarium owners, primarily because the mild winters permit the growth of these plants throughout the year. The natural high fertility of Florida's waters has also contributed to rampant aquatic plant growth.

Control of aquatic plants is necessary to prevent their interference with fish production, recreation, irrigation, and navigation. In south Florida, weed control is essential for maintaining water table levels and allowing efficient drainage of water in the event of a hurricane. Presently, attempts to prevent weed interference with water use are being studied, as are mechanical harvesting and techniques in water level manipulation. Due to high costs or lack of development of other methods,

herbicides provide the major means for aquatic plant management. However, a single chemical treatment costs from 50 to 150 dollars per ha, chemical residue may persist, and plant regrowth often occurs in less than 1 year. Past research has emphasized studies in control and has largely ignored the ecology and physiology of submersed plants. The absence of any substantial amount of physiologically oriented research has prompted a widely recognized plant physiologist to state that "perhaps rooted aquatic plants have an unusual metabolism" (11 p. 260). This statement clearly emphasizes our lack of knowledge of the metabolism of aquatic plants. Further questions concerning the presence or absence of a transpiration stream, nutrient uptake, carbon absorption, and translocation have not been resolved.

Research in other aspects of lake biology has been extensive. Algal physiology and biochemistry are well developed areas of specialization, and journals have reported this research for many years. The portion of primary productivity of lakes ascribed to the phytoplankton has been the subject of numerous research articles. However, the portion of productivity resulting from aquatic macrophytes frequently has been ignored, although they often constitute a much greater biomass than the phytoplankton (95). Recent reviews of aquatic macrophyte productivity and ecology indicate the importance of these plants to lake systems (29).

The apparent lack of interest in aquatic macrophyte research results from the less dominant role they once maintained, and because the classical research methods of lake biology are not readily adaptable to vascular plants. The introduction of exotic aquatic plants has compounded the problem because they often originate, or are found primarily in less developed tropical countries where research by necessity is concentrated

on food production, not on aquatic plant control, ecology, or physiology.

The submersed aquatic plant presently causing the most serious problems in Florida is hydrilla (*Hydrilla verticillata* (L.F.) Royle). This species was introduced to Florida around 1960 and has very rapidly spread to all parts of the state (14). It has become a much more serious weed than the native species of vallisneria (*Vallisneria neotropicalis* Marie-Vict.) or southern naiad (*Najas guadalupensis* (Spreng.) Magnus) were prior to its introduction (94). Hydrilla is extremely competitive, and soon after its introduction into a body of water it rapidly dominates the submersed flora. By 1970 hydrilla was estimated to occur in more than 30,000 ha of Florida's fresh waters (58), and presently is believed to infest in excess of 40,000 ha, or about 5% of Florida waters. Control measures are applied in only a small portion of the total area. The overall capital outlay for hydrilla control is difficult, if not impossible, to determine accurately because several dozen federal, state, county, and municipal agencies are active in aquatic weed control including weeds other than hydrilla. However, it is estimated that hydrilla control in Florida costs taxpayers between 4 and 6 million dollars annually.<sup>1</sup>

There has been little research reported on the physiology and ecology of the native species vallisneria and southern naiad, and even less published on the exotic hydrilla. The factors responsible for hydrilla's rapid dominance of aquatic systems will not be identified until more basic research is completed. When the growth and metabolism

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1 Dr. Alva P. Burkhalter, Florida Department of Natural Resources, February, 1974. Personal Communication.

of these plants are better understood, more effective management practices likely will result.

The primary objective of this research was to identify some of the metabolic characteristics of hydrilla which permit it to dominate our native submersed flora. Because growth and production are direct functions of photosynthesis, most of the experiments conducted were closely related to this physiological process.

#### Plant Biology and Distribution

Hydrilla, southern naiad, and vallisneria are common submersed vascular plants which root in the hydrosoil and often grow to the water surface. They are found throughout Florida and commonly inhabit any body of water from ditches to the largest lakes. When localized in small areas around a body of water, they are beneficial to fish and wildlife (51). However, prolific growth covering the water's surface results in numerous undesirable effects which necessitate some form of management.

The three plants studied are all monocots. Southern naiad belongs to the *Najadaceae* family, and vallisneria and hydrilla are members of the *Hydrocharitaceae* family. The importance of sexual reproduction in the natural propagation of these species has not been studied, but vegetative reproduction appears to be the major means of plant establishment.

Southern naiad (Figure 1) is monoecious and has opposite sheathing leaves 1.0 to 3.0 cm long and 0.5 to 1.0 mm wide. The stems are slender and branch profusely. The flowers are unisexual, and pollination is accomplished completely underwater (hydrophilous). Southern naiad has a very wide range in the western hemisphere extending from Quebec to the Rocky Mountains and southward across the equator into Argentina (3,26,59).

Figure 1. View of apical portion of southern naiad (*Najas guadalupensis* (Spreng.) Magnus) (2X).



Southern naiad is an important food for waterfowl and marsh birds and also provides shelter and food for fish. It can produce troublesome growth, and in the period of rapid agricultural development of southern Florida it caused problems in newly constructed irrigation and drainage canals. The need for control of southern naiad is completely overshadowed by the need for control of hydrilla which has replaced southern naiad communities where it had been introduced.

*Vallisneria* (Figure 2), commonly called eelgrass or tape grass, is also indigenous to Florida. It has no stem, but several simple leaves 15 to 22 mm wide arise from a rosette rooted to the hydrosoil. *Vallisneria* spreads vegetatively by stolons and rootstocks. The plants are dioecious, and pollination and "spiraling" of the peduncle after pollination show how well this species has adapted to the aquatic habitat (45,59).

Taxonomists have had difficulty in the classification of *Vallisneria* species. Fassett (26) briefly describes the history of the classification schemes that have been proposed. Recent Floras and the University of Florida Herbarium no longer recognize *Vallisneria americana* as a separate species but consider these plants to be the same as *Vallisneria neotropicalis* Marie-Vict. (48). The range of *Vallisneria neotropicalis*, now including the range formerly belonging to *Vallisneria americana*, includes most of eastern North America from Canada to Cuba.

*Vallisneria* is one of the most beneficial submersed aquatic plants for wildlife. Waterfowl and aquatic mammals eat nearly all parts of the plant, especially winter buds and rootstocks. The leaves harbor minute animals which fish prey upon, and they provide cover for spawning sport fish (59).

Figure 2. Leaves of vallisneria (*Vallisneria neotropicalis* Marie-Vict.).



Prior to the introduction of hydrilla, vallisneria was a serious problem in many lakes in central Florida. Presently, hydrilla also has replaced vallisneria in most of these lakes.

Hydrilla (Figure 3) is a new plant in the flora of North America and may soon become the most serious submersed aquatic weed problem in this country. Since its introduction some 15 years ago it has spread from Florida to Louisiana, Alabama, Georgia, Texas, and Iowa. Its spread through Florida is now nearly complete. It was found in Lake Seminole on the Florida-Georgia border in 1967 and in Lake George in north central Florida in 1968. Hydrilla was well established in Florida's largest lake, Lake Okeechobee, by 1972 and in Orange Lake by 1973. It is common in canals in southern Florida, including those of the Everglades. Major infestations around Orlando, in the Conway Lake chain, have caused severe control problems for several years. Reasons for its rapid movement from one area to another include its ability to survive prolonged periods of drying, production of vegetative propagules, and growth from fragmentation (56). Much of its spread is thought to be directly related to man's activities involving the movement of boats, motors, and trailers from one watershed to another.

Hydrilla flowers are unisexual, and each unilocular ovary produces two to three minute seeds. The plants can be either monoecious or dioecious. Leaves of hydrilla have serrated margins, are 6.0 to 15.0 mm long, and are found in whorls containing three to eight sessile leaves (32,48). Internodes may be over 20.0 cm long in deep water, but may be only a few millimeters long at the water's surface. Many aquatic plants produce various vegetative propagules, and hydrilla is no exception. Propagules are formed in the leaf axils of hydrilla stems

Figure 3. Node of hydrilla (*Hydrilla verticillata* (L.F.) Royle) showing several branches. (2X).



(turions) and also at the ends of subterranean rhizomes (tubers). These propagules, and possibly the underground roots and rhizomes, are the major source of the rapid reinfestation which occurs after control measures are applied (13).

Hydrilla, believed to originate in Malaysia, has spread throughout the tropics. It is causing serious problems in Lake Gatun and other regions of the Panama Canal (33,34). It is believed to have been introduced into Florida from Central America. Only the pistillate or female plant has become established in the United States. Monoecious (both sexes) and dioecious plants have been found in India and Indonesia, and seeds have been collected. Seeds brought back to the United States from these countries were found to be viable.<sup>2</sup>

When hydrilla was first found in Florida it was erroneously thought to be *Elodea canadensis* Michx. Several papers were published in the 1960's that reported results of research on *Elodea canadensis*, but it was soon recognized that the vegetative propagules and floral characteristics were unlike any known *Elodea* species. The plant was then thought to be a new *Elodea* species and was called Florida elodea. In 1965, specimens were sent to Harold St. John, a recognized authority on the *Hydrocharitaceae* family, and he properly identified the mystery plant (14).

#### Literature Review

The rapid movement of hydrilla throughout the State of Florida is recorded in taxonomic records in the University of Florida Herbarium. A true appreciation of the potential threat of hydrilla to Florida water

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2 Dr. George Allen, University of Florida, March 1974, Personal Communication.

resources can only be obtained by closely observing the domination of a waterway by this species. In the early 1960's, hydrilla was just becoming established in the canals of southern Florida. White (96) studied four of these canals extensively. The movement of hydrilla into two of the canals is reported by Blackburn *et al.* (15). In 1962, one of these canals had an 80% cover of southern naiad, but by 1974 it was completely covered by hydrilla.

In July 1972, an 0.08-ha earthen pond in Orange County, Florida, was divided into eight equal plots. Hydrilla, southern naiad, vallisneria, and *Chara* sp. were planted separately in each of two plots. One year later, over 90% of this pond contained hydrilla. Two years after planting, the pond was completely covered by hydrilla.<sup>3</sup>

The size of a waterway had little effect on the establishment of hydrilla. Rodman Reservoir is a large, shallow reservoir in north-central Florida. In August 1970, Hestand *et al.* (35) reported that hydrilla was just becoming established in the reservoir. In August 1971, hydrilla covered about 5 ha; however, hydrilla presently is the dominant species in the major part of the reservoir and is moving upstream, presumably with the aid of fishermen and motorboats. Presently, the area covered by hydrilla exceeds 200 ha.

Several studies have shown that, in most waters, light is a major limiting factor for the growth of submersed aquatic plants. Pearsall and Ulliyott (60) have emphasized that underwater light measurement and

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<sup>3</sup> Dr. David L. Sutton, Univ. of Fla., Agr. Res. Center, Ft. Lauderdale, Fla. January 1974. Personal Communication.

interpretation of results is difficult. Light intensity decreases with depth, but a complication arises because the absorption coefficient for radiant energy of a specific wavelength is not the same in different bodies of water, or even at different levels in the same body of water. For example, the differential penetration of light through distilled water is presented in Figure 4. This figure represents the maximum light penetration that can be expected at a given wavelength because transparency of most natural waters is substantially reduced by suspended and dissolved materials. Extensive studies of the physical and biological aspects of light penetration in lakes have been reported by Birge, Juday, and co-workers (7,8,9,10,43).

Pearsall and Hewitt (68) compared light penetration and vegetation limits in the lakes of Windermere in 1920 to those measured in 1933. The total radiation decreased during this period, and the lowest limit of rooted aquatic plants decreased from 6.5 m to a depth of 4.3 m. The percentage of full sunlight at the lower vegetation limits was 0.18 to 0.28 in 1920 and 0.11 to 0.18 in 1933. The names of the plants were not mentioned, but it was reported the blooms of Cyanophytes and Chrysophytes significantly reduced the penetration of light of wavelengths below 5000 Å. From related studies, Pearsall and Ullyott (69,70) reported a rapid extinction of blue light in Windermere, presumably due to the presence of phytoplankton. It was suggested that blue light of wavelengths less than 5000 Å may be a very important factor influencing organisms living in fresh water habitats. Their results indicated that blue light alone, or possibly the ratio of blue light to total light, limits growth of aquatic vegetation. This evidence was derived from data which showed that, in summer, phytoplankton

reduced blue light intensity at 4.3 m by more than 50% compared to periods when phytoplankton was less abundant. The coincidence of heavy plankton blooms with the period of potential maximum growth of submersed plants suggests that the abundance of plankton is one of several interacting factors which limit the growth of rooted aquatic plants.

The differential penetration of water by light resulted in the development of the "chromatic adaptation" theory proposed by Engelmann (25). This theory states that algae and other plants growing at different depths and in different waters will adapt their pigment composition to take advantage of differences in spectral composition. Thus, green algae predominate in shallow waters which allow red light penetration, red algae occur in deeper water where blue-green light predominates, and various forms of brown algae occur at intermediate depths. Dutton and Juday (24) reviewed chromatic adaption studies and presented four conclusions derived from the literature; (1) that chromatic adaptation results from differences in light quality; (2) that light quantity is the major factor controlling this phenomenon; (3) that both light quantity and quality are important in chromatic adaptation; and (4) that the medium, particularly the nitrogen content, exerts an influence on the pigment composition of algae. The differences of opinion concerning the cause of chromatic adaptation result from the many different kinds of plants used to study this response. There are the exceptions, however, and some plants show no chromatic adaptation at all.

Wallen and Green (89,90,91) studied the effect of light quality on several aspects of marine phytoplankton growth. They found that the diatom *Cyclotella nana* (Hustedt) and the green alga *Dunaliella tertiolecta* (Butcher) grew much better under blue light than under white or green

light. When these organisms were exposed to carbon-14 ( $^{14}\text{C}$ ) under the same light regimes, 60% of the absorbed  $^{14}\text{C}$  was in the ethanol-insoluble fraction of algae grown under blue or green light, whereas only 20% was in the same fraction when the plants were grown under white light. Both algae contained more chlorophyll a and less carotenoids when exposed to blue light. The chlorophyll a:b ratios of *Dunaliella* in green, white, and blue light were 2.08, 2.00, and 1.75 respectively. Also, the uptake of  $^{14}\text{C}$  as a function of water depth was studied in natural phytoplankton populations. The greatest amount of  $^{14}\text{C}$  activity at the water's surface was found in the ethanol-soluble fraction of phytoplankton, and in deeper water the greatest activity was found in the ethanol-insoluble fraction. Thus, the  $^{14}\text{C}$  in the ethanol-soluble fraction decreased with depth, and the amount in the ethanol-insoluble fraction increased with depth. Through an extensive series of laboratory studies, it was shown that these differences were attributable to light quality rather than light quantity.

The effect of light quality on the pigment content of vascular aquatic plants has not been studied as extensively as it has been with marine algae and phytoplankton. Dutton and Juday (24) attempted to correlate the presence of several pondweeds (*Potamogeton* spp.) with depth and color of plants. The pondweeds ranged in color from deep brown to bright green. They found no evidence of chromatic adaptation in either the pigment content or depth distribution of the plants. Further, they found no evidence of chromatic adaptation in vallisneria and slender naiad (*Najas flexilis* Willd.). The pigment responsible for the color changes among the pondweeds was thought to be rhodoxanthin. Although their studies were negative with respect to chromatic

adaptation, Dutton and Juday indicated that such might exist in these plants under different conditions. The lake they studied was exceptionally clear, having a value of only six on the platinum-cobalt scale. They also emphasized that chromatic adaptation would be especially important to new growth in early spring when aquatic plants are growing under a maximum depth of water.

Most light studies conducted on aquatic plants have been concerned with determining their light compensation points. The purpose of these studies was to elucidate the light limits for aquatic plant growth. Blackburn *et al.* (12) grew Brazilian elodea (*Egeria densa* planch.) and waterstargrass (*Heteranthera dubia* (Jacq.) MacM.) under lights of different spectral composition and at different intensities of white light. Brazilian elodea produced the most growth under daylight fluorescent bulbs at an illuminance of 108 lux. White light above 1345 lux resulted in the deterioration of Brazilian elodea due to solarization. Optimum growth of waterstargrass occurred at illuminances at or above 6350 lux. Most plants of both species died when grown for 12 weeks under blue light. Brazilian elodea and waterstargrass grew well under red light, but waterstargrass grew best under green light. Wilkinson (97,98,99) studied the effect of light on growth of coontail (*Ceratophyllum demersum* L.) as well as waterstargrass. The maximum growth of these species resulted when exposed to full sunlight (approx. 108 klux) and during the longest days of the year (June 10 to July 8). The optimum temperature for growth was 25 to 30 C. The light compensation point for both species was 2.0 to 3.0% of the illuminance of sunlight, but under red light was only 1.7% and 1.0 to 3.8% of the illuminance

of full sunlight for waterstargrass and coontail, respectively. The lower compensation point under red light supports the contention that spectral selectivity is a very important factor in aquatic plant growth. Among aquatic plants and various algae there are different responses to different light regimes. This fact does not necessarily support the chromatic adaptation theory, but does indicate that certain light regimes will favor the growth of one organism over another.

Several field studies on light compensation points and the growth limit light imposes on aquatic plants have been reported (42,44,54,55, 76,77,96). The wide variation observed in these studies reflects differences in water quality, time of year, plant species, and technique. The overall impression seems to be that aquatic plants have a wide range of adaptability under low light intensities.

The uptake of carbon by a few aquatic plants and many species of algae has been reported, but several important questions are yet unanswered. The direct uptake and subsequent utilization of bicarbonate ( $\text{HCO}_3^-$ ) in the photosynthesis of aquatic flora is an area of continuing controversy. The relative proportions of the various ionic forms of carbon in equilibrium in natural waters is a function of pH (Figure 5). It has been shown that aquatic plants prefer and readily utilize free carbon dioxide ( $\text{CO}_2$ ) in photosynthesis. The utilization of  $\text{HCO}_3^-$  in photosynthesis was suggested by Ruttner (75) and Arens (4) on the basis of the formation of carbonate ( $\text{CO}_3^{=}$ ) deposits on the leaves of some aquatic plants. These deposits were only found on the upper sides of the leaves. It was theorized that some aquatic plants absorb  $\text{HCO}_3^-$  resulting in an ion exchange which precipitates  $\text{CaCO}_3$ .

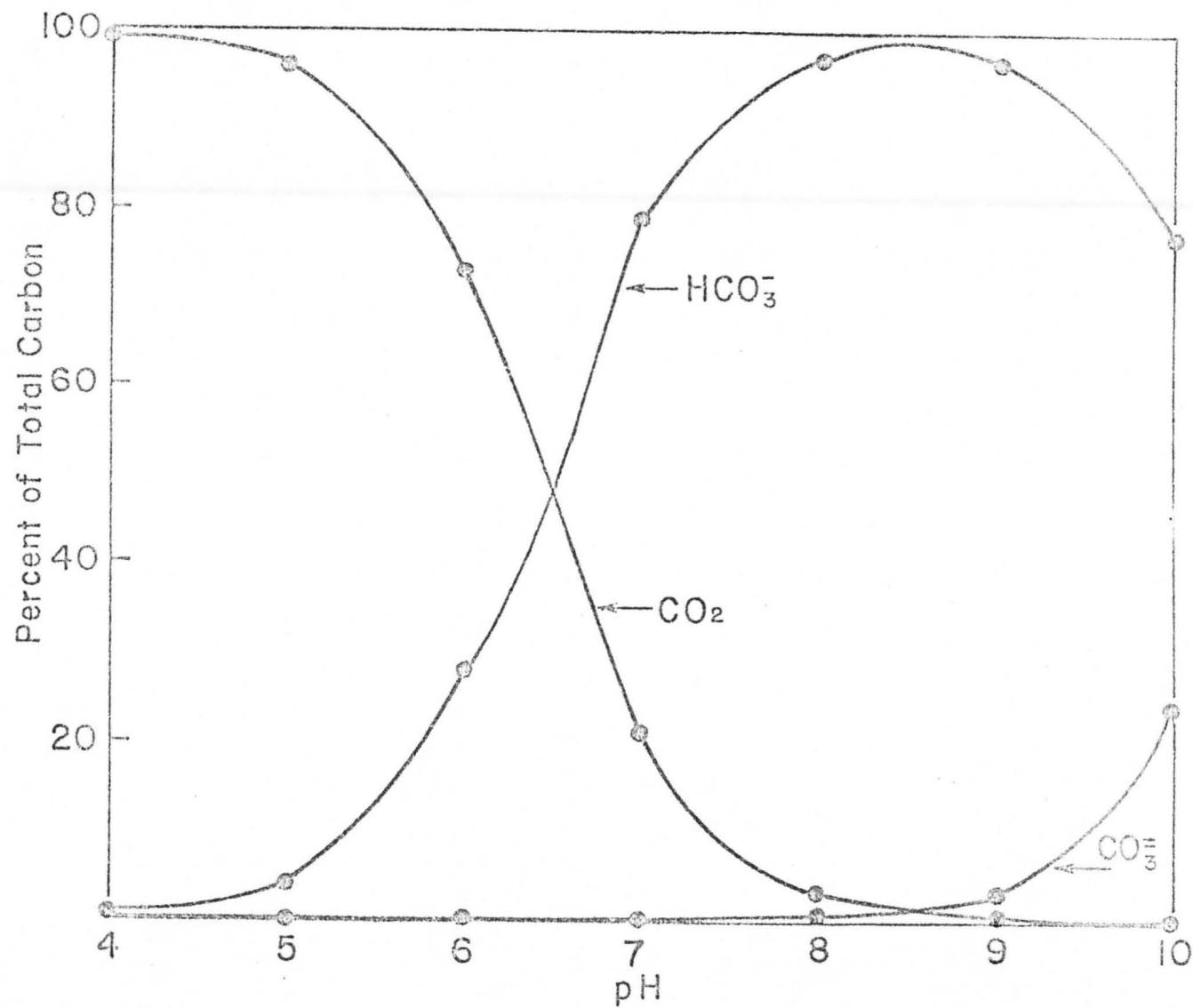


Figure 5. Proportions of  $\text{CO}_2$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$  in water at various pH values (39).

Steemann Nielsen (83) reported that broadleaf milfoil (*Myriophyllum heterophyllum* Michx.) was unable to utilize  $\text{HCO}_3^-$  but that the aquatic moss *Fantinalis* sp. utilized it effectively. By making two unfounded assumptions, Hood and Park (37) showed that *Chlorella pyrenoidosa* utilized  $\text{HCO}_3^-$  in photosynthesis. They assumed that the equilibrium formed between free  $\text{CO}_2$  and added  $\text{CO}_3^{=}$  and  $\text{HCO}_3^-$  in solution was a slow process. When radioactive  $^{14}\text{HCO}_3^-$  was added to a carbon-free system and  $^{14}\text{C}$  was taken up by *Chlorella* instantaneously, they concluded that *Chlorella* utilized  $\text{HCO}_3^-$ . A second method used to discriminate between  $\text{CO}_2$  and  $\text{HCO}_3^-$  uptake involved purging the culture solutions with  $\text{N}_2$  to remove  $^{14}\text{CO}_2$  faster than it could be formed by dehydration of  $\text{H}_2^{14}\text{CO}_3$ . Watt and Paasche (93) and Steemann Nielsen (84) sharply criticized the procedures, assumptions, and conclusions reached by Hood and Park. Watt and Paasche first showed that equilibrium between  $\text{CO}_3^=$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_2$  can be achieved in a very short period of time (80 sec at 25°C, pH 8.0). The production of  $^{14}\text{CO}_2$  from added  $^{14}\text{CO}_3^=$  or  $\text{H}^{14}\text{CO}_3^-$  is so fast that it invalidates the theory of instantaneous uptake of  $\text{H}^{14}\text{CO}_3^-$ . Watt and Paasche demonstrated that the free  $\text{CO}_2$  content of a solution cannot be lowered by air purging without lowering the  $\text{HCO}_3^-$  fraction in a similar proportion.

Osterlind (61,62,63,64,65,66) conducted a series of experiments on the  $\text{CO}_2$  and  $\text{HCO}_3^-$  uptake by the algae *Scenedesmus quadricauda* and *Chlorella pyrenoidosa*. He compared growth rates of these two algae in solutions of varying pH. He discovered the *Scenedesmus* had an optimum growth rate at 6.5, and that growth decreased to about half the optimum rate at pH 9.0. Because the  $\text{CO}_2$  content of the culture solution at pH 9.0 was much less than 1% of that of the solutions at pH 6.5, he concluded that *Scenedesmus* was utilizing  $\text{HCO}_3^-$ . Growth of *Chlorella* was

negligible at pH 9.0 and was apparently capable of utilizing only the available  $\text{CO}_2$ .

Carbon dioxide is available in very low concentrations at high pH's. The utilization of  $\text{CO}_2$  by plants at high pH's is thought by some to be much faster than  $\text{CO}_2$  can be supplied by the equilibrium established with  $\text{HCO}_3^-$ . Carbonic anhydrase is believed to catalyze the degradation of  $\text{HCO}_3^-$  into  $\text{CO}_2$  and hydroxide ( $\text{OH}^-$ ) ions. When the carbonic anhydrase content of *Scenedesmus* and *Chlorella* was assayed by Osterlind, no differences in content of the enzyme were found. Likewise, Steemann Nielson and Kristiansen (85) found no difference in the carbonic anhydrase content of *Elodea canadensis* (Michx.) and *Fontinalis*, an aquatic moss shown to utilize  $\text{HCO}_3^-$ . Osterlind (62) suggested that the ability of certain plants to utilize  $\text{HCO}_3^-$  may be due to differences in their protoplasmic membranes.

Further studies of Osterlind showed that 5-day old cultures of *Scenedesmus* readily assimilated  $\text{HCO}_3^-$ , but 10-day old cultures had nearly lost that capability. *Chlorella* cultures of any age were unable to utilize  $\text{HCO}_3^-$ . Both algae utilized  $\text{CO}_2$  in direct proportion to the  $\text{CO}_2$  concentration between 10 and 100  $\mu\text{M}$ . It was also discovered that "photoactivation" was necessary for *Scenedesmus* to maximize  $\text{HCO}_3^-$  uptake. *Scenedesmus* cultures were grown in light and at high pH for several hours. When growth of these cultures was measured, it was found to be considerably greater than cultures freshly placed at high pH, or cultures grown at high pH in darkness. Further experimentation showed that pH values of 3.0 to 9.0 had no effect on photosynthesis other than affecting the ratio of  $\text{CO}_2:\text{HCO}_3^-$ . Cyanide was found to be more inhibitory to photosynthesis when the  $\text{CO}_2$  content of the culture

medium was low. Osterlind (66) called cyanide a competitive inhibitor because greater inhibition occurred at high pH's where the  $\text{CO}_2$  content was very low.

Blinks (17) measured the effect of pH on photosynthesis for more than 20 species of marine algae. He found that seven species could maintain photosynthetic activity up to pH 10.0, but only one species required a photoactivation period. He suggested that  $\text{CO}_3^-$  deposition on leaves must result from a secondary factor such as some characteristic of the cell membrane. This was suggested because he discovered several algae that apparently utilize  $\text{HCO}_3^-$ , but fail to form  $\text{CO}_3^=$  deposits.

Shiyan and Merezko (79) studied the uptake of radiocarbon by *Potamogeton perfoliatus* L. and coontail in phosphate buffers of pH's 5.8 to 9.1. They found that a 30-minute exposure period was necessary to obtain significant results. Carbon uptake by both species was highest at lower pH's and was reduced 5 to 10 fold at pH 9.1. Carbon metabolism appeared to be altered by high pH. At pH 9.1, a higher percentage of absorbed  $^{14}\text{C}$  was found in more complex carbon molecules (organic acids, amino acids, proteins, lipids, starch and cellulose) than at pH 6.2 where most activity was localized in simple carbohydrates.

Paasche (67) made an extensive study of the uptake of  $^{14}\text{C}$  by one species of cocolithophorid. These are free-floating marine plants which form a delicate shell of  $\text{CO}_3^=$  through the reaction  $2\text{HCO}_3^- = \text{CO}_2 + \text{CO}_3^= + \text{H}_2\text{O}$ . He found that tris(hydroxymethyl)aminomethane ("Tris") buffer had no inhibitory effect on carbon uptake at pH's of 6.0 to 9.1. Sea water has a usual pH of 8.0 to 8.3; however, maximum carbon uptake occurred at pH 7.5. At pH 9.1, carbon uptake decreased to about 30% of maximum.

The question of  $\text{CO}_2$  limitation to growth at high pH's and the utilization of  $\text{HCO}_3^-$  is still a matter of controversy and produces theories and counter-theories. Recently, Shapiro (78) theorized that blue-green algae dominate eutrophic waters of high pH because these algae are more effective in utilizing  $\text{CO}_2$  than other algal groups. Goldman (30) refuted Shapiro's contention on the basis that the total carbon in equilibrium ( $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{=}$ ) is a massive reservoir for free  $\text{CO}_2$ . He also pointed out that pH changes affect the availability of various nutrients and may possibly affect enzymes which transport them.

Interest in  $\text{CO}_2$  and  $\text{HCO}_3^-$  uptake by plants is not limited to aquatic flora. In terrestrial plants,  $\text{CO}_2$  is absorbed from the atmosphere, but it is ultimately taken up by plant cells from aqueous solution. Raven (72,73) reviewed the significant literature on  $\text{HCO}_3^-$  utilization in plants and suggested possible metabolic sequences involved at the cellular level. He believes that  $\text{HCO}_3^-$  enters the cell by active transport, is dehydrated to  $\text{CO}_2$  by the enzyme carbonic anhydrase, and is fixed by carboxydismutase. Raven indicated that several problems of  $\text{HCO}_3^-$  utilization have yet to be resolved. The diffusion of  $\text{HCO}_3^-$  through plant membranes is a slow process, and no evidence of active transport has been found. In addition, carbonic anhydrase is present in plants, but there is no evidence that plants capable of utilizing  $\text{HCO}_3^-$  have a higher carbonic anhydrase content than plants which apparently cannot utilize  $\text{HCO}_3^-$ . All plants are capable of fixing  $\text{CO}_2$ , and it diffuses rapidly across plant membranes. The flux of  $\text{CO}_2$  formed from the dehydration of  $\text{HCO}_3^-$  inside a cell would be away from the plant toward the culture solution of high pH which has a high affinity for  $\text{CO}_2$ . This is a potential short circuit, analogous to the  $\text{CO}_2$  leakage that short circuits the " $\text{CO}_2$  pump" in four-carbon ( $\text{C}_4$ )

plants. The fixation of  $\text{CO}_2$  from  $\text{HCO}_3^-$  would be greatest if the dehydration of  $\text{HCO}_3^-$  occurred near or in the chloroplast. Evidence supporting this spatial arrangement is the localization of carbonic anhydrase in the chloroplasts of typical pentose phosphate ( $\text{C}_3$ ) plants. Localization of  $\text{HCO}_3^-$  dehydration near the chloroplast places released  $\text{CO}_2$  close to the carboxylative enzyme but increases the long diffusion path of  $\text{HCO}_3^-$ . Raven, using information from scant and conflicting literature, formulated a likely explanation for  $\text{HCO}_3^-$  utilization. His conclusions were in agreement with the idea of  $\text{HCO}_3^-$  utilization in single cell algae where diffusion paths to chloroplasts are minimal compared to vascular plants. The lack of significant cuticular material and the presence of chloroplasts in the epidermis also would facilitate  $\text{HCO}_3^-$  utilization in submersed aquatics.

Meyer (53) determined the daily cycle of photosynthesis in coontail by measuring changes in oxygen content of the surrounding water. There was a rapid rise in photosynthesis during the morning hours with maximum rates occurring between 10 a.m. and noon. Photosynthesis rate was closely correlated with solar intensity. The skewing of photosynthesis curves toward the morning hours was attributed to internal factors which exert an influence on the daily course of photosynthesis.

The internal factors described by Meyer probably included the accumulation of  $\text{CO}_2$  in the aerenchyma of coontail. Laing (47) and others (6) showed that on sunny days a negative  $\text{O}_2$  gradient and a positive gradient of  $\text{CO}_2$  occurred between the leaves and roots of aquatic plants. Concentration gradients between roots and leaves were much less pronounced in the early morning. This indicated that the  $\text{O}_2$  content in the aerenchyma had dropped to a uniform low level at night, and  $\text{CO}_2$  had increased to

about equal concentrations in the roots and leaves. Hartman and Brown (31) measured the  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$ , and  $\text{CH}_4$  content of aerenchyma in elodea (*Elodea canadensis* Michx.) and coontail throughout a daily cycle of photosynthesis. Their work showed that research on determining light compensation points in submersed aquatic plants by measuring photosynthesis via  $\text{O}_2$  evolution may be erroneous. It was shown that, at low light intensities, no increase in the dissolved  $\text{O}_2$  content of surrounding water could be detected, although the internal  $\text{O}_2$  concentrations had increased.

Carbon dioxide is important in photosynthesis, but in addition acts as a growth regulator. Several aquatic plants produce one type of leaf under water and an entirely different type leaf above water (heterophyly). Bristow (18) and Bristow and Looi (19) showed that the amphibious leaves of several aquatic plants developed many of the characteristics of submersed leaves when exposed to a stream of air containing 5%  $\text{CO}_2$ . They concluded that concentrations of free  $\text{CO}_2$  higher than those in air may be essential for the normal growth and development of submersed amphibious plants. Dale (23) showed that the  $\text{CO}_2$  content of water surrounding the roots of Brasilian elodea has a pronounced effect on root production. Additional  $\text{CO}_2$  stimulates root production, but the effect of lowered pH as a result of added  $\text{CO}_2$  was not determined.

There has been much conjecture but little research on other sources of photosynthetic carbon besides inorganic carbon. Natural waters contain an abundance of organic complexes which may provide carbon to photosynthesizing organisms. Smith *et al.* (80) showed that marine planktonic algae utilized the carbamino complex of alanine in preference to inorganic forms of  $\text{CO}_2$ . Steemann Nielson (84) criticized the results of Smith *et al.* and discounted the possibility that alanine

is preferred to CO<sub>2</sub> for use in photosynthesis.

Literature relating to physiological research of vascular aquatic plants is widely scattered and covers a wide range of topics. Fortunately, a broad base of aquatic research has been reported for various algae; however, it is difficult to make conclusions concerning aquatic plants based on algal research.

In recent years, a few studies have been completed on plants very closely related to those in this report. Stanley (81) and Stanley and Naylor (82) have made an extensive study of photosynthesis and carbon metabolism in eurasian watermilfoil (*Myriophyllum spicatum* L.). The CO<sub>2</sub> compensation point of eurasian watermilfoil was near zero. As water temperature was increased from 10 to 35 C, photosynthesis also increased and was maximum at 35 C. The initial photosynthetic products were glycerate-3-P and glycolic acid, indicating this plant to be a typical C<sub>3</sub> plant. Assimilate accumulation reduced photosynthesis rates. In addition, 0.5 M "Tris" buffer inhibited carbon uptake by about 50%.

Hough and Wetzel (38) developed an assay for photorespiration in aquatic plants. They allowed axenic cultures of slender naiad to absorb <sup>14</sup>C for 30 minutes, then measured the release of <sup>14</sup>C from these plants in light and dark vessels. A flow of water through the vessels was maintained, and aliquots were taken at regular intervals for determination of <sup>14</sup>C activity. They obtained little evidence of photorespiration in slender naiad, but they felt that they could induce photorespiration by high oxygen tensions (25 mg O<sub>2</sub> l<sup>-1</sup>). In none of their experiments was photorespiration significantly greater than dark respiration. Some of the problems encountered in this particular study may have been laminar water flow near the plants, or refixation of photorespired CO<sub>2</sub>. It is

possible that photorespired  $\text{CO}_2$  never leaves the plant but is moved in and out of a gas pool in the aerenchyma system. The low carbon compensation points of aquatic plants favors either no photorespiration at all, or recycling of photorespired  $\text{CO}_2$  entirely within the plant.

Ikusima (40) found the maximum rate of photosynthesis at light saturation in hydrilla to be  $38 \text{ mg O}_2 \text{ g dry wt}^{-1} \text{ hr}^{-1}$ . In general, the photosynthetic activity of submersed macrophytes studied by Ikusima was from only 10 to 50% of that of land plants on a leaf area basis. Two types of profile structures were found in vallisneria and pondweed communities. The profile of the linear-leaved vallisneria correlated closely with typical grass communities. Generally, aquatic plants correspond to herb-type communities by producing more biomass at the top of the plant. The concentration of pondweed leaves at the surface is exaggerated by the floating habit of the shoot tips. Ikusima (42) made an intensive study of a *Vallisneria denserrulata* community in a ditch 1 m deep. The biomass ranged from a low of  $82 \text{ g dry wt m}^{-2}$  in May, to a maximum of  $212 \text{ g dry wt m}^{-2}$  in August. In the study period from May to October, the chlorophyll content of vallisneria ranged from  $0.51 \text{ g m}^{-2}$  to  $1.7 \text{ g m}^{-2}$ , and the leaf area index (LAI) varied between 4.8 to 9.3. Photosynthesis of vallisneria was calculated on a leaf area basis and at light saturation was  $2.1$  to  $3.6 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ . The photosynthetic activity of vallisneria seldom reached the potential rate at light saturation because of light extinction in the water. The rate of photosynthesis in the upper stratum was greatest; however, respiration seemed equal throughout the depth of the community.

A fundamental problem in aquatic research has been the lack of accurate, standardized methods. Biomass determination is an important parameter, but is very difficult to obtain accurately. Fosberg (27) discussed the limitation of studies on sub-aquatic vegetation imposed by the lack of an accurate sampling device. He reported on the various types of sampling techniques and described an apparatus he designed for sampling aquatic standing crop. He found the standing crop of coontail in two lakes was 280 and 360 g dry wt  $m^{-2}$ . The water depths at the sampling sites were 2 m and 3 m respectively. By successive sampling throughout the summer growing period, he obtained growth rates for coontail of 2.8 g dry wt  $m^{-2} day^{-1}$ , and for the alga *Nitella* sp., 2.5 g dry wt  $m^{-2} day^{-1}$ .

Polisini and Boyd (71) determined the standing crop and nutritive value of several aquatic plants; however, they did not determine the standing crops of the submersed species because of the difficulty in obtaining accurate samples. It was estimated that the standing crops of the submersed species studied were less than 0.5 kg dry wt  $m^{-2}$ .

Westlake (95) reviewed the literature on plant productivity of several different terrestrial, aquatic, and marine communities. He concluded that the littoral zones of rivers and lakes containing emergent vegetation were among the most productive systems. In contrast, submersed communities were among the least productive. The maximum biomass reported for several submersed communities was less than 1.0 kg dry wt  $m^{-2}$ . In addition, Westlake (95) reported that the terminology used by investigators was often conflicting and difficult to interpret on a comparative basis. The term biomass

has been used to describe the mass of plants not including the roots, as well as the weight of all the plant parts. The term standing crop should be used for the former example, with the term biomass correctly used in the latter case. Further, Westlake suggested a consistent use of standard terminology and techniques that will improve future research and produce more comparable data.

## CHAPTER II

### CHLOROPHYLL, CARBOHYDRATE, AND MORPHOLOGICAL CHARACTERISTICS OF NATURAL PLANT POPULATIONS

#### Introduction

Comparative studies of aquatic plants in their natural habitat are difficult because of variability in the physical and chemical nature of natural waters. This section presents a survey of the chlorophyll content of plants from three different habitats. Further, the carbohydrates and selected morphological characteristics of two species at a single site were studied in detail.

#### Methods and Materials

##### Chlorophyll Studies

Plants were collected from plastic growth pools at Ft. Lauderdale in April 1972. The pools were planted 18 to 24 months previously and had not been recently fertilized. The apical 20 cm of each species were harvested, washed in tap water, and the chlorophyll content was determined on 4-cm sections taken from the midpoint of the harvested plant piece. The method of analysis was that of Arnon (5). Plants from canals near Ft. Lauderdale were collected also in April 1972 and analyzed for chlorophyll content. The method of selecting tissue was the same as for the pools, except that sections of southern naiad and hydrilla were separated into leaf and stem fractions. Additional samples were taken to determine dry weight.

The chlorophyll content of hydrilla and vallisneria at different water depths was determined using plants collected from adjoining earthen

ponds in Orange County, Florida. Plants were pulled from the hydrosoil and 5-cm sections from depths of 0.0, 0.5, 1.0, and 1.5 m were placed in jars containing pond water. The jars were placed in ice in a closed ice chest. The plants were harvested in late afternoon on September 20, 1973, and analyzed the following morning.

Chlorophyll content of hydrilla as a function of iron content of the culture medium also was investigated. Plants were grown 14 days in 0.1-strength Hoagland's solution (36) under controlled environment conditions as described by Sutton *et al.* (88). The iron concentrations of nutrient solutions were adjusted to 0, 2, 4, 8, 16, 64, and 128 ppmw ( $\text{mg l}^{-1}$ ). Iron in the form of Sequestrene 330 was added to the 3.8-l culture jars.<sup>4</sup> Two 6-cm apical sections of hydrilla (150 mg dry wt) were planted in 5  $\text{cm}^2$  plastic pots. Each jar contained four pots. At the end of 14 days, the plants were removed from the culture jars and rinsed in tap water. Representative plant sections (about 40 mg fresh wt) from each pot were weighed and their chlorophyll content determined. The remaining plant material was dried and ground in a Wiley mill through a 40-mesh screen. The iron content of the tissue was then determined by dry ashing at 550 C. The ash was dissolved in 5 ml of 5N HCl and filtered through Watman No. 2 filter paper. The filter paper was rinsed with an additional 5 ml 5N HCl and then with 90 ml distilled water. The iron content of the filtrate was determined by atomic absorption spectrometry. The four pots in each treatment solution were considered replications for statistical comparison.

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<sup>4</sup> Sequestrene 330 is a trade name for sodium ferric diethylenetriamine pentaacetate, which contains 14.2% Fe as  $\text{Fe}_2\text{O}_3$ .

### Morphology Studies

The production of roots relative to top growth was determined experimentally for hydrilla, southern naiad, and vallisneria. Plants (1-g fresh wt) were planted in 10 cm of acid-washed quartz sand in 3.8-l jars containing 0.1 strength Hoagland's solution supplemented with 50 ppm NaHCO<sub>3</sub>. After 6 weeks, the nutrient solution was decanted and replaced. The jars were maintained under a greenhouse bench for a total of 12 weeks, after which they were separated into root and shoots and dry weights determined.

The accurate determination of leaf area index (LAI) and biomass for submersed aquatic plants is almost impossible unless the area under study can be drained. Earthen ponds (0.8 ha) in Orange County were monitored over a 2-yr period and provided for LAI and biomass determinations on hydrilla and vallisneria. The ponds were planted in July 1971, and were drained in January 1974. During this period, hydrilla and vallisneria had become well established. The LAI of vallisneria was determined by harvesting four complete plants and placing the leaves on blueprint paper which then was exposed to ultraviolet light. The dry weight of the vallisneria leaves was also determined. By determining the dry weight of a known area of paper, it was possible to determine the area of leaf cut-outs. The ratio of area to dry weight of traced plants multiplied by the dry standing crop weight would result in total leaf area. The leaf area of terrestrial plants is expressed in terms of one surface only and this rule was applied to these plants also.

The LAI of hydrilla was determined on the basis of only two plants because of the difficulty in working with many small leaves. Further, it was necessary to harvest whole plants because leaf size and internode

length increase with depth. All of the leaves were removed from two plants and their branches. The leaves of each plant were mixed and a sub-sample consisting of about 200 leaves was taken. These leaves were placed on blueprint paper, covered with glass to hold them flat, and exposed to ultraviolet light. The impressions were cut out with a small scalpel and weighed. The traced leaves were dried and weighed, as was done with *vallisneria*, to determine leaf weight to area ratio. The remaining leaves and stems were dried and weighed to determine leaf area to biomass ratio.

After the ponds (1.5 m deep) were drained, eight replicate samples of each species were taken at random for biomass determinations. The sampler was a 1-m<sup>2</sup> frame covered with window screen and equipped with a door in the center of the frame. After the frame was pressed down on the plants, the door was opened and a 902-cm<sup>2</sup> sample was easily cut away with a long knife. After the top growth was harvested, the hydrosoil was cut out to a depth of about 15 cm to obtain the roots. The plants and roots were then rinsed and dry weight determined.

Several plants of *vallisneria* and *hydrilla* were harvested from the Orange County ponds and cut into 10-cm portions from the hydrosoil to the water surface. The plant portions were dried and weighed to determine the profile of the standing crop. The data are expressed on a percent of the total plant weight at each 10-cm interval.

#### Carbohydrate Studies

The effect of depth on carbohydrate composition of *vallisneria* and *hydrilla* was determined in September 1973, and January 1974, in plants from the Orange County ponds. The sampling was conducted in a manner similar to that used for chlorophyll analysis; however, the samples were

immediately frozen and maintained as such until they could be analyzed. The carbohydrate content of southern naiad was determined for only January 1974. Southern naiad was collected from water 1.5 m deep in Rodman Reservoir. The determination of the carbohydrate components is described by Carter *et al* (21).

The frozen plants were thawed and approximately 0.5 g was extracted in boiling 80% ethanol. Extractions were repeated until the tissue was cleared of most pigmentation. The tissue was dried, weighed, and homogenized in 0.1 M acetate buffer, pH 4.8. An aliquot of the homogenate was then digested with amyloglucosidase at 55 C to convert starch to glucose. Glucose content was then determined colorimetrically by the glucose oxidase method.<sup>5</sup>

The combined ethanol fractions (containing reducing sugars and sucrose) were heated to remove the alcohol. The remaining aqueous solution was brought to volume with distilled water. The Nelson-Somogyi method then was used to determine the reducing sugar and sucrose content of the extract (60,86,87). Differentiation between reducing sugar and sucrose content was accomplished by hydrolyzing one set of duplicate samples with invertase. The respective sugar contents were determined colorimetrically then by comparison to standard curves.

#### Results and Discussion

##### Chlorophyll Studies

The chlorophyll content of hydrilla, southern naiad, and vallisneria grown in plastic pools is presented in Table 1. The total

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5 Glucostat, Worthington Biochemical Corp., Freehold, N.J.

Table 1. Chlorophyll content of hydrilla, southern naiad, and vallisneria grown in plastic pools.

Species	Chlorophyll content (mg g fr wt <sup>-1</sup> ) <sup>a/</sup>			a/b ratio
	a	b	c	
Hydrilla	0.5730 b	0.3181 b	0.8911 b	1.80 a
Southern Naiad	0.4431 a	0.2239 a	0.6670 a	1.69 a
Vallisneria	0.5965 b	0.3522 c	0.9487 c	1.98 b

<sup>a/</sup> Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of four replications.

chlorophyll content of all three species was less than  $1.0 \text{ mg g fr wt}^{-1}$ . *Vallisneria* had the greatest amount of chlorophyll, and southern naiad contained the least. *Vallisneria* also had a higher chlorophyll a to chlorophyll b ratio (a:b ratio) than either hydrilla or southern naiad; however, this ratio for all species was below 2.0.

Hydrilla and southern naiad grown in plastic pools had considerably lower chlorophyll contents and a:b ratios than plants growing under more natural conditions (Table 2). The chlorophyll content of *vallisneria* plants did not seem to be adversely affected by long term growth in plastic pools. It was recognized long ago that "healthy" aquatic plants should be used for research purposes (16). Thus, it is important that plants grown in plastic pools be properly maintained to obtain useful research results.

The chlorophyll contents of hydrilla and southern naiad leaves and stems are also presented in Table 2. The chlorophyll contents of the leaves of these two species were much higher than the chlorophyll contents of the stems. In addition, the chlorophyll components (a and b) were different in these plant parts. Hydrilla stems contained a greater proportion of chlorophyll b than hydrilla leaves. The a:b ratios for hydrilla stems and leaves were 1.57 and 2.23, respectively. The chlorophyll components of the leaves and stems of southern naiad were reversed. The a:b ratios of southern naiad leaves and stems were 1.86 and 2.18, respectively. The percent dry weights of these submersed species indicated that 90 to 92% of their fresh weight was water.

The importance of the different chlorophyll composition of hydrilla and southern naiad leaves and stems is unknown. However, chlorophyll analyses of hydrilla tissue taken from water depths up to 1.5 m indicate

Table 2. Chlorophyll content of hydrilla, southern naiad, and vallisneria collected from the surface of drainage canals in southern Florida.

Species	Dry wt %	Chlorophyll content (mg g fr wt <sup>-1</sup> ) <sup>a/</sup>			a/b ratio
		a	b	total	
<b>Hydrilla</b>					
whole plant	9.67	1.1847 c	0.5532 c	1.7379 c	2.14 c
stem	7.41	0.2373 a	0.1514 a	0.3887 a	1.57 a
leaves	12.26	1.8685 e	0.8351 d	2.7036 e	2.23 c
<b>Southern Naiad</b>					
whole plant	7.94	1.0655 c	0.5171 c	1.5826 c	2.06 bc
stem	6.11	0.3703 a	0.1701 a	0.5404 a	2.18 c
leaves	9.71	1.4996 d	0.8063 d	2.3059 d	1.86 ab
<b>Vallisneria</b>					
whole plant	8.29	0.6728 b	0.3292 b	1.0020 b	2.04 bc

<sup>a/</sup> Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Tests. Each value is the mean of four replications.

that the chlorophyll composition of this species may be an important photosynthetic factor.

The chlorophyll composition of hydrilla and vallisneria plants grown in water 1.5 m deep is compared in Table 3. Plant samples for chlorophyll analysis were taken from water depths of 0.0 (surface), 0.5, 1.0, and 1.5 m. The total chlorophyll content of hydrilla decreased 50% from the surface to a depth of 0.5 m. The total chlorophyll content of vallisneria decreased by only 12% over the same depth. In general, the chlorophyll content of both species decreased with increased depth. The important difference between the two species was the difference in their a:b ratios. In vallisneria the ratios remained constant at all water depths sampled. However, the a:b ratio of hydrilla decreased from 1.77 at the water surface to only 1.15 at 1.5 m. Both the chlorophyll a and chlorophyll b content decrease with increasing depth in hydrilla, but the chlorophyll a content decreases faster than the chlorophyll b content. The differential change in chlorophyll composition found in hydrilla suggests a possible "chromatic adaptation".

Hydrilla stems have a low a:b ratio in comparison to hydrilla leaves (Table 2). Hydrilla sampled for chlorophyll analysis had longer internodes at greater depths. Thus, the low a:b ratio at 1.5 m may have resulted in part by the greater proportion of stems at this depth. However, stems from the surface of canals had an a:b ratio of 1.57 (Table 2). Therefore, the low a:b ratio (1.15) of hydrilla sampled at a depth of 1.5 m does not seem to reflect a simple change in the proportion of stems in the samples.

Iron has been suggested as one of the limiting nutrients of hydrilla growth in Florida's natural waters (74). Growth and total chlorophyll

Table 3. Chlorophyll content of hydrilla and vallisneria as a function of water depth.

Species	Depth (m)	<u>Chlorophyll content (mg g fr wt<sup>-1</sup>)<sup>a/</sup></u>			a/b ratio
		a	b	total	
Hydrilla	0.0	0.7835 d	0.4420 c	1.2252 d	1.77 cd
	0.5	0.3787 c	0.2408 b	0.6194 c	1.57 b
	1.0	0.2887 b	0.1784 a	0.4667 b	1.54 b
	1.5	0.1736 a	0.1508 a	0.3253 a	1.15 a
Vallisneria	0.0	0.5296 c	0.3012 c	0.8270 c	1.96 cd
	0.5	0.5011 c	0.2392 b	0.7402 bc	2.09 d
	1.0	0.4062 b	0.2262 b	0.6323 b	1.94 cd
	1.5	0.0842 a	0.0411 a	0.1227 a	2.00 d

a/ Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of four replications.

content of hydrilla were studied in plants grown in water with varying iron concentrations (Table 4). No increase in hydrilla growth was evident after 2 weeks growth in the treatment solutions. The total chlorophyll content increased two-fold as the iron concentrations of the treatment solutions increased from 0 to 16 mg Fe  $l^{-1}$ . The iron content of hydrilla tissue was maximum in solutions of 16 mg Fe  $l^{-1}$  and then gradually decreased in solutions of higher iron concentration. Iron concentrations above 16 mg Fe  $l^{-1}$  inhibited hydrilla growth.

No significant differences were found in hydrilla growth or chlorophyll content between 0 and 2 mg  $l^{-1}$  treatments. However, the data suggest that a reduction in variability by a longer growth period or an increase in the number of replicates would result in significant differences between the two lowest treatment solutions.

The importance of higher proportions of chlorophyll b in hydrilla has not been studied. The absorption spectra of chlorophyll b is maximal at light wavelengths of  $4200\text{ }^{\circ}\text{A}$  to  $4800\text{ }^{\circ}\text{A}$  (100). Hydrilla could be efficiently utilizing these wavelengths if light penetration in Florida waters is similar to the light penetration in distilled water (Figure 4). In light limiting systems, the pigment changes in hydrilla could have a pronounced effect on production.

The a:b ratios of the aquatic plants studied were lower than corresponding values for terrestrial plants. Black (11) categorizes the a:b ratio of  $C_3$  plants between 2.4 and 3.2, and  $C_4$  plants between 3.3 and 4.5. The range of a:b ratios found for the submersed plants in this study was 1.15 to 2.23. This also indicates that chlorophyll b is an important pigment in aquatic plant production. Several other pigments as carotenoids and xanthins and light penetration in Florida waters should be studied to ascertain the importance of chromatic

Table 4. Growth, iron content, and total chlorophyll content of hydrilla grown for 2 weeks in solutions of various iron concentrations.

Treatment Concn. ppm	Growth <sup>a/</sup> mg dry wt	Iron content <sup>a/</sup> mg g dry wt <sup>-1</sup>	Total chlorophyll <sup>a/</sup> mg g fr wt <sup>-1</sup>
0	130 b	1.3 a	1.495 ab
2	162 b	5.7 b	1.814 b
4	180 b	6.1 bc	1.805 b
8	166 b	8.0 c	1.978 b
16	128 b	14.0 f	2.748 c
32	52 a	12.6 ef	3.078 c
64	15 a	12.0 de	1.741 b
128	37 a	10.5 d	1.038 a

a/ Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of four replications.

adaption in aquatic vascular plants.

#### Morphology Studies

Root production in aquatic vascular plants ranges from plants which have no roots (coontail) to plants which form thick, turf-like sod on the hydrosoil (*vallisneria*). If two aquatic plants had equal photosynthetic capability and only one produced an excess of non-photosynthetic tissue, the production of standing crop by the latter would be reduced.

The production of roots of hydrilla, southern naiad, and *vallisneria* was compared after young plants were grown for 12 weeks in 3.8-l jars (Table 5). Total growth, from highest to lowest, of the three species was southern naiad, *vallisneria*, and hydrilla. The ranking in respect to shoot growth was southern naiad and hydrilla, followed by *vallisneria*. The total growth of *vallisneria* was about as high as the other species, but the high proportion of roots produced (15.7%) resulted in less production of top growth.

The growth characteristics of hydrilla and *vallisneria* in earthen ponds in Orange County, Florida, are presented in Table 6. The standing crop biomass (top growth) data from the ponds were very different from results obtained in 3.8-l jars, illustrating the difficulty in simulating natural growth under controlled conditions. The standing crop biomass of *vallisneria* grown in the ponds was almost three times greater than the standing crop biomass of hydrilla (400 vs 161 g m<sup>-2</sup>, respectively). The total biomass of *vallisneria* was also much greater than the total biomass of hydrilla. However, roots accounted for 40.6% of the biomass of *vallisneria*, but only 12.4% in hydrilla. All of the standing crop of *vallisneria* was leaf tissue, but 56.5% of hydrilla

Table 5. Production of roots and shoots (mg dry wt) by hydrilla, southern naiad, and vallisneria grown for 12 weeks in 3.8-l jars.<sup>a/</sup>

Species	Roots	Shoots	Total	Roots (%)
Hydrilla	8.5	353.4	369.1	2.5
Southern Naiad	7.8	402.5	410.3	2.0
Vallisneria	58.9	314.1	373.0	15.7

<sup>a/</sup> Each value is the mean of four replications.

Table 6. Morphological characteristics of hydrilla and vallisneria as determined from natural unmixed populations in earthen ponds 1.5 m deep in Orange County, Florida.

Characteristics	Hydrilla	Vallisneria
Dry Weight ( $\text{g m}^{-2}$ )		
standing crop	161.4	400.1
roots	22.6	273.6
biomass	184.0	673.7
Components of Biomass (%)		
leaves	31.1	59.4
stems	56.5	-
roots	12.4	40.6
Surface Area		
$\text{dm}^2 \text{ g dry leaf}^{-1}$	8.25	2.18
leaf area index (LAI)	4.76	8.74
LAI range <u>a/</u>	4.0-6.2	6.5-10.8
Production ( $\text{kg ha}^{-1}$ )		
wet standing crop	17678	48262
dry standing crop	1614	4001
dry standing crop range <u>a/</u>	1280-2213	2976-4960

a/ Range determined on eight replications.

standing crop was stem tissue. Hydrilla leaves are small and thin. The area per gram of dry leaf tissue was 8.25 and  $2.18 \text{ dm}^2$  for hydrilla and vallisneria, respectively. The LAI of hydrilla was only half of the LAI of vallisneria. The LAI of hydrilla (4.76) compares with the LAI of corn (*Zea mays L.*) (1), and the LAI of vallisneria (8.74) corresponds with LAI's of waterhyacinth (*Eichornia crassipes* (Mart.) Solms) (46) and ryegrass (*Lolium perenne L.*) (20,57). Loomis and Williams (49) found that a LAI of at least 3.0 is necessary for 100% interception of radiant energy. Gessner (28) determined the LAI of turtle grass (*Thalassia testudinum* Banks ex Konig), a saltwater vascular plant, closely related to vallisneria. In an intertidal zone, turtle grass had a LAI of 18.6. It was not specified if this value represents one leaf surface or both. If it represents both leaf surfaces, the LAI of vallisneria is nearly the same as the LAI of turtle grass. The LAI of vallisneria found in this study is similar to the LAI of vallisneria reported by Ikusima (41).

The dry standing crop of hydrilla and vallisneria was much lower than values normally found for terrestrial or emergent aquatic plants. Standing crop is a function of depth, but dry matter accumulation remains low in submersed aquatic plants growing in deep water because of light extinction. The high water content of these species is the major factor which makes mechanical harvesting an uneconomical control procedure.

The horizontal distribution of hydrilla and vallisneria grown in the Orange County ponds is compared in Figure 6. The depths at which half of the standing crop was distributed above and below were 0.45 m

for hydrilla and 1.05 m for vallisneria. Vallisneria leaves did not reach the water surface. The tips were necrotic and appeared that either animals were feeding on the leaves, or more likely, vallisneria was unable to withstand the high light intensity at the surface (solarization).

Hydrilla formed an extensive canopy by floating on the water. This is a major competition factor which permits hydrilla to rapidly dominate a particular area. Few vascular plants, if any, could survive under such a hydrilla canopy when, even without hydrilla shading, light is a limiting factor for growth.

The location of the meristem is believed to be the factor governing the horizontal distribution of aquatic plants (41). The meristem of vallisneria is located at the base of the plant, while hydrilla has its meristem at the shoot apex.

#### Carbohydrate Studies

The starch content of hydrilla and vallisneria was determined at depths of 0.0 (surface), 0.5, 1.0, and 1.5 m in September 1973, and January 1974 (Figure 7). The starch content of plant roots only was determined in January 1974. The starch content of hydrilla tissue taken from the surface and a depth of 0.5 m was similar in September and January. However, samples harvested in September from lower depths contained nearly three times more starch than the January samples. Thus, hydrilla seems to be storing starch in the lower plant portions in the summer.

Vallisneria generally had a higher starch content than hydrilla. However, vallisneria appeared to be storing starch in the lower leaf portion in January instead of in September.

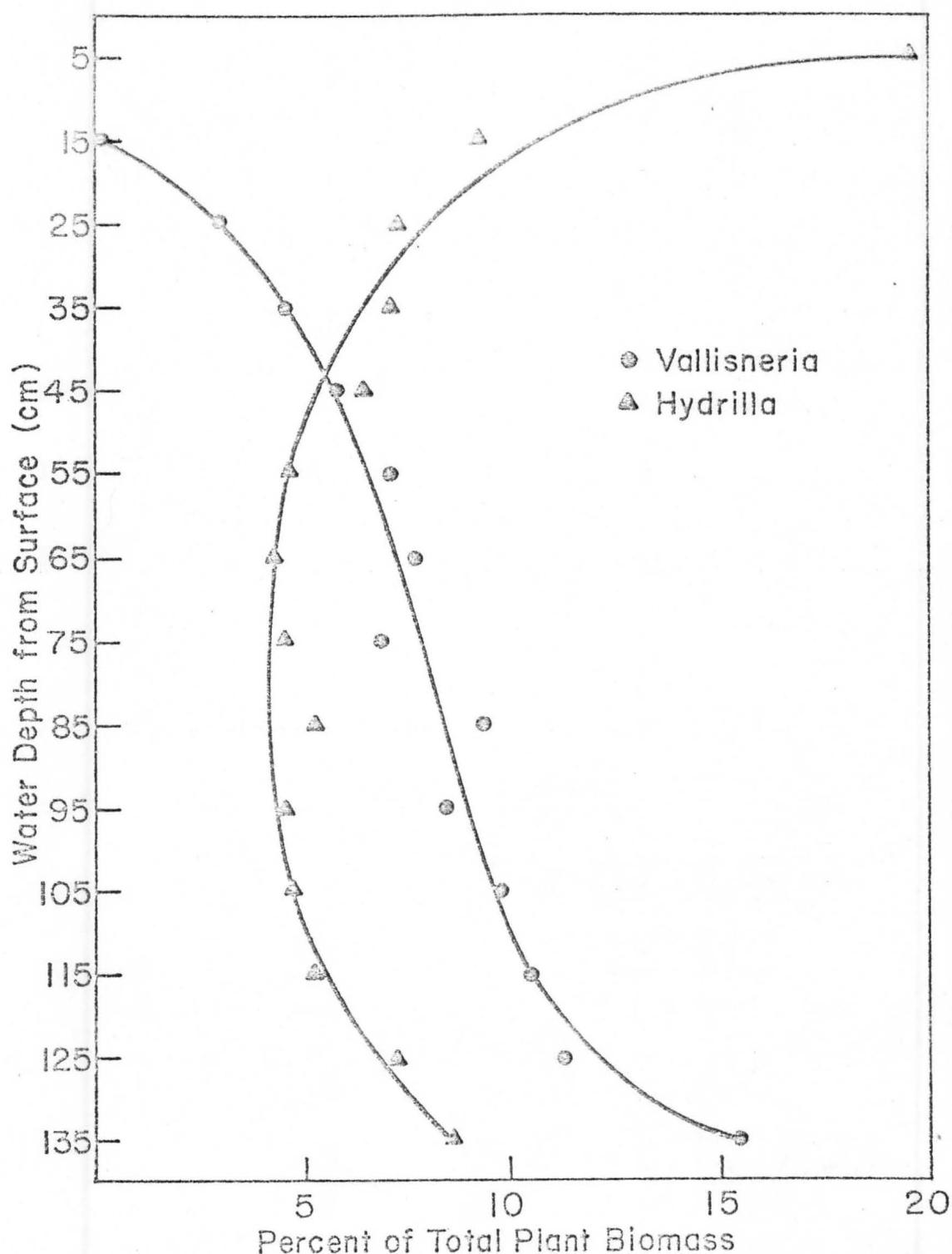


Figure 6. The depth distribution of hydrilla and vallisneria grown in ponds 1.5 m deep.

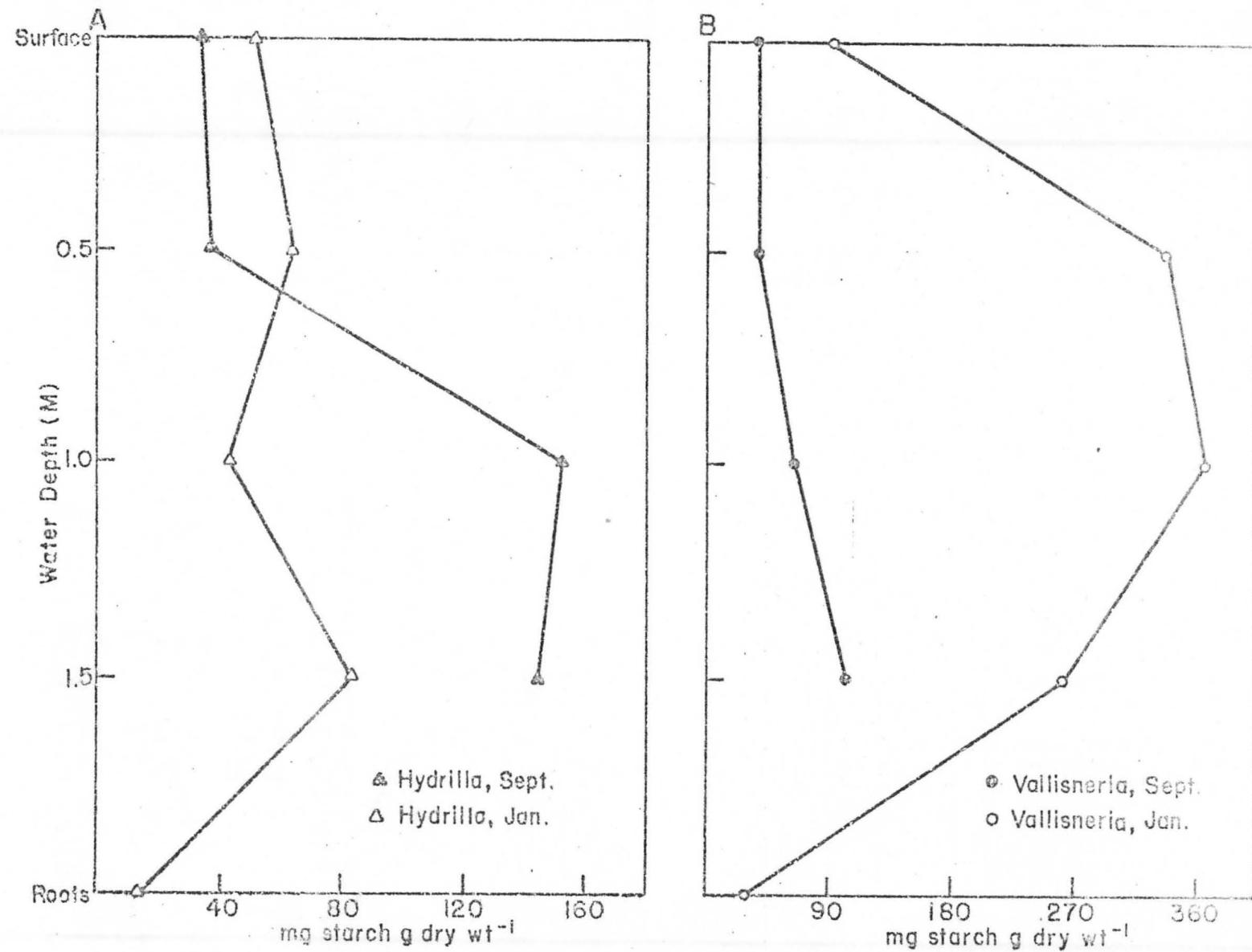


Figure 7. Starch content of hydrilla (A) and vallisneria (B) as a function of depth.

The sucrose and reducing sugar content of hydrilla and vallisneria was studied in the same manner as their starch contents. The sucrose content of both species was approximately the same in January, and no particular differences with depth were found (Figure 8). The sucrose content of the plants was higher in September and decreased with increasing depth. The obvious error in the sucrose content of vallisneria taken in September at 1.0 was apparently caused by mislabeling the 1.5 and 1.0 m samples. Allowing for this error, the sucrose content of both species was similar.

The reducing sugar content of both plants was lowest at all depths sampled in January (Figure 9). The reducing sugar content of September hydrilla samples was highest at 0.5 m. In January, vallisneria accumulated the greatest amount of reducing sugars near the base of the foliage in proximity to the meristem.

Total non-structural carbohydrates (TNSC) is a summation of starch, sucrose, and reducing sugars. The TNSC content of hydrilla and vallisneria samples is presented in Figure 10. Vallisneria contained more TNSC than hydrilla. The TNSC content of vallisneria was highest in January, whereas it was highest in hydrilla in September.

The non-structural carbohydrate components of southern naiad were studied in January 1974 (Figure 11). The data are very similar to hydrilla data for the same month. A final comparison of TNSC of the three species is presented for the January sampling date.

The high accumulation of starch in the January vallisneria tissue may be due to several environmental factors. The cooler water in January might favor more efficient photosynthesis. This may explain the occurrence of vallisneria primarily in springs and large lakes in

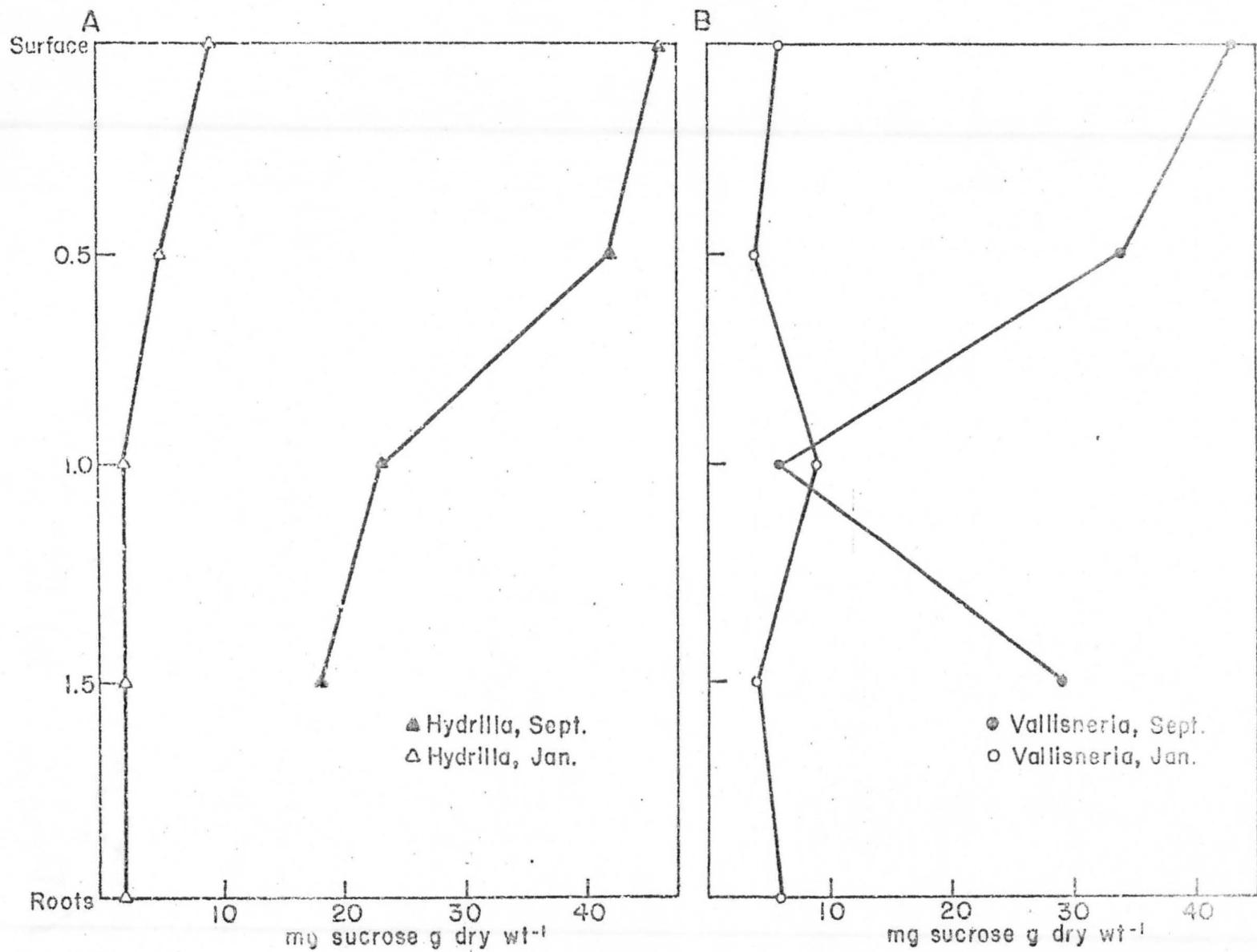


Figure 8. Sucrose content of hydrilla (A) and vallisneria (B) as a function of depth.

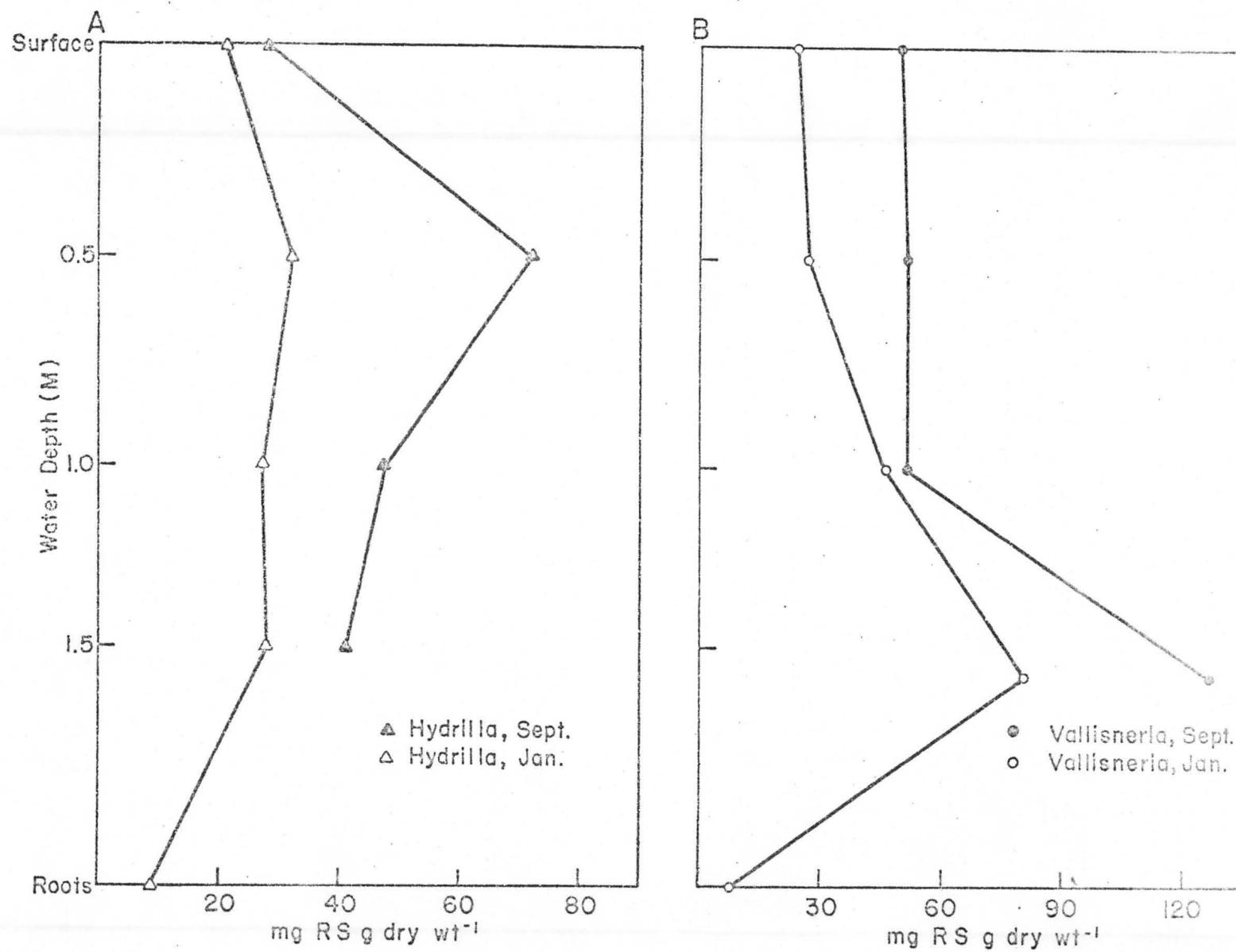


Figure 9. Reducing sugar content of hydrilla (A) and vallisneria (B) as a function of depth.

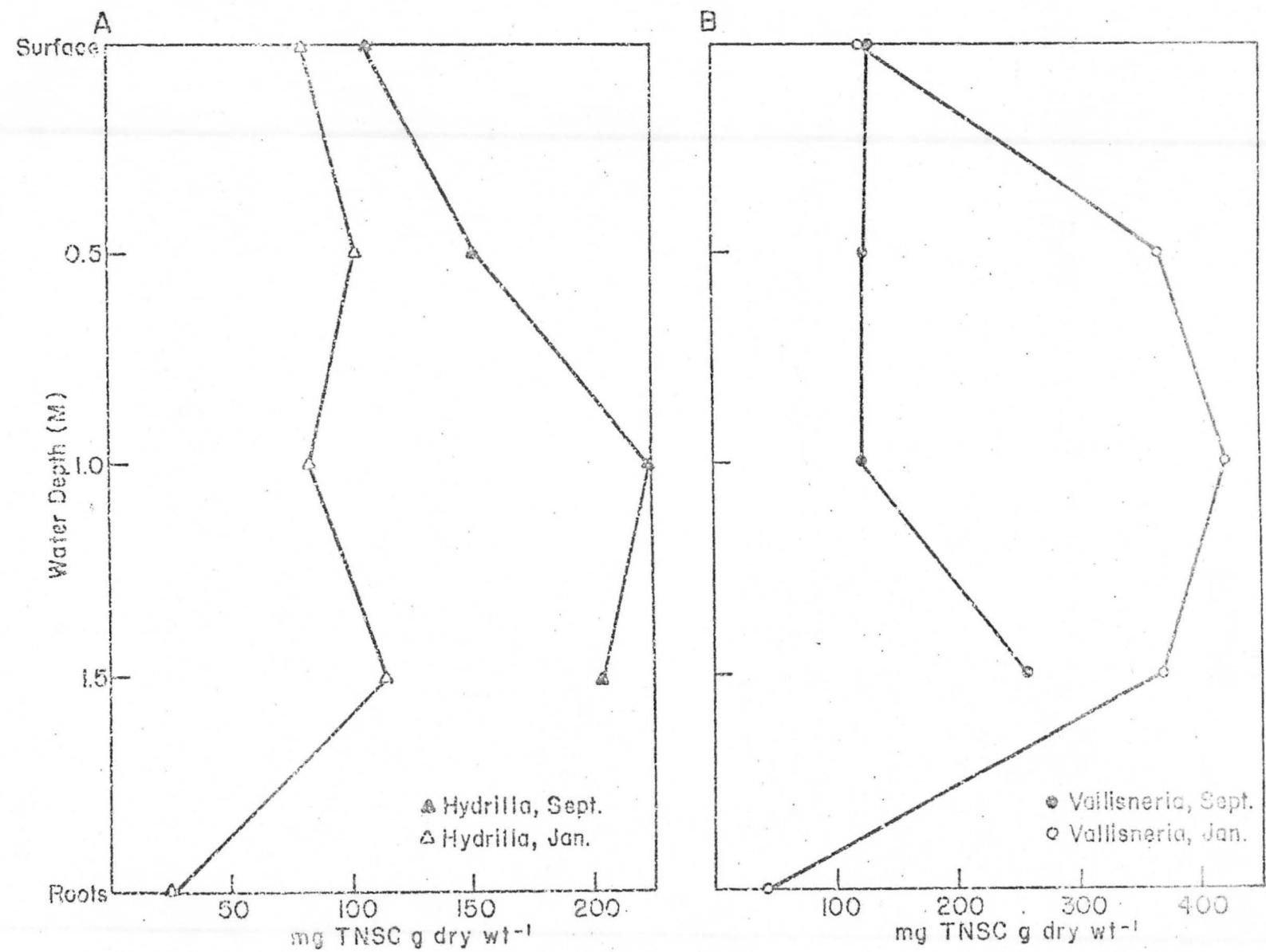


Figure 10. Total non-structural carbohydrate (TNSC) content of hydrilla (A) and vallisneria (B) as a function of depth.

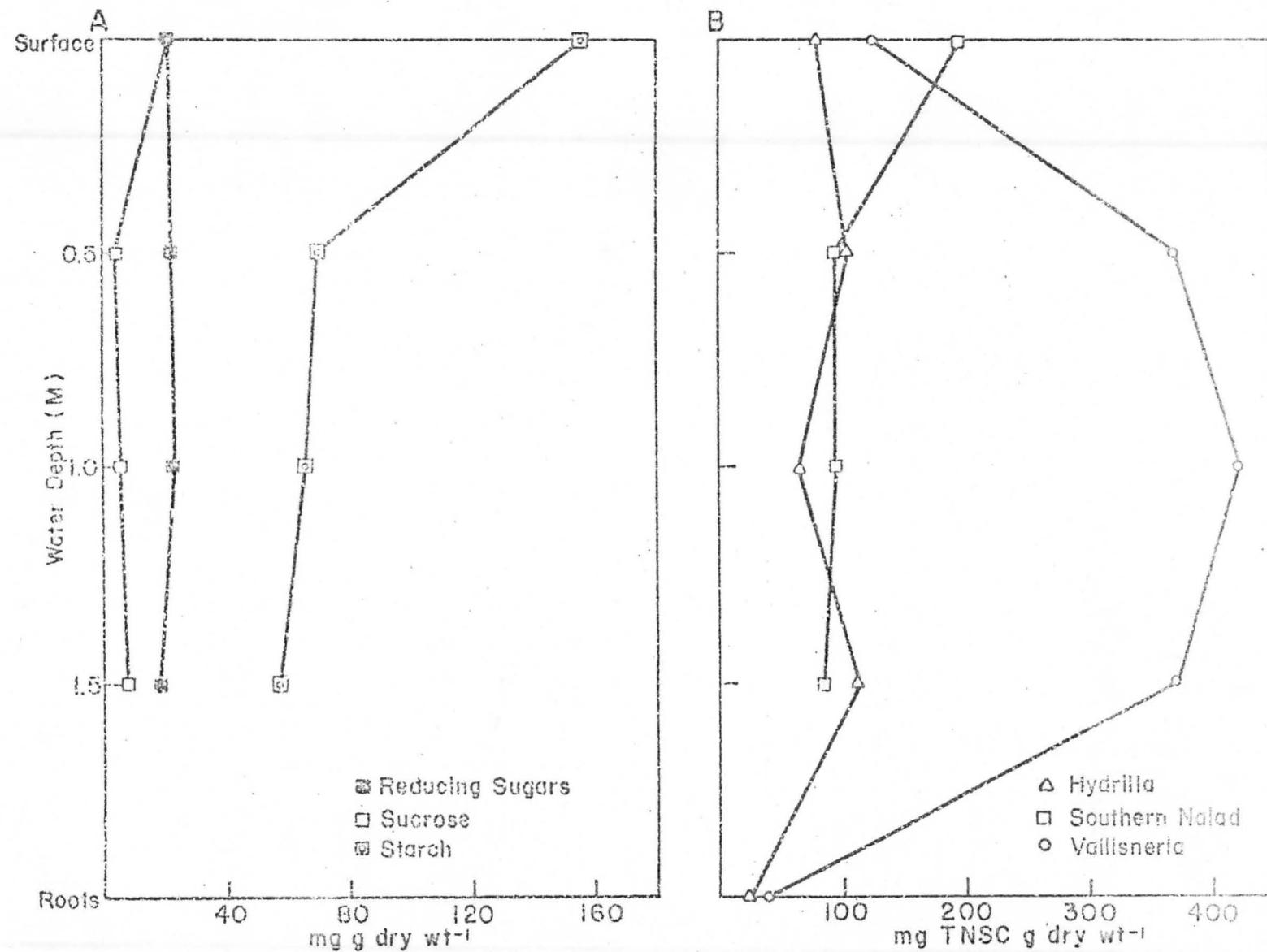


Figure 11. Carbohydrate components of southern naiad (A), and a comparison of total non-structural carbohydrate (TNSC) content of the three species in January 1974 (B).

Florida. In addition, *vallisneria* is not widely distributed in southern Florida.

Further speculation is that photosynthesis may proceed at equal rates in summer and winter, but respiration is slowed by the cooler winter temperatures, resulting in an accumulation of starch. In summer, respiratory activity would increase, and starch storage would no longer occur.

The literature contains no information on the carbohydrate status of these or most other submersed aquatic plants. The data presented here suggest that the carbohydrate content and composition of aquatic plants vary throughout the year. In addition, major differences occur between plant species. Carbohydrates are among the primary structural components of plants and are responsible for the formation of underground rhizomes and propagules. The production of underground propagules would be intimately associated with the carbohydrate status of the above ground plant portions.

Denying accumulation of carbohydrates, or preventing sugar translocation at critical periods of underground growth, could lead to more efficient plant management. More complete studies on this and related subjects need to be done on submersed aquatic plants.

## CHAPTER III

### FACTORS AFFECTING THE ABSORPTION OF CARBON IN PHOTOSYNTHESIS

#### Introduction

There are many biochemical, physical, and chemical factors which affect photosynthesis of submersed vascular plants. Past research has emphasized the physical aspect of the aquatic environment. This chapter, however, describes studies of the effect of photosynthesis on water chemistry and the effect of pH on carbon uptake. In addition, carbon compensation points were determined.

#### Methods and Materials

##### Plant Culture

Plants were collected originally from ponds and canals in central and south Florida and planted at Gainesville in outdoor pools. The pH of water in the culture pools remained at 7.5 to 8.5 throughout the year. Excess plant material was harvested periodically, and the pools were fertilized to maintain healthy plants and prevent accumulation of algae. Plants used in laboratory experiments were selected from the pools on the basis of general appearance and uniform size.

##### Effect of Photosynthesis on Water Chemistry

Nutrient solutions (0.1-strength Hoaglands) containing carbon were prepared by bubbling through respired  $\text{CO}_2$  and adding  $\text{NaHCO}_3$ . The initial concentrations of  $\text{HCO}_3^-$  and  $\text{CO}_2$  were determined by titration with 0.02N HCl to pH 4.5 and with 0.02N NaOH to pH 8.3, respectively (2).

Erlenmeyer flasks (250-ml) were filled with 230 ml of this culture solution. Approximately 2.0 g fr wt of hydrilla, southern naiad, and vallisneria were placed in separate, tightly stoppered flasks. The apical portions of hydrilla and southern naiad were used, but it was necessary to use complete vallisneria plants because vallisneria leaves soon die after they are separated from their roots. Plants were washed 30 minutes in running tap-water to remove as much epiphytic algae as possible. Four replications (flasks) of each species were harvested 1, 2, 4, 6, 8, and 10 days after initiation of the experiments. The  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{=}$ , and  $\text{OH}^-$  contents of the culture solutions were determined for each flask and averaged to obtain daily values. A separate set of flasks containing no plant material served as controls to monitor any changes in water chemistry not related to photosynthesis.

The flasks were placed in a growth chamber with provided 160 ueinsteins  $\text{m}^{-2} \text{ sec}^{-1}$  of light energy from incandescent and fluorescent lamps. The photoperiod was 12 hr light-12 hr dark, and the temperature was maintained at 30 C. On the harvest dates, flasks were removed and the water analyzed near the end of the 12-hr light period.

#### Effect of pH on Carbon Uptake

The uptake of carbon within the pH range 3.0 to 9.3 was studied. Solutions between pH 3.0 and 7.0 were made with 50 mM phosphate buffer, and between 7.0 and 9.3 with 25 mM "Tris" buffer. Solutions of pH 7.0 were prepared with each of the buffers, and these were used to compare the possible effects of the buffers on carbon uptake. The buffer concentrations were chosen because they were the lowest concentrations that resulted in a pH change of less than 0.05 when  $\text{NaHCO}_3$  (29.76 umoles) was

added to 49 ml of buffer solution.

Test tubes 25 mm in diameter and 150 mm long were filled with 49 ml of the respective buffer solutions (no nutrients added). The tubes were purged with N<sub>2</sub> to remove free CO<sub>2</sub> and were then kept under N<sub>2</sub>. Plant sections 12 cm long were rinsed in flowing tap-water for 30 minutes and then placed in the tubes. Two replications (tubes) were run at each pH for each species. After the plants were placed into the test tubes, 1 ml of 29.76 mM NaH<sup>14</sup>CO<sub>3</sub> (specific activity 9.08 uCi mmole<sup>-1</sup>) was added. The test tubes were stoppered tightly, inverted several times to mix the contents, and immediately placed into a water bath at 30 C. Two mercury vapor and four Sylvania Gro-Lux lamps provided light intensity of 500 ueinsteins m<sup>-2</sup> sec<sup>-1</sup>.

After a 30-minute light period, the plant sections were removed from the test tubes, rinsed for 3 minutes in tap water, dried, and weighed. After drying, the plant sections were homogenized in 5 ml of 25 mM "Tris" buffer, pH 7.8. The homogenate was decanted into a 50-ml test tube. The homogenizer was then rinsed with a additional 5 ml of buffer. The combined homogenate and rinse was mixed on a vortex mixer, and a 0.5 ml aliquot was placed into a liquid scintillation vial containing scintillation fluid composed of 100 g of naphthalene, 7 g of 2,5-diphenyloxazole (PPO), and 0.3 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in 1.0 l dioxane. Samples were counted at least twice at 12 C. The radioactivity of the samples was converted to disintegrations per minute (dpm) with a programmed absolute activity analyzer. The analyzer was programmed with the counting efficiency of various automatic externalization ratios determined with standard samples.

The problem of tissue self-absorption was minimized by diluting the sample from which the aliquot was taken for counting (92).

Generally, aliquots containing 1.0 to 3.0 mg dry wt of tissue were counted. When the radioactivity of the culture solutions was desired, 0.5 ml aliquots were counted.

The loss of  $^{14}\text{CO}_2$  from solutions of low pH by exchange with unlabelled atmospheric  $\text{CO}_2$  was thought to present a problem in determining the uptake of carbon by the plants. A series of solutions having a pH of 3.0 to 9.3 was prepared in test tubes. Plants were not placed in the tubes, but 0.5-ml aliquots were taken at various times to determine the rate of  $^{14}\text{C}$  dilution. The test tubes were placed in a hood at room temperature and were tightly stoppered except when aliquots were taken.

#### Carbon Dioxide Compensation Points

The  $\text{CO}_2$  compensation points for the three species were determined in stoppered flasks containing plant tissue, buffered nutrient solution, and  $^{14}\text{C}$ -labelled  $\text{NaHCO}_3$ . Erlenmeyer flasks (125-ml) were filled with 123 ml of buffered, 0.1-strength Hoagland's solution (25 mM "Tris" pH 7.8). Washed plant sections weighing approximately 0.15 g dry wt were placed separately by species into the flasks, and 2.0 ml of 29.26 mM  $\text{NaH}^{14}\text{CO}_3$  stock solution (specific activity 7.70 uCi  $\text{mmole}^{-1}$ ) were added to each flask. The flasks were stoppered tightly and placed in growth chambers under the same environmental regime as was used for determining the effect of photosynthesis on water chemistry. Three replicate flasks of each species and control flasks which contained no plants were removed from the growth chambers 1, 2, 4, 6, 8, and 10 days following initiation of the experiments. Plant sections were then rinsed in 200 ml of tapwater for 3 minutes and dried. Three 0.5-ml water samples were

taken from each flask and counted. Three additional 0.5 ml water samples were placed in empty scintillation vials and acidified with 3 drops of glacial acetic acid to convert all  $\text{HCO}_3^-$  and  $\text{CO}_3^{=}$  to free  $\text{CO}_2$ . The acidified vials were placed in an oven (70°C) to dry in order to remove all  $\text{CO}_2$ . After the vials were completely dry, they were filled with scintillation solution, shaken, and the remaining radioactivity determined. The chemical composition of the substances retaining radioactivity was not determined, but they were presumably various carbon metabolites exuded by the plants. To correct the  $\text{CO}_2$  compensation points, the radioactivity remaining in the acidified samples was subtracted from the radioactivity of the aqueous samples. Dried plant samples were homogenized, diluted, and mixed. The radioactivity was determined in the same manner as described previously. Radioactive recovery was determined by comparing the radioactivity added to the flask with the sum of the radioactivities of plants, culture solutions, and rinse water. In all instances involving  $^{14}\text{C}$  uptake, it was possible to account for at least 90% of the added radioactivity.

#### Results and Discussion

##### Effect of Photosynthesis on Water Chemistry

The effect of photosynthesis on the carbon content of culture solutions is presented in Figure 12. Data were taken for a period of 10 days, but only the first 6 days are presented because the major changes in water chemistry occurred during this time. Data collected from the *vallisneria* experiment are presented in Figure 12 and are representative of results obtained with *hydrilla* and southern naiad. The principal factor affecting the presence of carbon-containing ions at any given time was the amount of plant tissue present in the flasks.

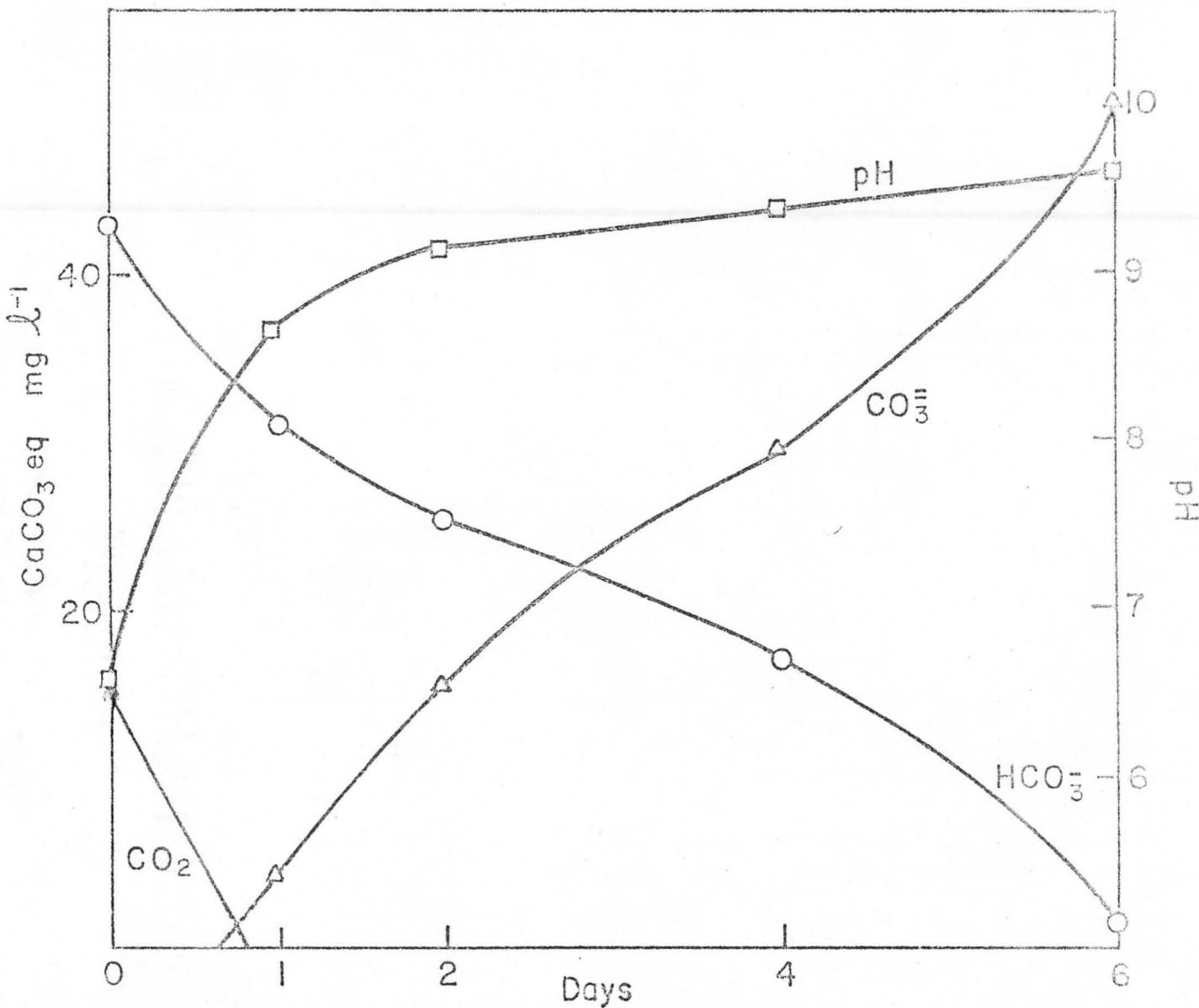


Figure 12. The effect of photosynthesis on water chemistry of culture solutions in 250-ml sealed vessels.

Free  $\text{CO}_2$  was utilized very rapidly. Detectable levels of  $\text{CO}_2$  were not found on the first day following the initiation of the experiments. The rapid disappearance of  $\text{CO}_2$  was accompanied by a rapid pH rise in the culture solutions. Decreased  $\text{HCO}_3^-$  content corresponded with increased  $\text{CO}_3^{=}$  content of the culture solutions. The plants apparently were utilizing  $\text{CO}_2$  from  $\text{HCO}_3^-$  according to the reaction,  $2\text{HCO}_3^- = \text{CO}_2 + \text{CO}_3^= + \text{H}_2\text{O}$ . As the pH increased to 10.0 or slightly higher on days 8 and 10, the production of  $\text{CO}_3^=$  slowed and low levels of  $\text{OH}^-$  were detected. These changes in water chemistry were characteristic of all three species.

By the tenth day of the experiments, the plants were nearly dead, for their leaves were necrotic and almost transparent. Also, colonies of algae began to appear in some of the flasks by the tenth day. Maintaining algae-free cultures was difficult, particularly with southern naiad. Whenever flasks contained visible algae, the results were discarded. The reason for plant mortality is not known. Carbon in a suitable form for photosynthesis, or the availability of some other nutrient, may have become limiting.

#### Effect of pH on Carbon Uptake

One of the problems encountered in studying the uptake of carbon by aquatic plants is the presence of varying ionic species containing carbon in solutions of different pH. Another difficulty is the dilution of dissolved  $^{14}\text{C}$  in water by exchange with atmospheric  $\text{CO}_2$ .

The  $^{14}\text{C}$  loss from buffer solutions of different pH was studied to allow for the correction of data from pH studies. These results are presented in Figure 13. Initially, all test tubes contained 50 ml of buffer solution which had a radioactivity of  $13,800 \text{ dpm ml}^{-1}$ .

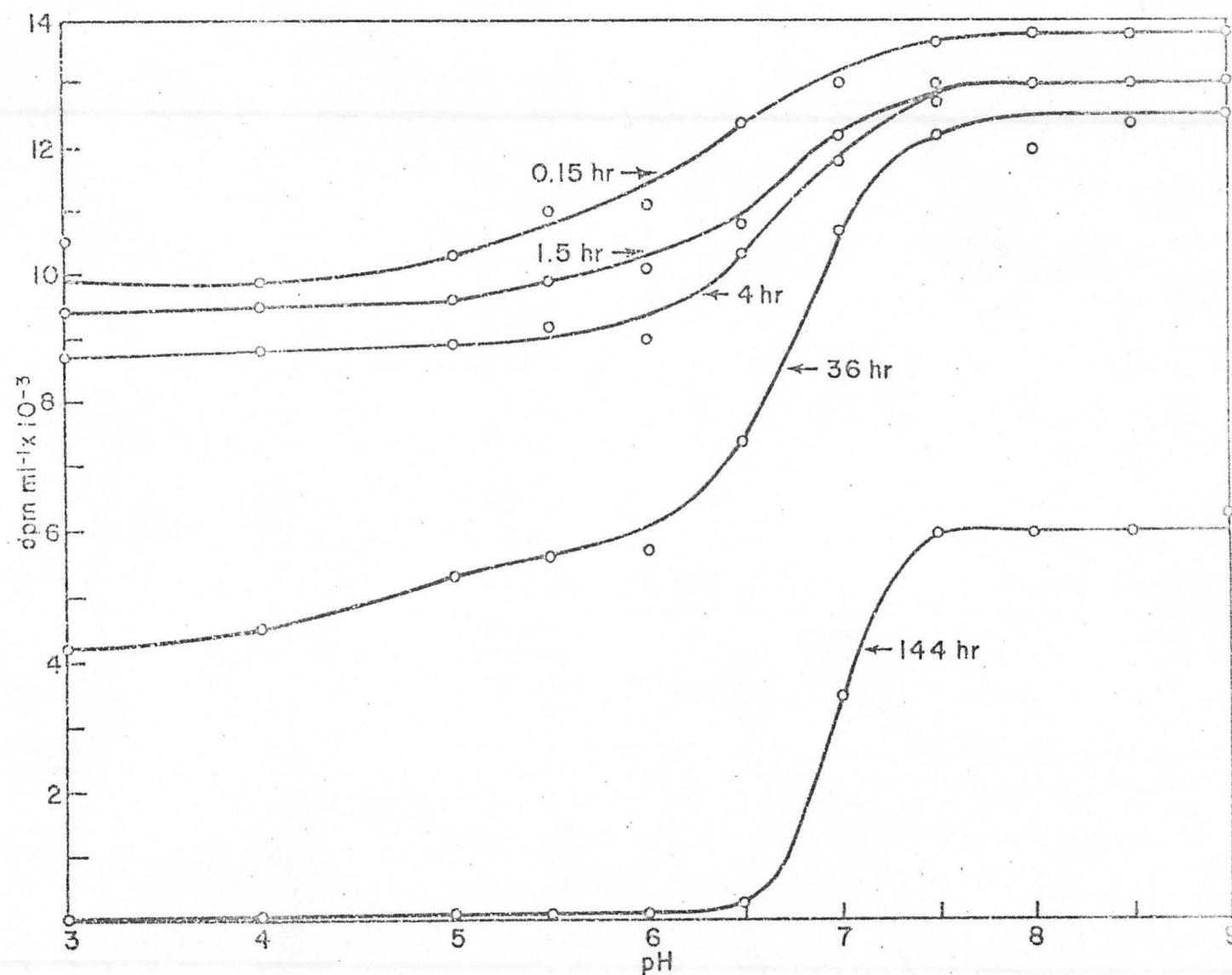


Figure 13. Loss of  $^{14}\text{C}$  from 50-ml test tubes containing buffer solutions at various pH's as a function of time.

Periodically, the stoppered test tubes were opened, and the radioactivity was determined. The radioactivity in solutions of low pH decreased very rapidly when  $\text{NaH}^{14}\text{CO}_3$  was initially added to the tubes. After 0.15 hr, solutions of pH 3 and 4 had lost about 14% of their initial radioactivity. The loss of  $^{14}\text{C}$  from tubes of high pH was evident after 1.5 hr, but the loss of radioactivity at high pH's was considerably less than the loss at low pH's. After 36 hr, solutions of pH 3.0 had lost nearly 67% of the added radioactivity, while solutions of pH 9.0 had lost only about 11%.

Plant sections were placed in freshly prepared solutions of the same pH and the uptake of  $^{14}\text{C}$  was measured (Figure 14). In order to minimize  $^{14}\text{C}$  dilution with atmospheric  $\text{CO}_2$ , the treatment solutions were prepared immediately before the plants were placed in them. The total time between adding radioactive  $\text{NaH}^{14}\text{CO}_3$  and the termination of the experiments was about 1 hour.

Hydrilla, southern naiad, and vallisneria absorbed more  $^{14}\text{C}$  in solutions of low pH where a greater proportion of carbon present was free  $^{14}\text{CO}_2$  (Figure 14). Southern naiad absorbed the greatest amount of  $^{14}\text{C}$  in solutions of pH 4.0 to 5.0; above pH 5.0 there was a progressive and rapid decline in  $^{14}\text{C}$  uptake. Vallisneria absorbed the least  $^{14}\text{C}$  of all the species studied, and uptake gradually decreased as the pH of the buffer solutions increased above 4.0. Overall, hydrilla had the highest rate of photosynthesis or  $^{14}\text{C}$  uptake. Carbon uptake by hydrilla was maximum in solutions near pH 6.0. The proportion of  $\text{CO}_2:\text{HCO}_3^-$  at pH 6.5 is about 1:1. Whether this is what caused a high uptake at these pH's, or whether the activity of some enzyme is maximum at these pH's is not known. At pH values above 8.0 to 8.5, the uptake

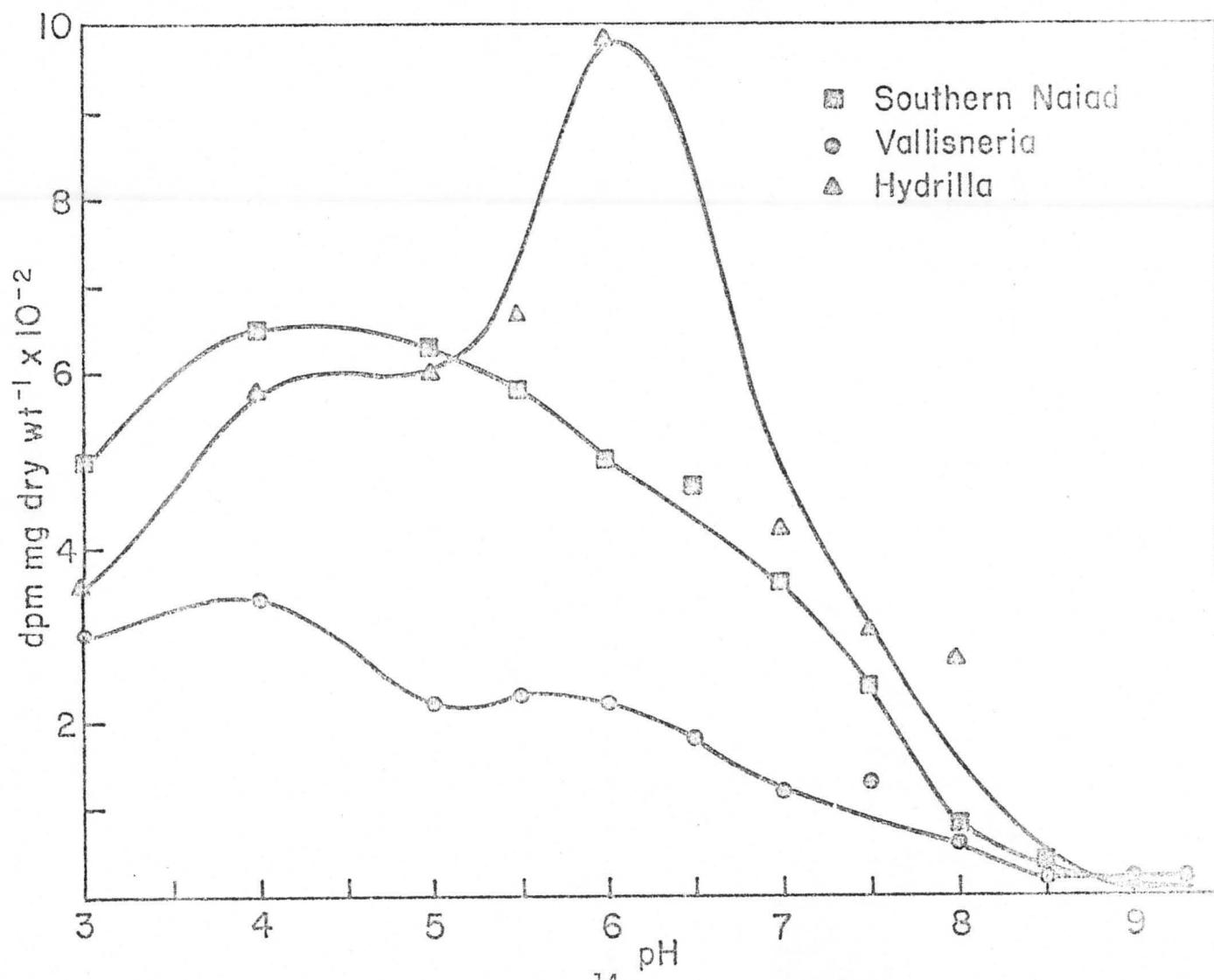


Figure 14. The effect of pH on the  $^{14}\text{C}$  uptake of hydrilla, southern naiad, and vallisneria.

of  $^{14}\text{C}$  by all species essentially ceased. However, if  $^{14}\text{C}$  were plotted against the proportion of free  $\text{CO}_2$  in solution, it would be evident that these species utilize free  $\text{CO}_2$  most easily. These results are similar to those obtained by Paasche (67) when he studied the uptake of carbon at various pH's by a coccolithophorid.

Osterlind (64) found that a photoactivation period was required by *Scenedesmus* in order for photosynthesis to occur in solutions of high pH. It is possible that a photoactivation period would increase the uptake of  $^{14}\text{C}$  by hydrilla, southern naiad, and vallisneria in solutions of high pH. The low carbon absorption above pH 8.0 by hydrilla was unexpected. Deposits of  $\text{CO}_3^=$  have been reported to occur on hydrilla leaves, and this plant is commonly found in waters of pH 10.0 or higher (50).

Hydrilla's growth in waters of high pH does not necessarily mean that  $\text{HCO}_3^-$  is being utilized directly in photosynthesis. In the Orange County ponds, the pH of the water was above 10.0, but  $\text{CO}_3^=$  deposits on hydrilla leaves were not evident.<sup>5</sup> It is possible that  $\text{CO}_2$  supplied by algal respiration and sediment oxidation was sufficient to maintain hydrilla in a canopy where rapid growth was no longer necessary. High respiration rates in these ponds was indicated by results obtained when the diurnal oxygen content of the water was measured. The aerenchyma in hydrilla probably stores  $\text{CO}_2$  at night, and this may be an important carbon source the next day.

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When the Orange County ponds were first planted with hydrilla, the pH of the water was 7.5 to 8.0. The pH has increased the past 3 years to about 10.5. Hydrilla is presently well established in the ponds, and water quality that is measured is the result of three years of hydrilla growth. Hydrilla probably is not growing at a maximum rate now that a canopy has formed, but does have to produce enough growth to maintain its canopy. If the ponds were cleared with herbicide, the additional  $\text{CO}_2$  derived from decaying vegetation likely would result in a high rate of regrowth. Much of this reasoning is speculative, and further studies need to be completed. The rapid increase in  $^{14}\text{C}$  absorption by hydrilla between pH's 5.5 and 6.0 also should be examined more closely.

#### Carbon Dioxide Compensation Points

The uptake of  $^{14}\text{C}$  in closed flasks and the resulting  $\text{CO}_2$  compensation points for hydrilla, southern naiad, and vallisneria are presented in Figures 15-17. Initially, the flasks contained 58.5 umoles (39.3 mg  $\text{l}^{-1}$ ) of  $\text{NaH}^{14}\text{CO}_3$ , and the radioactivity was 11,800 dpm  $\text{ml}^{-1}$ . After 6 to 8 days, the radioactivity of the solutions attained a uniformly low level of radioactivity (300 dpm  $\text{ml}^{-1}$ ). The amount of  $\text{NaH}^{14}\text{CO}_3$  in solution decreased to about 1.49 umoles (1.00 mg  $\text{l}^{-1}$ ). The  $\text{CO}_2$  compensation points for all three species were about the same. After correcting for the relative proportions of carbon in solution, the results indicated  $\text{CO}_2$  compensation points of  $0.52 \text{ mg l}^{-1} \text{ CO}_2$ , or on a volume basis,  $8.01 \text{ ul l}^{-1}$ . For comparison to the  $\text{CO}_2$  compensation points of terrestrial plants, the latter value expressed on a volume basis should be used. There was no visible evidence of algal growth in any of these flasks during the 10-day experiments; therefore, the results represent the compensation points

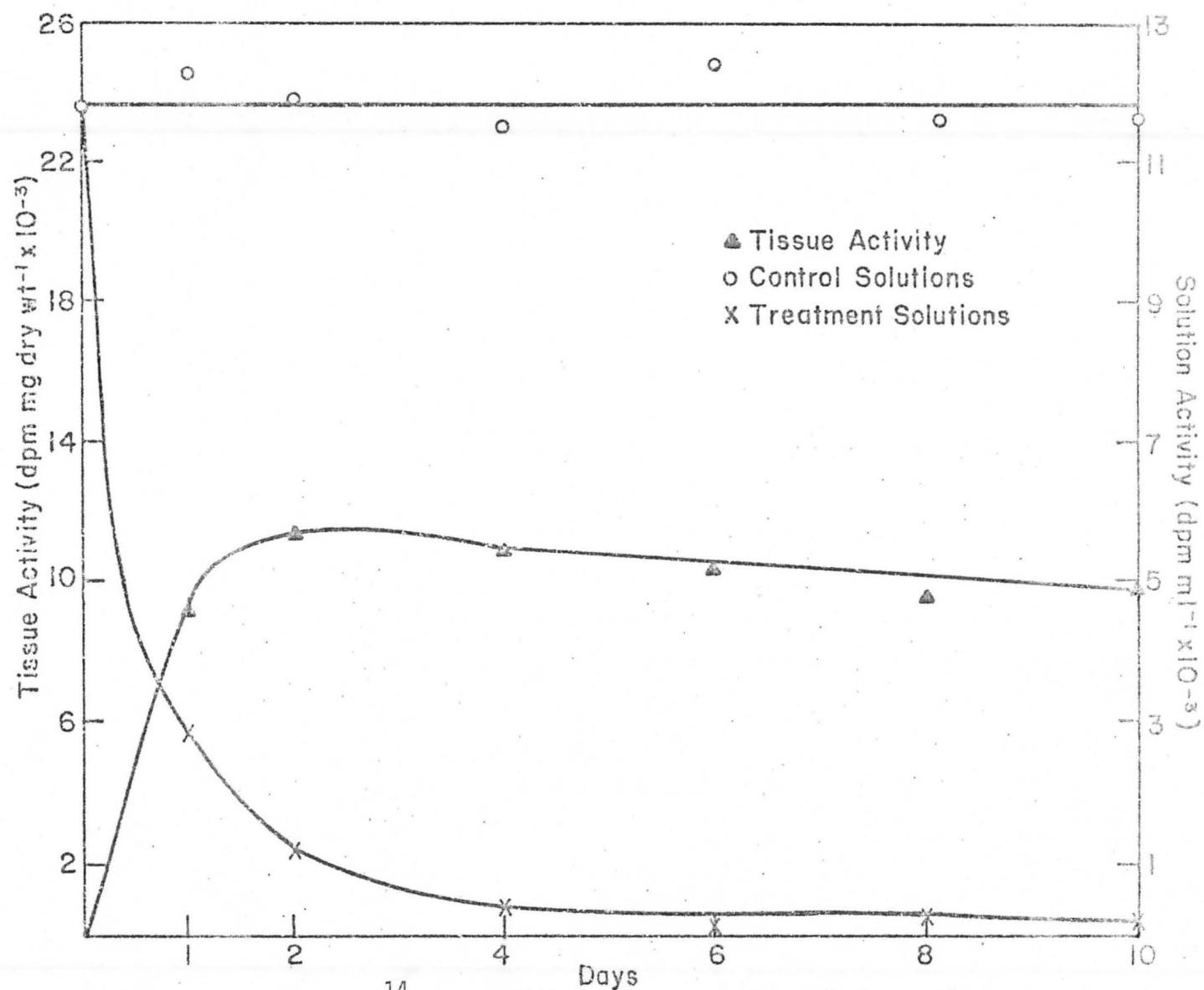


Figure 15. Uptake of  $^{14}\text{C}$  by hydrilla contained in sealed vessels.

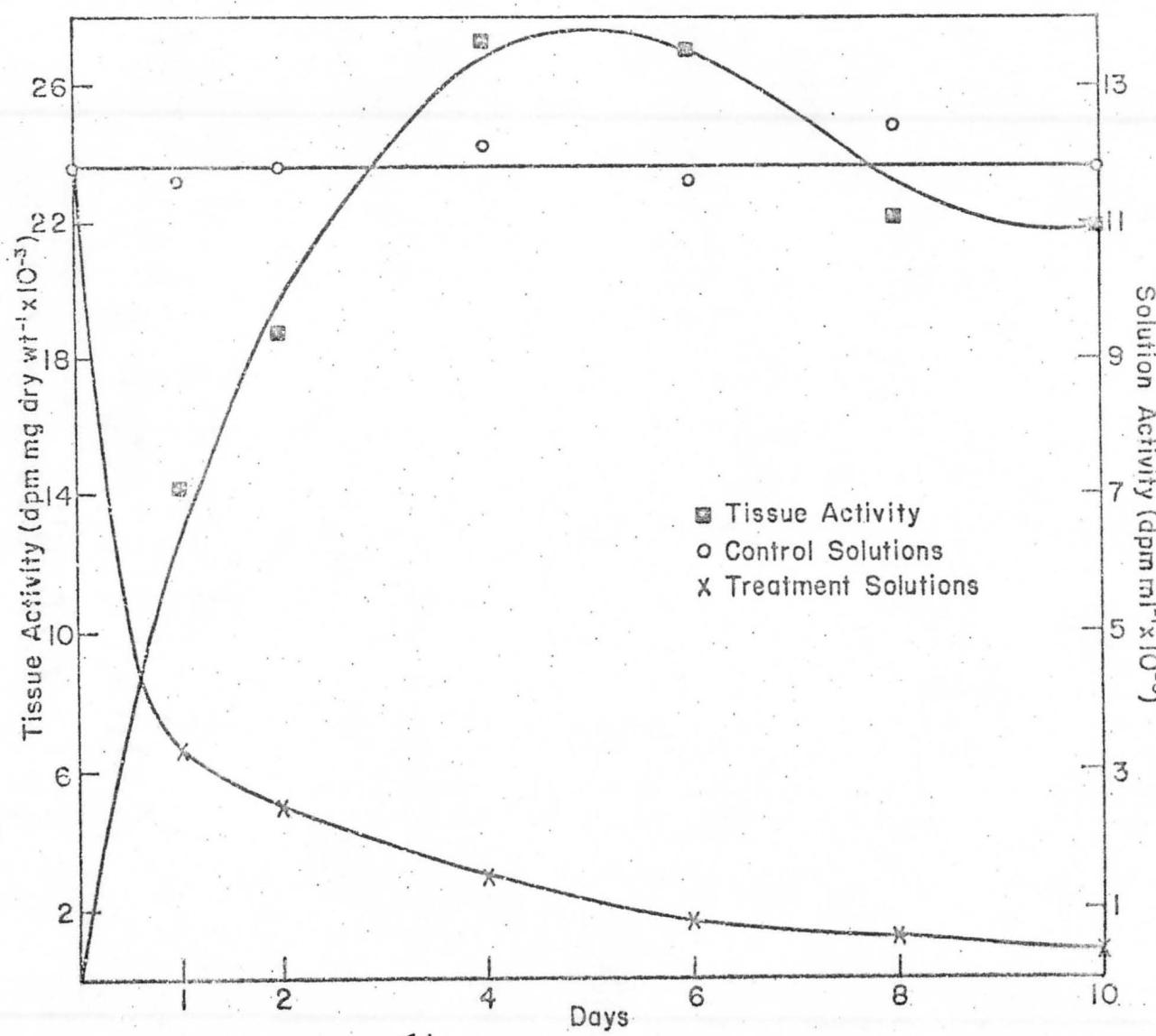


Figure 16. Uptake of  $^{14}\text{C}$  by southern naiad contained in sealed vessels.

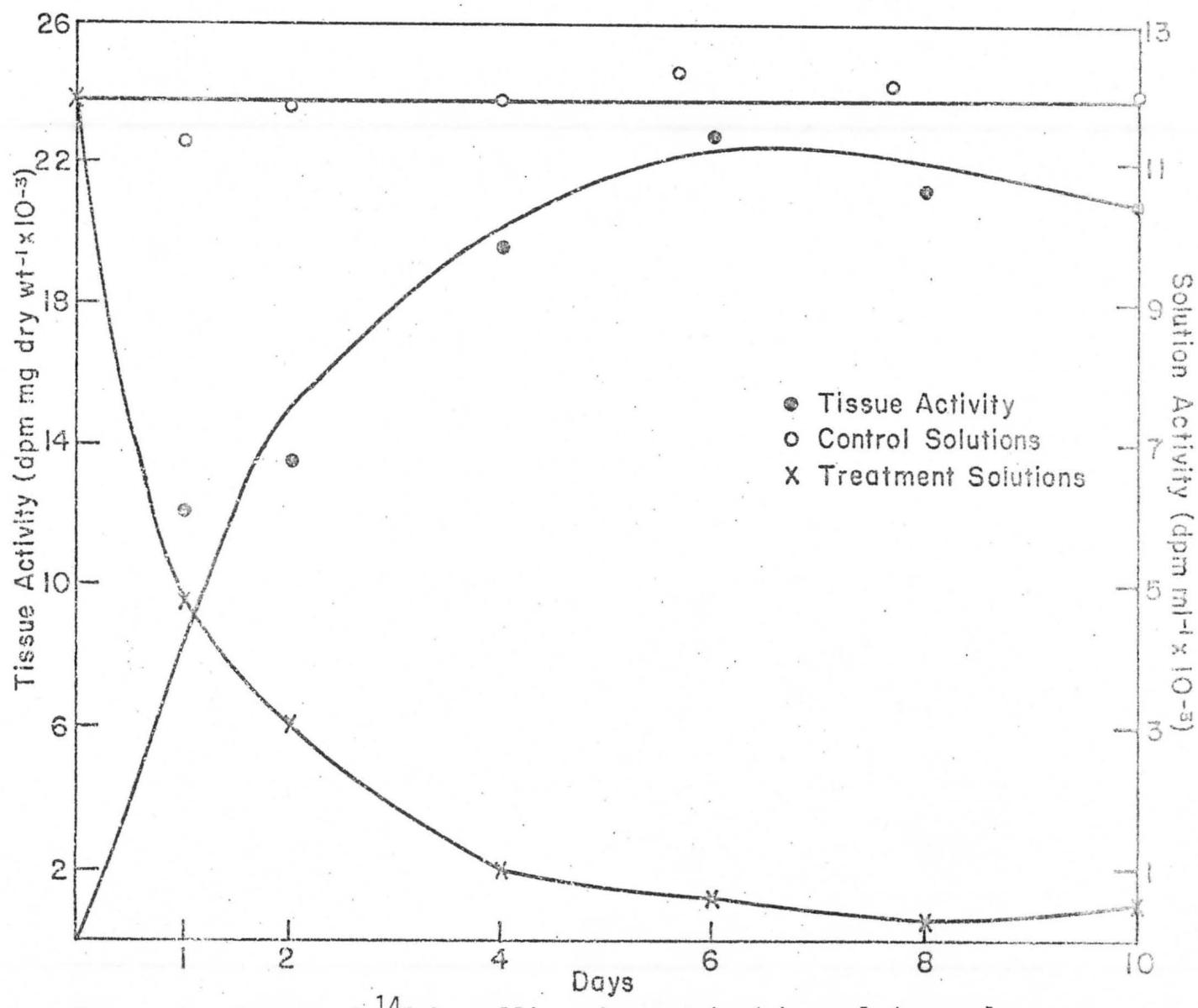


Figure 17. Uptake of  $^{14}\text{C}$  by vallisneria contained in sealed vessels.

of the vascular plants and not algae. When working with aquatic plants from natural conditions, there are always some algae present. It was doubtful, however, that the  $\text{CO}_2$  concentrations of these solutions were significantly affected in the first 6 days by algae.

The radioactivity of the plants (dry weight basis) varied between species because the results were not corrected for differences in plant weights used (tissue dilution). The radioactivity of the plants increased rapidly until about the fourth day and then decreased after the sixth day. The decrease in radioactivity of the plant tissue was caused by the increasingly poor condition of the plants past the sixth day. Some of the tissue were becoming necrotic, resulting in  $^{14}\text{C}$  leakage. The loss of various carbon metabolites from the plant tissue is presented in Figure 18. Aliquots taken from the solutions were acidified and dried to remove  $^{14}\text{CO}_2$ ,  $\text{H}^{14}\text{CO}_3^-$ , and  $^{14}\text{CO}_3^=$ . Thus, values in Figure 18 are measures of the loss of  $^{14}\text{C}$  metabolites from the plant tissue. These data also give an idea of the relative capability of these plants to survive in closed systems. The greatest amount of radioactivity was lost by southern naiad and vallisneria. The radioactivity lost by hydrilla during the experiments was very low. This illustrates how well hydrilla can survive conditions which are relatively unfavorable to the growth of other aquatic plants.

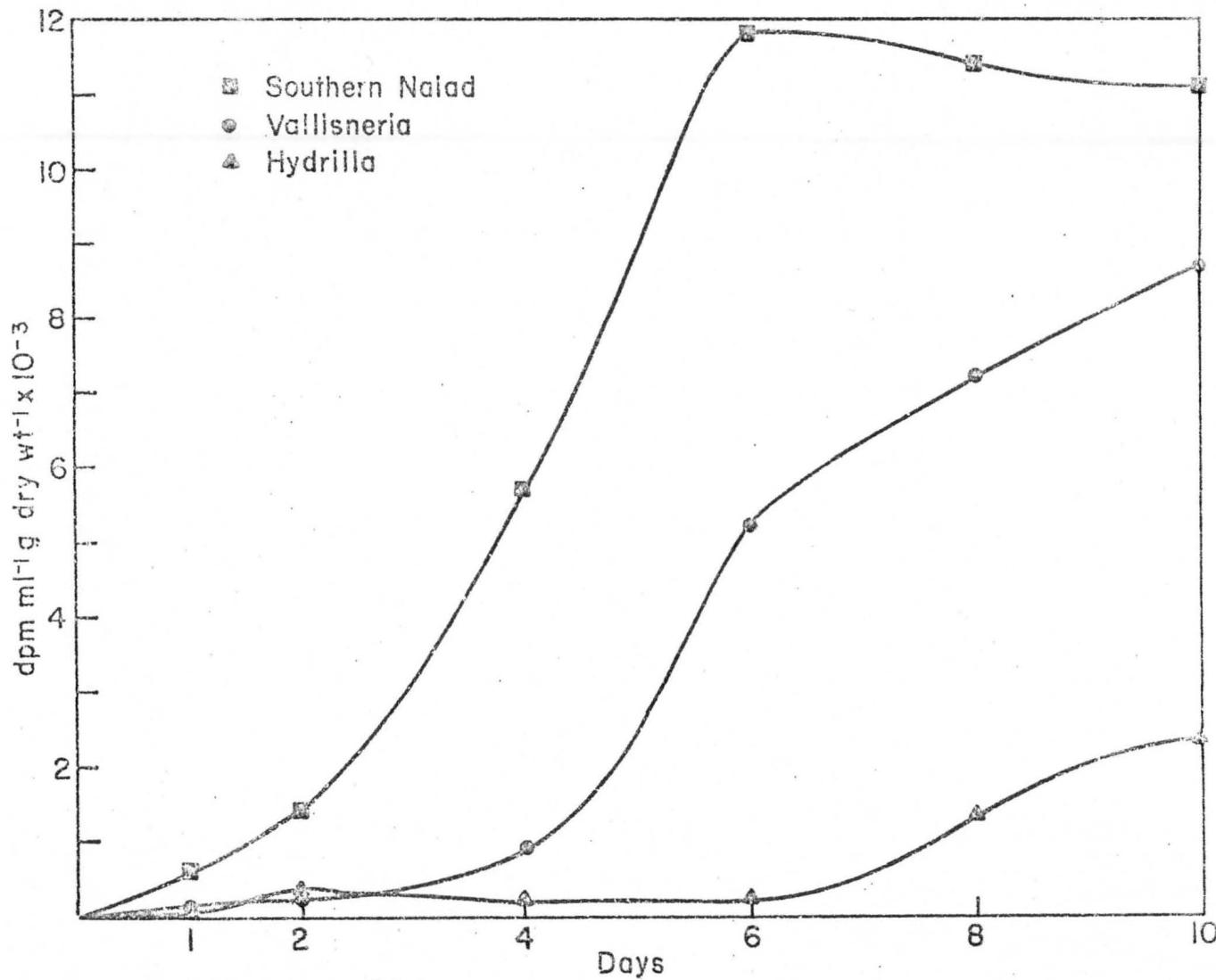


Figure 18. Loss of  $^{14}\text{C}$  metabolites by hydrilla, southern naiad, and vallisneria contained in sealed vessels.

CHAPTER IV  
PHYSIOLOGICAL COMPARISONS AND SUMMARY

Introduction

The objective of this chapter is to summarize the photosynthetic characteristics of aquatic plants. Although few studies have been reported on the physiology of aquatic plants, it is evident from these data that they have several distinct characteristics which are very different from their terrestrial counterparts. In order to understand their uniqueness, some general comparisons are listed.

Discussion

Properties of Aquatic Plants

A comparison of some photosynthetic properties of hydrilla, southern naiad, and vallisneria is outlined in Table 7. Probably the most important factor which allows hydrilla to dominate other aquatic plants is its relatively high rate of photosynthesis. Vallisneria had twice the LAI of hydrilla. However, the photosynthetic rate of hydrilla was three to four times higher than that of vallisneria. The potential advantage of chromatic adaptation in hydrilla also could be an important photosynthetic property. Photosynthetic rates in these plants are much lower than rates found in terrestrial plants. An increased level of chlorophyll b would not be as important in plants which have high rates of CO<sub>2</sub> fixation, but this may have great significance in aquatics. The canopy formed by the growth of the apical meristem of hydrilla would deprive light to plants already growing in a light-limiting situation.

Table 7. Comparisons of the photosynthetic properties of hydrilla, southern naiad, and vallisneria.

Factor	Hydrilla	Southern Naiad	Vallisneria
Leaf area index (LAI)	4.8	nd <sup>a/</sup>	8.7
Dominant apical meristem	yes	yes	no
Possible chromatic adaptation	yes	nd <sup>a/</sup>	no
Chlorophyll content (field) (mg g fr wt <sup>-1</sup> )	1.7	1.6	1.0
Photosynthetic rate pH 6.0 (mg C g dry wt <sup>-1</sup> hr <sup>-1</sup> ) pH 7.8	1.41	0.71	0.30
Root production (field) (%)	12.4	nd <sup>a/</sup>	40.6
Root production (lab.) (%)	2.5	2.0	15.7

a/ Not determined.

The high LAI of vallisneria would be an advantage in its competition with hydrilla. However, the low content of chlorophyll, low rate of  $\text{CO}_2$  fixation, and high production of non-photosynthetic tissue (roots) prevents vallisneria from maintaining itself in the presence of hydrilla.

The photosynthetic characteristics of terrestrial and submersed aquatic plants are compared in Table 8. The presence of ribulose di-phosphate (RuDP) carboxylase as the primary carboxylating enzyme and products of photosynthesis (63,64) of submersed aquatic plants are indicative of a typical  $\text{C}_3$  plant. However, the low  $\text{CO}_2$  compensation points demonstrated for these plants are more typical of  $\text{C}_4$  plants. On the basis of this anomaly, Black (11) stated that little is known about the metabolism of aquatic species. The literature relates several more unusual characteristics of aquatic plants that prevents their categorization into a typical  $\text{C}_3$  or  $\text{C}_4$  scheme. The maximum rate of photosynthesis is only 2 to 4 mg  $\text{CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ . Because of this, the growth rates and dry matter production are also very low. The growth of submersed plants seems very rapid to investigators who study them; however, they contain over 90% water and the resulting dry matter accumulation is very low. The presence of photorespiration has not been demonstrated in submersed aquatics. The low light intensities and low  $\text{O}_2$  tensions in water do not favor the photorespiratory process. It is possible that the mechanism of photorespiration exists; however, the presence of aerenchyma tissue and low  $\text{CO}_2$  compensation points indicates that the  $\text{CO}_2$  is not lost by the plant but is re-assimilated rapidly.

The properties of aquatic plants which are like those of  $\text{C}_3$  plants are carboxylation predominately by RuDP carboxylase and photosynthetic products typical of the pentose-phosphate pathway. The characteristics

Table 8. Photosynthetic characteristics of terrestrial and submersed aquatic plants.

Characteristic	$C_3$ <sup>a/</sup>	$C_4$ <sup>a/</sup>	Submersed Aquatic	Aquatic Species <sup>b/</sup>	Reference
Primary carboxylating enzyme	RuDP	PEP RuDP	RuDP	Hy,N,V	<u>c/</u>
$CO_2$ compensation point (ppm $CO_2$ )	30-70	0-10	0-8	M,Hy,N,V	63,64
Maximum rate of photosynthesis ( $mg\ CO_2\ dm^{-2}\ hr^{-1}$ )	15-40	40-80	2-4	V	68
$CO_2$ loss by photorespiration	Yes	No	No	N	65
Optimum temperature for $CO_2$ fixation (C)	15-25	30-47	25+	M,He,C	63,64,22
Maximum growth rate ( $g\ m^{-2}\ land\ area\ day^{-1}$ )	16-23	17-44	2-3	C,Ni	60,62
Leaf chlorophyll a:b ratio	2.4-3.2	3.3-4.5	1.8-2.3	Hy,N,V	
Dry matter production (tons $ha^{-1}\ yr^{-1}$ )	20-25	22-56	10	C	62

a/ Data for terrestrial plants adapted from Black (11).

b/ Abbreviation code: Hy-hydrilla; He-Heteranthera; V-Vallisneria; N-Najas; Ni-Nitella; M-Myriophyllum; and C-Ceratophyllum

c/ Preliminary enzyme analysis by this laboratory shows the RuDP carboxylase content of aquatic plants is much higher than the PEP carboxylase content.

which suggest C<sub>4</sub> classification are their CO<sub>2</sub> compensation points, apparent lack of photorespiration, and high optimum temperature for CO<sub>2</sub> fixation. The rates of photosynthesis, dry matter production, maximum growth, and the a:b ratios are lower than those found in either C<sub>3</sub> or C<sub>4</sub> plants.

If the photosynthetic rates of aquatic plants were higher, they probably would fit into the C<sub>3</sub> category fairly well. The C<sub>4</sub> characteristics would then be adaptations to the low CO<sub>2</sub> content and low light intensity of the aquatic habitat.

The low photosynthetic rates found in submersed plants invites speculation. Water certainly would not limit growth, and nutrients likewise probably would not be a limiting factor. Therefore, light has to be the major factor limiting aquatic growth. Further evidence of this is the practice of fertilizing farm ponds to promote the growth of algae which prevent growth of aquatic plants (presumably by shading). The light saturation level of submersed plants is probably very low as well as rates of photosynthesis at light saturation.

#### Summary

The object of these studies was to determine why hydrilla can rapidly dominate the submersed vascular flora of a body of water. The nature of the problem was such that several parameters were examined in three submersed species. The lack of previously reported research in this area necessitated studies of three plants in order to obtain valid comparisons.

Hydrilla had a higher rate of photosynthesis than the other species. The higher proportion of chlorophyll b in hydrilla also may be an important factor for growth. The morphology of the hydrilla canopy and

hydrilla's reproductive potential cannot be discounted in the expression of dominance over other species. The lack of a significant production of roots by hydrilla favors rapid growth of the upper portions of the plant. No single factor can explain the dominance demonstrated by hydrilla, but the physiological characteristics presented here, and possibly others, make hydrilla well adapted for continued growth and expansion in natural and man-made waterways.

Submersed aquatic plants are most similar to C<sub>3</sub> plants, with the exception of their extremely low productivity which probably results from growth under low light intensities. Their low CO<sub>2</sub> compensation points and lack of photorespiration are undoubtedly further examples of the adaptation of plants to their environments.

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#### BIOGRAPHICAL SKETCH

William T. Haller was born June 28, 1947 in Watertown, New York. Elementary and high school education was obtained at LaFargeville Central School. He received the New York State Regents Diploma in 1965. The requirements for the Bachelor of Science degree were completed at Cornell University in June 1969. He entered graduate school at the University of Florida in June 1969, and was awarded the degree Master of Science in Agriculture in June 1971. In April 1972, he received the Fred H. Hull Research and Achievement Award for outstanding research in Agronomy. He completed his Doctor of Philosophy degree in Agronomy in August 1974. He is married to the former Jean L. Caswell and has two sons. Professional memberships include the Ecological Society of America, Weed Science Society of America, and the Hyacinth Control Society.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



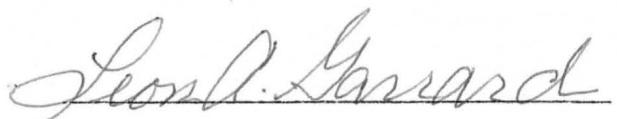
Sherlie H. West, Chairman  
Professor of Agronomy and  
Assistant Dean for Research

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



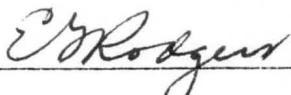
David L. Sutton, Co-Chairman  
Assistant Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Dean of the college of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1974

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Dean, Graduate School