# GAS CIRCULATION THROUGH THE YELLOW WATERLILY

By

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# ABSTRACT

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The gases in the lacunar system of aquatic vascular plants are important to the metabolism of these plants. Current models hold that individual gases diffuse within these gas spaces according to gradients in their partial pressures. These gradients are generally thought to be the product of gas metabolism by the plant — the varying uptake and release of $CO_2$ and $O_2$ in a static gas phase. In this study of *Nuphar luteum*, attention was focused on the interaction between the biological processes of the plant and the physical exchange processes between the plant and its environment.

The internal gas spaces in the yellow waterlily act as a pressurized flow-through system. Air enters the youngest emergent leaves against a small gradient in total pressure. This pressurization phenomenon is the result of a purely physical process of molecular effusion, driven by gradients in temperature and water vapor between the atmosphere and the lacunae of the youngest emergent leaves. As the leaves mature and grow in size, they become more porous and lose their capacity to maintain the positive pressures. Since the lacunae of these leaves are continuous with the lacunae of the younger leaves,

the older leaves act to vent the elevated pressures of the younger leaves.

The pressures generated in the young emergent leaves are small (less than 0.002 atm), but they drive a fluid flow of gas down their petioles to the rhizome, and from the rhizome up the petioles of the older leaves to the atmosphere. Using gas tracer methods, it was found that up to 60 ml/min of air (21% $O_2$) was transported down a single petiole to the rhizome buried in anaerobic sediment. The efflux from the rhizome to the atmosphere carried gas rich in $CO_2$ and $CH_4$. Much of the $CO_2$ was fixed by plant photosynthesis, while the $CH_4$ passes through the leaves to the atmosphere at an ecologically significant rate.

I, having built a house, reject
The feud of eye and intellect,
And find in my experience proof
One pleasure runs from root to roof,
One thrust along a streamline arches
The sudden star, the budding larches.

The force that makes the winter grow
Its feathered hexagons of snow,
And drives the bee to match at home
Their calculated honeycomb,
Is abacus and rose combined.
An icy sweetness fills my mind,

A sense that under thing and wing
Lies, taut yet living, coiled, the spring.

Jacob Bronowski
"The Abacus and the Rose"
Science and Human Values

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All those students of science I have known or read whose inspiration was contagious,

My parents, whose spirit imbues my search for understanding.

## TABLE OF CONTENTS

Page

CHAPTER 3 (cont'd)

APPENDICES

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

It is generally accepted that aquatic vascular plants have evolved from terrestrial ancestors. Even though these higher plants originally evolved from earlier equatic forms, no more than 1% of extant angiosperms are aquatic (Sculthorpe, 1967). The original invasion of land by plants was accompanied by a number of adaptations to the new environment. Some of these adaptations, most notably the reproductive strategies that evolved for life on land, are not easily lost on re-invasion of the aquatic habitat. There are, however, other adaptations that must take place in order to support life in this "new" environment.

In general, the transition from the terrestrial environment to the aquatic has meant a move from a relatively dry, aerated habitat to a wet, gas-poor one. As a result, aquatic plants have tended to lose their adaptations against water loss (stomata are commonly absent or nonfunctional; cuticle is reduced). On the other hand, they have developed adaptations to counter the shortage of metabolic gases in their environment. Aquatic plants are permeated by an extensive system of internal gas spaces. These adaptations are characteristic of obligate aquatic plants. Similar changes in morphology can be reversibly induced within the lifetime of an amphibious plant by moving it from one habitat to the other.

Considerable attention has been focussed on the developmental aspects of the growth of lacunae in aquatic plants. Ecologically, the dual embryonic origins of these gas spaces (lysigenous and schizogenous, cf., Sifton, 1945 and 1957) suggest that the various plants have converged in morphology in order to achieve an effect fundamental to metabolism in the aquatic habitat.

In spite of the widely reputed role of gases in the metabolism of these plants, there is very little understood about the dynamics of the gases in these internal spaces or lacunae. It is generally accepted that the primary role of the lacunae is the delivery of oxygen to respiring plant parts buried in the anaerobic sediment (Hutchinson, 1975). Such an oxygen transport system may also be involved in detoxifying the highly reducing rhizosphere (Armstrong, 1975). Some recent work (Wium-Anderson, 1971; Sondergaard and Sand-Jensen, 1979) has demonstrated that these gas spaces may also serve to transport carbon dioxide from the sediments to the photosynthesizing leaves of certain plants.

Only in a very few cases have researchers studied the exchanges of gases between the plant and its environment. It has generally been believed that gases diffuse through the stomata of emergent leaves in accordance with models of simple molecular diffusion. It is also considered that a similar, but slower, diffusive exchange occurs between gases in the submerged organs of plants and the gases dissolved in the surrounding water. Oxygen has received the most attention in this respect, particularly the loss of oxygen from roots to the environment (Armstrong, 1964, 1978). Except for a hypothetical biochemical pathway for the active transport of oxygen in plant tissue (Mitsui, 1964), oxygen transport in the plant is treated as a problem of gaseous diffusion in a passive gas phase. This phase is, however, made up of other gases which have their own dynamics within the lacunae. The gas mixture is a fluid, and as a result the behavior of a single gas cannot be a priori separated from the dynamics of the gas mixture as a whole. Furthermore, the behavior of this gas mixture can only be understood within a framework that takes into consideration of physical nature of the external environment. Such an investigation is fundamental to the understanding of the dynamics of gases in the lacunae.

The effects of a number of physical environmental parameters on plant growth have been studied. Temperature, light intensity and quality, turbulence, and sediment characteristics influence the metabolism of aquatic plants (Gessner, 1952; Sculthorpe, 1967). Although it has obvious importance in aquatic systems, pressure is a physical parameter that has received surprisingly little attention. Lab studies have shown that very small hydrostatic pressures (less than 1 atm) dramatically impair the growth and reproduction of several aquatic plants (Gessner, 1952, 1961). These observations have led to the widespread generalization that vascular plants tend not to grow at depths greater than 10 m (2 atm pressure). This generalization is contradicted by the fact that such plants are found at considerably greater depth in certain environments (Gessner, 1961), but no theoretical explanation has been forthcoming.

It is unlikely that the observed effects of hydrostatic pressure on plant growth are a direct result of pressure per se, since terrestrial plants can withstand gas pressures of over 100 atm without apparent injury (Scholander et.al., 1966). Furthermore, the effects of hydrostatic pressure are absent in the lower aquatic plants (algae, mosses, and liverworts) which lack lacunae (Hutchinson, 1975).

Clearly, hydrostatic pressure exerts a direct effect on gas relations by changing partial pressures and therefore gas solubilities. These changing relations are bound to have effects on the dynamics of the gases in the lacunae. These dynamics are the product of a number of processes: metabolic gas-consuming and -producing processes within the plant; diffusive and fluid dynamic processes in the internal and external gas phases.

A comprehensive understanding of the gas dynamics in the lacunae requires the concepts of biology and physics. It is the purpose of this dissertation to develop a physical framework incorporating the dynamics of the environmental gases with those of the lacunar gases.

In Chapter 1, a physical perspective of the littoral habitat is developed, with special attention to the emergent growth habit. Several general (and testable) predictions are made about the possible behavior of gases in the lacunae of these plants.

In Chapter 2, the considerations of the first chapter are applied in a study of some aspects of the gas dynamics in Nuphar luteum ssp. macrophylla. The emphasis is placed on the gases dissolved in the interstitial water of the sediment, and the interaction between these gases and those in the buried parts of the plant. During the study, it became clear that there was a pattern of discrepancy between the observations and the initial predictions.

In Chapter 3, the further investigation of these discrepancies required a study of the physical exchange processes between the plant and the atmosphere. By means of a purely physical process, the pressures of the gases in the plant are increased over barometric, thereby generating a gas flow-through system of great benefit to the plant.

# CHAPTER 1

# THE LITTORAL HABITAT

From a purely physical perspective, the littoral habitat can be broken up into three distinct phases. A phase is generally defined as a region of uniformity in a system, separated from other homogeneous parts of the system by boundary surfaces. Since the focus of this research is on gases, obviously the atmosphere is one of the phases under consideration. The gases of the atmosphere constantly exchange with the gases dissolved in the surface of the second major phase - the water column. Strictly speaking, the third phase, the sediment, is made up of many phases - the interstitial water, the solid phases presented by the various types of particulate matter comprising the sediment, and the gas phase presented by bubbles trapped in the interstices of the sediment. For the purposes of this study, the sediment can be treated as a single phase, recognizing that it is not truly homogeneous. In order to understand the value of partitioning the environment in this way, it is necessary to further describe the physical characteristics of the three major phases. Primary consideration will be given to the gases of the atmosphere and the sediment phases, since the exchanges between these phases and the plant dominate the gas dynamics within the plant.

## The Atmosphere

The gas composition of the atmospheric phase is $N_2$ (78%) and $O_2$ (21%), and its total pressure is 1.0 atm at sea level. Thus, the partial pressures of $N_2$ and $O_2$ are 0.78 atm and 0.21 atm, respectively. Three other gases are present in significant quantities — Ar (0.9%), $CO_2$ (0.03-0.04%), and $H_2O$ (1-4%). Water vapor is an important component of the atmosphere even though it is rarely considered as a component (most listings are for dry air). The amount of water vapor in the atmosphere depends very much on local air temperatures and the availability of liquid water for vaporization (by evaporation or transpiration). The changes in the phase equilibria of water are responsible for much of the earth's weather pattern, and as will be discussed later in this thesis, they also play a fundamental role in the gas dynamics in the yellow waterlily.

## The Sediment

The sediment is extremely important in the growth of rooted plants (Bristow and Whitcomb, 1971; McRoy and Barsdate, 1970; Twilley et.al., 1977). It is clear that these plants derive most of their mineral nutrition from this source. It is also becoming apparent that gases dissolved in the interstitial water (i.e., carbon dioxide) may be important in the metabolism of a number of plants (Wium-Andersen, 1971; Sondergaard and Sand-Jensen, 1979).

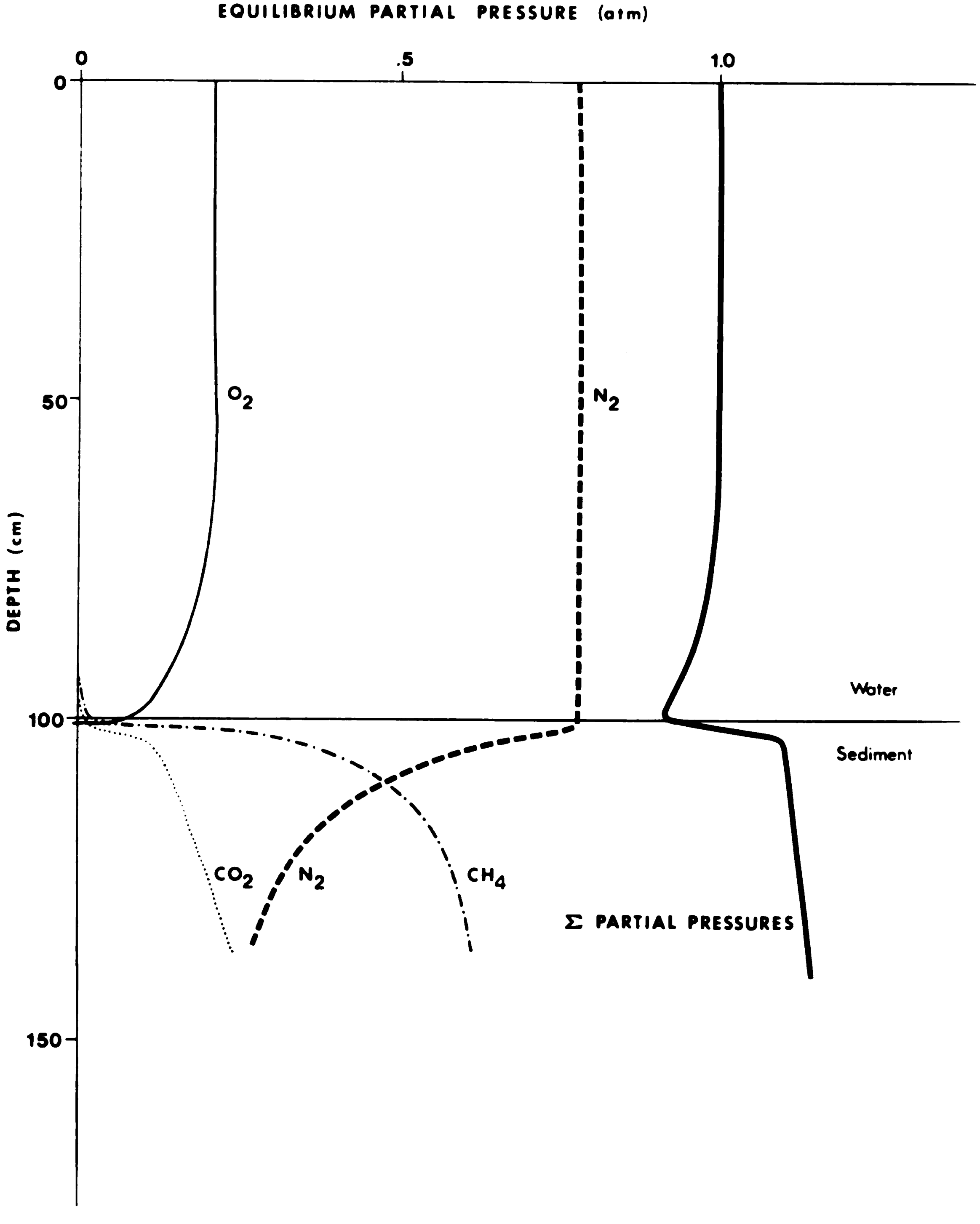
Most of the volume of the sediment is water; the volume percentages are variable depending on the nature of the sediment matrix. These depend on the geological characteristics of the surrounding terrain, and on the extent and character of biological processes occurring in the vicinity.

The matrix presented by the particulate matter of the sediment allows the accumulation of dissolved gases by restricting mixing with the overlying water. It also has the secondary effect of trapping bubbles of gas in the sediment interstices. The continuous production of gases in the sediment inevitably leads to ebullition into the water column. This process sometimes transports significant quantities of gas to the atmosphere (Strayer and Tiedje, 1978; Robertson, 1978), and may also act to accelerate the transport of dissolved compounds from the interstitial water into the water column by turbulent mixing (Ohle, 1958).

The compositions of individual bubbles lodged in the sediment are determined, of course, by the partial pressures of the gases dissolved in the neighboring water. These pressures are in turn determined by kinetic properties, such as: the nature of the sedimenting particulate matter, the metabolism of sediment organisms, diffusive exchange within the sediment and across the sediment-water interface, and groundwater flow processes.

The vertical distribution of gases in sediments has been the subject of a number of studies (Kaplan, 1974; Reeburgh, 1969). Typically, the partial pressures of atmospheric gases decrease with increasing depth in the sediments. Oxygen usually disappears within millimeters of the sediment surface; nitrogen and argon generally decline much more slowly, and never really disappear. Conversely, carbon dioxide and methane concentrations increase sharply below the surface of the sediment. The distribution of gases in the littoral habitat has been generalized in Figure 1.

Figure 1.-- A generalized distribution of the principal gases in the plant environment.
Below the sediment-water interface the equilibrium partial pressures of $N_2$ and $O_2$ fall dramatically. In contrast, the partial pressures of $CH_4$ and $CO_2$ increase sharply below the interface. The presence of bubbles trapped in the sediment interstices demonstrates that the sum of the partial pressures of the dissolved gases equals the hydrostatic pressure at that depth.


EQUILIBRIUM PARTIAL PRESSURE (atm)
0
.5
1.0
0
50
100
150
DEPTH (cm)
$O_2$
$N_2$
Water
Sediment
$CO_2$
$N_2$
$CH_4$
Σ PARTIAL PRESSURES

## The Water Column

In the water column, there are also a number of processes that significantly influence the composition of the dissolved gases. The dominant factor is the continual exchange of gases with the atmosphere, and as a result the partial pressures of the gases in the water approach their partial pressures in the atmosphere (i.e., $N_2$, 0.78 atm; $O_2$, 0.21 atm; Ar, 0.01 atm).

The absolute solubility of gases is an inverse function of water temperature, so the amounts of gases dissolved per unit volume of water tend to decrease with increasing termperature while the equilibrium partial pressures remain constant.[1] (Appendix A)

There are also other processes that tend to move the water away from this equilibrium. Photosynthesis by plants dependent on dissolved carbon dioxide (either as $CO_2$, $H_2CO_3$, or $HCO_3$) tends to reduce the partial pressure of $CO_2$ below atmospheric (0.03 atm).

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[1] The choice of dimension in the discussion of dissolved gas data must reflect the purpose behind the data analysis. The convention of using mass/vol seems to be a poor choice in many applications. This approach presumably follows the convention commonly applied to the solution of solids in liquids. With solids, the notion of mass has conceptual value; this is not true for gases. It is much easier to think of gases in terms of pressures, volumes, or moles; it's virtually impossible to think of gases in terms of weights. It's tempting to draw an analogy with the differences between the Imperial system and the metric system of mensuration. Conceptualization can often be hampered by adherence to traditional methods (cf., Appendix A).

Consider, for example, dissolved oxygen profiles for a lake. These are universally presented in mg $O_2$/l $H_2O$. As discussed in Appendix A, these values are of limited significance without temperature data, and even with such data it is difficult to make much more than relative comments about the dynamics of the gases. However, if the same information were presented in atm $O_2$ (the equilibrium partial pressure of $O_2$ in water), it becomes immediately clear how far the gas composition of the volume of water in question has diverged from the equilibrium composition it seeks at lake turnover.

Photosynthesis in the water column also acts to increase the partial pressure of $O_2$ in this phase. Conversely, respiration tends to decrease the partial pressure of $O_2$ and increase that of $CO_2$.

Since $CO_2$ is more soluble than $O_2$, each molecule of $CO_2$ contributes relatively less to its partial pressure in the aqueous phase. As a result, aerobic respiration near or at the sediment surface may tend to decrease the sum of the partial pressures of the dissolved gases. This hypothetical outcome is represented in Figure 1 by the slight depletion in the sum of partial pressures just above the sediment surface.

In hardwater lakes, the partial pressure of $CO_2$ in the surface waters often exceeds atmospheric. This phenomenon results from geochemical kinetic processes involving the degassing of a large carbonate pool derived from groundwater and sediment respiration processes.

The pressure relations of the gases in the water column are very important to submersed vascular plants, but appear to be less significant in the growth of emergent plants. This investigation of the dynamics of gases in the yellow waterlily suggests that during the summer growth period, the gases dissolved in the water column are of little significance to the plant. These gases do appear to be important, however, during those times of the year when the entire plant is submerged (roughly November - April). Then the plants put out morphologically distinct leaves that appear to act as efficient exchangers of gas between the lacunae and the water column (Arber, 1920).

## The Lacunar Interphase

Given the very simple principles underlying this discussion, it is possible to make some very interesting and significant predictions about the interaction of the plant with its environment (vis-a-vis gases). Since the plant under study is a waterlily, the predictions outlined here will be framed in their terms.

The waterlily spends nearly half of its life without surface leaves, from November through April in Michigan. It may or may not have submersed leaves during that time, but even if it does, these leaves are few and only project a short distance out of the sediment. The roots and the rhizome form the vast majority of the plant bulk (dry wt., volume, etc.), and these parts are for the most part buried in the sediment. The low rates of plant metabolism during the winter months (due to low light intensity and low temperatures) imply that the plant is unlikely to be carrying out processes that greatly alter the composition of the gases in the lacunae of the rhizome. The gas spaces of the lacunae of the roots and rhizome can be likened to a large, continuous bubble held in the sediment by a membrane of plant tissue that is permeable to gas diffusion. The cumulative effect of these diffusion processes means that the gas inside the lacunae should ever-increasingly resemble the gas in the bubbles trapped in the sediment interstices. That means that the volume percentages of methane and carbon dioxide should be relatively high, and those for atmospheric gases should be low. Furthermore, this convergence in gas composition also means that the total pressure of the gases in the lacunae should approach the pressure in the external bubbles (1 atm + hydrostatic pressure). As will be discussed in the following chapter, these predictions are upheld by observations made in the course of this study. This research has lead to the first published discussion of pressure equilibration between a plant and its anaerobic environment (Dacey and Klug, 1979).

During the summer growth period, the plant extends above the lake surface, and allows rapid diffusive exchange with the atmosphere. Considering the fact that the bulk of plant biomass is still buried in the sediment, significant rates of exchange between the plant and the sediment water should be expected. In a sense, the gas spaces of the lacunae represent an extension of the atmosphere into the sediment. In Appendix B, the possibilities for movement of gas to and from the sediment water are discussed from a purely physical perspective. In that appendix, the term "interphase" is borrowed from mineralogy to emphasize the role of the lacunar gases as an "interfacial phase". The continuity of this interphase suggests a possible net flux of gas in one direction or the other, depending on a number of physical and biological factors. Such a net flux could have great significance in plant metabolism.

In the next chapter, the idea that the plant may act as a source or sink for atmospheric gases is investigated. It is in this study that a pattern of discrepance becomes apparent, leading to the development of hypotheses fundamentally altering modern conceptions of the factors regulating the distribution of gases in these plants.

# CHAPTER 2

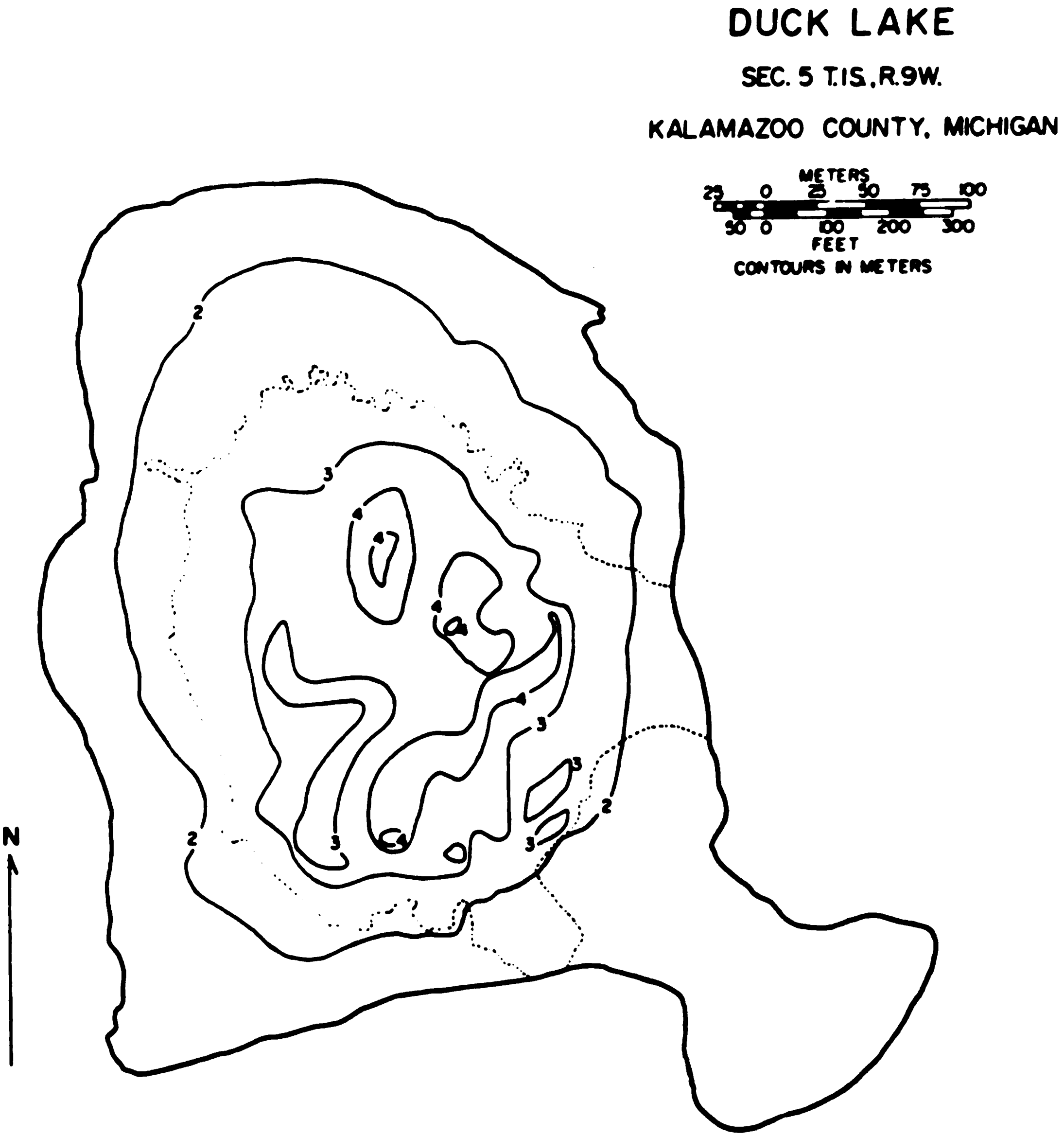
# LIFE BETWEEN PHASES

As described by Sculthorpe (1967), there is typically a zonation of plant growth habits along the shores of lakes: Emergent plants in the shallow water, floating-leaved in deeper water, and submersed plants in still deeper water. Although there are exceptions in any littoral zone, it does point out a relationship between the plants and their environment that is relevant to this study. It suggests that the waterlilies, as floating-leaved plants, should represent the deepest extension of atmospheric gases (at roughly atmospheric pressure) into the submerged sediment. Although most submersed plants do actually reach the surface when they flower (a vestige of their terrestrial origin), they cannot be said to grow with their leaves in the atmosphere. This is important, because the effects predicted in Chapter 1 should be most easily observed in plants extending from deep sediments to the surface.

<u>Duck Lake</u> (Kalamazoo Co., Michigan: $42^{\circ}24'30''$N, $85^{\circ}22'57''$W)

The plants used in this study grew along the perimeter of Duck Lake, from the shoreline out to a depth of roughly 2 m. Duck Lake is a kettle lake (mean depth 2 m; maximum depth 4 m), with no surface inflows or outflows (Figure 2). The total area of the lake

Figure 2.--Duck Lake
The dashed line represents the outer extent of *Nuphar* and *Nymphaea* growth in the lake. The map is adapted from Miller (1972).



DUCK LAKE

SEC. 5 T.1S.,R.9W.

KALAMAZOO COUNTY, MICHIGAN

is 12.6 hectares; roughly half of that is covered by a dense stand of waterlilies (mainly Nuphar luteum, and some Nymphaea odorata). These plants grow along most of the lake perimeter, while most of the central area of the lake is occupied by a dense population of Ceratophyllum demersum, coontail. Some physical and chemical parameters of the central region of the lake for 1968-1969 are presented by Miller (1972).

## Nuphar luteum

The yellow waterlily perennates in lakes by means of a horizontal creeping rhizome at or just below the surface of the sediment. The rhizome may be up to 10 cm in diameter and several meters long, accounting for roughly 80% of the plant's biomass during summer growth (Twilley et.al., 1977).

The rhizomes branch extensively, as shown in Figure 3, and each growing shoot apex bears a rosette of leaves (and flowers[1]) that rise up through the water column on long petioles. In Duck Lake, the leaves commonly float on the lake surface for variable lengths of time, and then rise up above the lake surface. This further elongation of the petiole does not appear to influence the gas exchange properties of the leaves, so throughout this discussion, leaves are called "emergent" once they have reached the lake surface. This result is presumably due to the fact that the stomata of the waterlilies are found only on the upper surface of the leaves (Sculthorpe, 1967).

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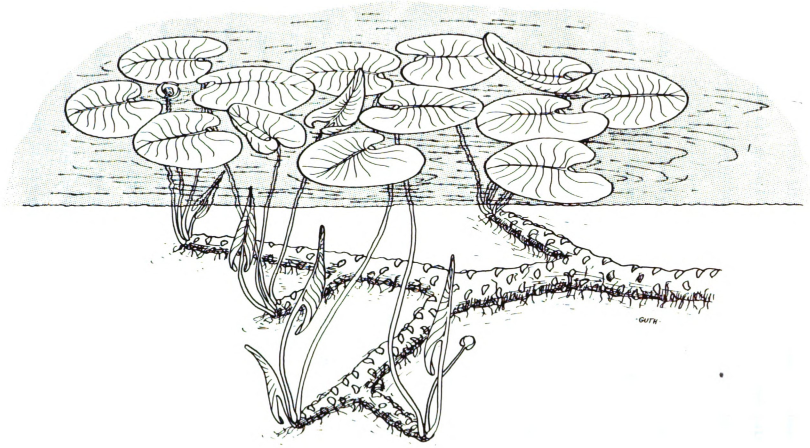
[1] It is an unusual feature of many waterlilies, including Nuphar, that their solitary flowers are neither axillary or terminal; they are borne on leaf sites in the parastichies (phyllotactic helices). (cf., Weidlich, 1976)

Figure 3.--Growth habit of the yellow waterlily during early spring growth.


GUTH

As the branching rhizome grows forward through the sediment, adventitious roots grow down into the sediment. Some of these roots are contractile, and act to pull the buoyant rhizome down into the sediment. These roots appear to grow as far as a meter into the sediment.

Much of the rhizome is leafless, and bears only the leaf scars from previous years' growth and, of course the adventitious roots (cf., Figure 3). The branching rhizome appears to be the dominate means of plant reproduction within a lake. As the rhizome elongates at its apices, it decomposes at an indeterminate rate in its older parts. The eventual decay of branching points leads to the vegetative reproduction of an individual plant into two.

### The Waterlily as Experimental Plant

In order to understand the dynamics of the gas phase within a plant, it is necessary to get analyzable samples from discrete regions of the plant. Much of the published data on these dynamics has depended on the extraction of gases from isolated plant parts under reduced pressure. The large volumes of gas within the tissue of the yellow waterlily make them ideal for gas sampling. Samples can be withdrawn by syringe, and can subsequently be injected directly into a gas chromatograph for compositional analysis. Furthermore, this sampling can be done *in situ*, avoiding the serious disturbances to plant integrity and metabolism that other methods cause.

The actual percentage of plant volume occupied by gas in the waterlily approaches 60% in the petioles, and lies between 20% and 40%

in the roots and rhizome.[1] Not surprisingly, the large volumes occupied by gases are a reflection of the role of gases in plant metabolism. As has already been mentioned, the dynamics of gases have been studied in a number of plants, but one of the most prominent is a study by Laing (1940, 1940b) on "*Nuphar advenum*" in lakes in the vicinity of Ann Arbor, Michigan.

The classification of waterlilies is confused, particularly in the genus *Nuphar*. Under the classification system of E.O. Beal (1956), the plant described by Laing would be called a subspecies of *N. luteum*. Under Beal's classification system the dominant yellow waterlily in Duck Lake would probably be called *N. luteum* ssp. *macrophylla*. Specimens have been placed in the herbaria of the W.K. Kellogg Biological Station and of Michigan State University, East Lansing.

## Plant Gas

Gases were normally withdrawn from the plants by means of a remote-port system according to the protocol described in the caption

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[1] Cores of plant tissue were taken from plants with a cork-borer. The gas volume-percentages of the cores were determined by two different methods. In one case, the cores were flushed with a gas mixture of known composition (i.e., 10% $CH_4$). The cores were then forced into bottles of known volume, the bottles were stoppered, and the gases were allowed to equilibrate inside the bottles. The equilibrium concentrations of $CH_4$ in the bottles were used to calculate the volume of $CH_4$ in the tissue cores, and therefore, the total gas volume in the cores.

The second method relied on the change in specific gravity of fresh and water-flooded cores. The cores were weighed in air, then placed under water and a vacuum was applied (roughly 0.05 atm for several hours). The pressure in the chamber was returned to atmospheric, forcing water into the lacunae vacated by the expanding gas. The weight increments were used to estimate the initial gas volume in the cores.

to Figure 4, and the sample syringes were stuck into numbered butyl rubber stoppers. Samples were normally held for no more than 4 hours before analysis.

This system allowed repeated sampling over several days from various points around the plant, without repeatedly puncturing the plant whenever samples were to be taken. Since most sampling sites were underwater, water leaks tended to be a problem, particularly in the rhizome. The eventual flooding of tissue at the sampling point was retarded by sealing the sampling needle with silicone robber in the manner illustrated in Figure 4.

## Sediment Gas

Sampling of sediment gas was carried out in two distinct fashions. The quicker and more direct method involved the trapping of bubbles dislodged from the sediment. A large funnel was stoppered at its spout, filled with water, and inverted over the sediment. It was then forced down into the sediment and vigorously agitated, thereby dislodging bubbles from the sediment interstices. Samples were withdrawn from the inverted funned by syringe and analyzed by gas chromatography.

The second method involved the analysis of dissolved gases collected from equilibrated dialysis samplers (Molongoski and Klug, in prep.; Hesslein, 1976; Winfrey and Zeikus, 1977). As soon as the dialysis sampler was withdrawn from the sediment and quickly rinsed, the equilibration ports were covered with a molded silicone rubber sheet (General Electric RTV-60) to stop gas exchange with the atmosphere. This operation was completed within 15 seconds of the sampler's

Figure 4.--Remote port sampling system.

Gas samples were withdrawn from various parts of the plant *in situ* through a 23 gauge needle and microbore tubing leading to a gas-tight port at the surface. The total volume of this system was roughly 0.5 ml, so a preliminary sample up to 1 ml was withdrawn to flush the system and rinse the sample syringe. The needles inserted into plant tissue were usually supported by a Tygon sleeve and silicone rubber sealant (G.E., RTV-11) to prevent damage to the plant and retard leakage of water into the plant.

arrival at the lake surface. Since only those ports that contained water used for atmosphere-sensitive analyses were covered by the rubber sheet, water temperatures were quickly taken along the length of the sampler. Water samples were then withdrawn through the rubber sheet by means of "home-made vacutainers" (Figure 5), and the pH of the water in the ports was measured by peeling back the rubber sheet from each individual port at the time of analysis.

After several hours of equilibration at a known temperature, the pressures of the flasks were measured by water manometry. The composition of the gas in the headspace was determined by withdrawing gas samples through the stopper, making sure to avoid atmospheric contamination. The samples were analyzed by gas chromatography.

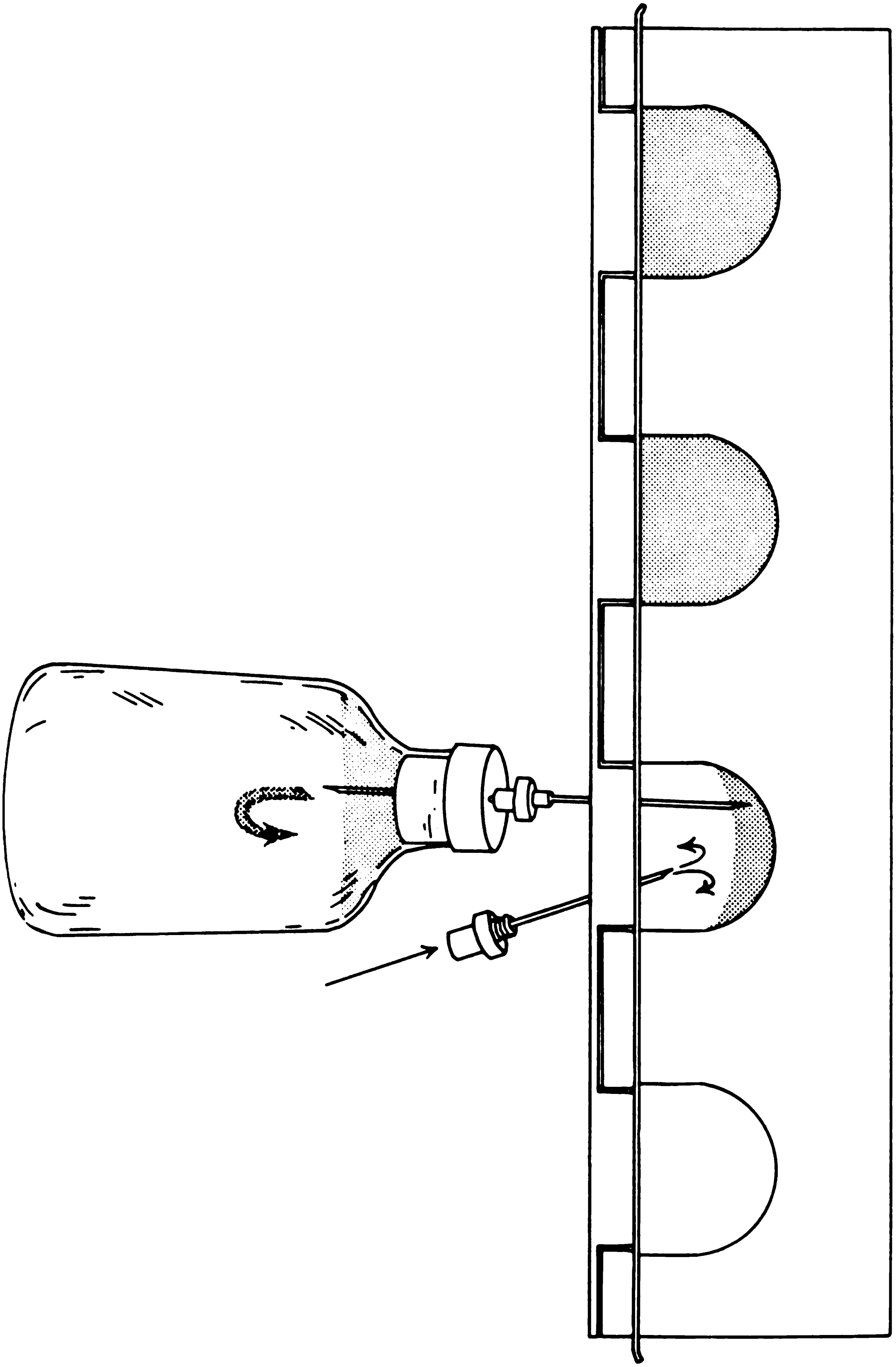
All headspace estimates were then used together with the partitioning coefficients (Henry's law constants) of the various gases at the equilibration temperature to calculate the total amount of each gas in the original water sample.

The $CO_2$ concentrations measured in the headspace cannot, however, be used as direct estimates of the $CO_2$ content of the pore water. During the equilibration between the headspace and the water sample, there is an equilibration of the bicarbonate buffer system in the water which tends to force more $CO_2$ into the gas phase.

The estimation of $CO_2$ partial pressures in the sediment water requires that the sample water be acidified in order to get an estimate of the total dissolved inorganic carbon pool size, $C_T$. The sample water was acidified by injecting 2 ml 3% $H_3PO_4$ into the serum bottle. The bottle was allowed to equilibrate for several hours, the pressure was again measured (this time by mercury manometer, since a

Figure 5.--Home-made vacutainers.

These were designed to speed and simplify the collection of atmosphere-sensitive water samples. In this application, 58 ml serum bottles were stoppered and rinsed with helium by means of needles through the thick butyl rubber stoppers. The helium atmosphere in the bottle was then evacuated to a predetermined pressure, thereby determining the volume of water to be withdrawn by the sampler from the port. When samples were to be taken from the dialysis port, the rubber sheet was punctured by two needles. One of these needles also punctured the stopper on the serum bottle and the vacuum in the bottle draws up the predetermined volume of water. During this study, the vacutainers were tared before sample collection, and reweighed after collection to accurately determine the volume of water actually drawn into the bottle.



considerable amount of de-gassing takes place from the water). The $CO_2$ content of the headspace was then determined by gas chromatography or IR analysis. The estimate of $C_T$ can then be used together with the pH and temperature measured at the time of sampling from the dialysis sampler to calculate an algebraic estimate of the partial pressure of $CO_2$ in the pore water.

As suggested earlier, the two methods for determining gas composition in the pore water have different applications. The inverted funnel method yields good estimates of $CH_4$, Ar, and $N_2$, without any regard for the depth distribution of the gases. This method consistently underestimates the pressures of $CO_2$ because of its high solubility in water, tending to dissolve inside the funnel.

The dialysis sampler gives good estimates of $CH_4$ and $CO_2$. Atmospheric gases, such as Ar and $N_2$, can be estimated by this method, but these values depend on complete rinsing of the bottle with helium and an absence of leaks of atmospheric gas into the bottle after evacuation (before sampling). The dialysis sampler has the general advantage of giving precise depth profiles of the dissolved gases. However, in this study these profiles are of limited utility, since the distribution of the exchange surface presented by the plant in the sediment is not known. The roots of the waterlily extend up to a meter into the sediment, and it is reasonable to conclude that the actual gas exchanges that occur are a weighted function of the depth profile of the gases.

## Chromatographic Analysis

Gas samples were withdrawn in ½ and 1 ml Glaspak tuberculin syringes and were analyzed by gas chromatography. $CH_4$ and $C_2H_6$ (Chapter 3) analyses were performed on a Varian 600 with a flame ionization detector; gas separations were achieved in 1 meter of 1/8th inch column packed with Porapak N.

Analyses of total gas composition (i.e., $N_2$, $O_2$, $CO_2$, $CH_4$, and Ar) were accomplished with a Carle BGC-8500 fitted with two columns (each 1 meter long) in series, and four valves to facilitate separation of component gases. $CO_2$ and $H_2O$ were separated from the other constituent gases on the first (Porapak Q) column. The valving arrangement allowed these gases to bypass the downstream column, passing directly to the detector (thermistor TC). These gases could also be backflushed to vent, as was usually done in the case of water vapor. The downstream column (Molecular Sieve 80/100) achieved the separation of $O_2$/Ar, $N_2$, and $CH_4$. This packing does not separate $O_2$ and Ar, so an external column of hot copper filings was used in series to remove $O_2$ from samples, thereby allowing a determination of Ar (and thus, by difference, $O_2$). Since Ar was always present at levels less than 1%, it was ignored in the determination of $O_2$ when $O_2$ concentrations exceeded 10%. When $O_2$ concentrations were less than 10%, the levels of Ar become a significant source of error, so the $O_2$-reduction technique was used.

Quantification of the partial pressure of water vapor was achieved on the same chromatograph, using a 1 meter column of Porapak QS.

Room air was used as a standard for $N_2$ (78%), $O_2$ (21%), and Ar (0.9%) when their composition was near atmospheric. $O_2$ was the only one of these gases that deviated significantly from atmospheric. At concentrations less than 10% $O_2$, a standard curve for $O_2$ was constructed by the serial addition of room air (21% $O_2$) to a helium-rinsed bottle of known volume. $CO_2$ standard curves were constructed in like fashion using pure $CO_2$ (Linde, 99.8% minimum). Methane standards were supplied by Linde (10%, Custom Grade; 540 ppm, Custom Grade) and standards outside these ranges were made by adding known volumes of pure $CH_4$ (Linde, 99% minimum) to a bottle of known volume.

Gas samples were injected into the chromatograph by two different means: on-column injections of 0.4 ml and gas sample loop injections of 0.2 ml (via gas sampling valve). The latter method was preferable as a result of its decreased variability — its coefficient of variation was found to be roughly half that for the on-column injection. It has the disadvantage of requiring larger volumes of gas (at least 1 ml) in order to rinse the sampling loop prior to injection. The loop was used whenever possible in this study.

### Spring Emergence of the Waterlily

In Chapter 1, it was pointed out that gases tend to equilibrate according to their partial pressures. As a result, a gas phase in equilibrium with the gas-saturated sediment water will tend to take on the composition and pressure of the bubbles in the sediment water. This phenomenon, never before described in the ecology of aquatic plants, was observed by measuring the pressure and composition of the gas phase in the lacunae of rhizomes that had lain dormant in the sediment throughout the winter. A water manometer was employed to measure the pressure in plants situated in about 40 cm water (rhizome depth). Gas samples were also taken from these rhizomes and bubbles were taken from the sediment pore water; these data, all means of two or more samples, are presented in Table 1.

Table 1 Late Winter Plant and Sediment Gases

| Sample Source | $CO_2$ | $CH_4$ | $N_2$ | $O_2$ | Pressure (cm water over barometric) |
|---|---|---|---|---|---|
| Petiole #1 | 6% | 35% | 56% | 1% | >10 cm |
| Petiole #2 | 3% | 37% | 53% | 2.2% | 23.5 cm |
| Petiole #3 | 4% | 36% | 53% | 1.3% | 27 cm |
| Petiole #3 (after bubbling overnight) | 5% | 38% | 53% | 0.6% | 27 cm |
| Sediment Bubbles | 5% | 40% | 51% | - | 40 - 50 cm |
| Sediment Bubbles | 4% | 47% | 51% | - | 40 - 50 cm |

The most important point illustrated by the data in Table 1 is the fact that the pressurization of the gas phase in the rhizome results from an equilibration of the lacunar gases with the interstitial water gases. In all the samples shown, the pressure exerted by $O_2$ in the lacunar gas phase (1% is equivalent to 10 cm water) is less than the pressure increment in that phase. This proves unequivocally that photosynthesis does not play a significant role in this pressurization.

Except for carbon dioxide, the concentrations of the other principal gases in the plant lie between the composition of the sediment and the atmosphere. This is exactly what would be expected in such a case, where parts of the waterlily extend up into the water column.

It is impossible to say from these data whether the apparent concentration of carbon dioxide is due to plant respiration or simply an artifact of the gas collection procedure. As described above, the funnel collection method always underestimates $CO_2$ concentrations in comparison with the dialysis samples. It's likely, therefore, that $CO_2$ concentrations in the late winter sediment also exceed the concentrations found in the lacunae.

The highest pressures observed in plant tissues were seen in the first emerging leaves of Nymphaea odorata. When such leaves were torn, sustained streams of bubbles were forced from the torn site, indicating that the pressure in the gas phase in the plant exceeded the hydrostatic pressure at the depth of the leaf. Data on such leaves are presented in Table 2.

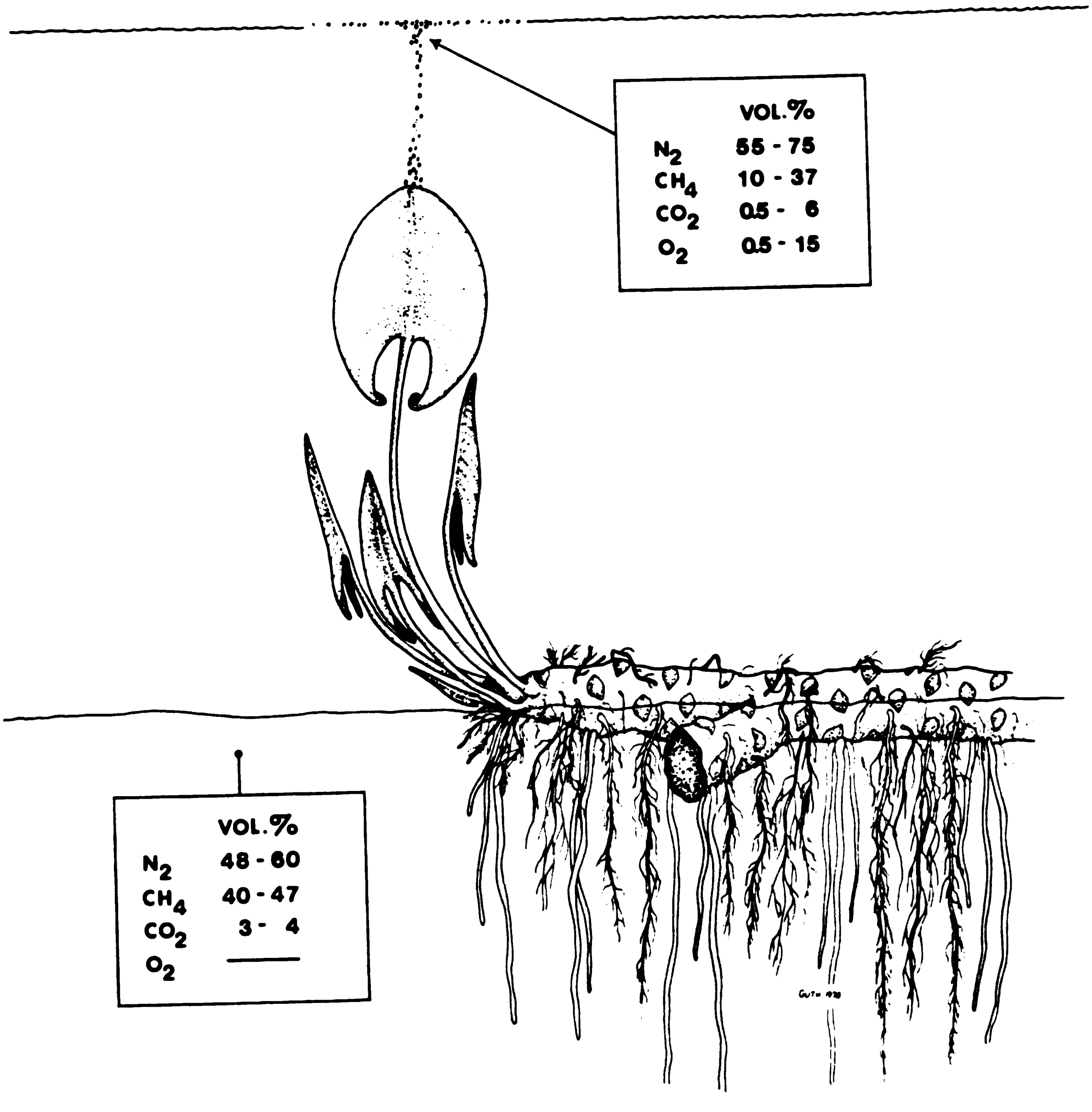
Table 2 Gas Compositions in Late Winter Nymphaea

| Sample Source | $CO_2$ | $CH_4$ | $N_2$ | $O_2$ | Pressure (cm water over barometric) |
|---|---|---|---|---|---|
| Torn Leaf | 5% | 39% | 53% | 2% | 70 cm |
| Torn Leaf | 5% | 35% | 52% | 2.5% | 60 cm |
| Torn Leaf | 4% | 34% | 57% | 2.4% | 40 cm |
| Sediment Bubbles | 4% | 40% | 51% | — | 80 - 100 cm |
| Sediment Bubbles | 3% | 42% | 60% | 1% | 70 - 100 cm |

Tables 1 and 2 show that there is a strong tendency for the gas in the lacunae to equilibrate with the gases dissolved in the interstitial water while the plant is submerged in winter. As the leaves grow towards the surface in spring, they encounter ever-decreasing hydrostatic pressure, and the gases inside the leaf tend to burst out through any tears in the leaf surface. (Figure 6) Once such a leaf is torn, bubbles may stream from the opening for several hours. Given the growth habit of Nuphar, it's likely that the volume of bubbles that emerge from any such leaf reflect the volume of the rhizome being vented. The submerged interconnections of the various shoot apices also leads to the result that not every emerging leaf exhibits this bubbling effect. Presumably, once one leaf has vented to the atmosphere, bubbles will not stream in this fashion from any other leaf connected by a common rhizome.

This pressurization phenomenon can be duplicated during the summer by removing all the leaves from an isolated section of rhizome. In Table 3, pressure and compositional data are shown for the gases in one such rhizome growing in about 140 cm water. The dash marks in the table mean that no data are available.

Figure 6.—Spring leaf emergence.
The gas composition of the sediment is expressed in terms of the composition of equilibrated bubbles in the sediment. During winter, the internal gas phase of the rhizome has approached equilibrium with the dissolved gases in the sediment. As the first leaves rise towards the surface in spring, sustained streams of bubbles escape from torn leaves.



|  | VOL.% |
|---|---|
| $N_2$ | 55 - 75 |
| $CH_4$ | 10 - 37 |
| $CO_2$ | 0.5 - 6 |
| $O_2$ | 0.5 - 15 |

|  | VOL.% |
|---|---|
| $N_2$ | 48 - 60 |
| $CH_4$ | 40 - 47 |
| $CO_2$ | 3 - 4 |
| $O_2$ | ——— |

Table 3 Pressurization of a Leafless Rhizome

| Day # | Hour | $CO_2$ | $CH_4$ | $N_2$ | $O_2$ | Pressure (cm water) | Depth of shallowest leaf |
|---|---|---|---|---|---|---|---|
| 1 | 1200 | 9% | 10% | 74% | 5% | 0.0 cm | —<br>(= not determined) |
| 1 | 1800 | 9% | 11% | 72% | 5% | — | — |
| 2 | 1200 | 10% | 14% | 71% | 2% | 50 cm | — |
| 3 | 1800 | 11% | 20% | 63% | 6% | 118 cm | — |
| 4 | 1800 | 11% | 23% | 61% | 4% | 111 cm | — |
| 5 | 1100 | 12% | 25% | 60% | 1% | 64 cm | 36 cm |
| 6 | 1700 | 13% | 27% | 58% | 1% | 54 cm | 30 cm |
| 7 | 1500 | — | 26% | 54% | 2% | 38 cm | 29 cm |
| 9 | 1500 | 11% | 32% | 56% | 2% | 31 cm | — |
| 10 | 1200 | 12% | 31% | 54% | 1% | 22 cm | 6.5 cm |
| 11 | 1100 | 12% | 31% | 52% | 1% | 0.0 cm | at surface |

It is important to realize that the rapid increase in gas pressure is not simply due to the flooding of the plant tissue through the cut surfaces of the petioles. The lacunae of the yellow waterlily are constructed so as to restrict the flow of water into this tissue. Furthermore, in a similar experiment on another rhizome, bubbling was observed to flow from one of the cut-off petioles several days after the leaves had been removed. This type of ebullition shows that the pressurization mechanism lies in the buried parts of the plant, since the lacunae of the petiole are occupied by gas phase.

Since the only way the pressure can be diminished in such a rhizome is for it to lose gas to the water column, the pressure in the rhizome correlates with the depth of the shallowest petiole or leaf. In the case of Table 3, the petioles were all cut off very near the rhizome. As the first leaf rose toward the lake surface, it allowed the escape of gases, presumably by ebullition. Once the first leaf reached the surface, the pressure in the rhizome dropped toward barometric pressure.

## Leaf Efflux of Methane

From the data in Table 3, it is clear that methane shows a strong tendency to enter the lacunae of the roots and rhizomes of the yellow waterlily. A cursory survey of the distribution of methane in the lacunae during the summer showed it to be present throughout most of the plant, with the highest concentrations in the roots and the lowest in the leaves (Figure 7). This gradient from roots to leaves suggests a diffusive flux of methane into the atmosphere.

The rate of flux was measured by tying gas-impermeable Saran bags over individual leaves, tying them closed around the petiole of the leaf (Figure 8). A study into the ecological significance of this flux through the plant showed that it accounted for 75% of the methane escaping from the littoral sediment of Duck Lake during two weeks in August (Dacey and Klug, 1979). The remainder escaped by ebullition directly from the sediment. Previously, investigators had considered ebullition to be the major transport mechanism for the escape of methane to the atmosphere, followed by a slower surface exchange (driven by a gradient of methane partial pressure between the surface water and the atmosphere). The observed $CH_4$ flux through the plant introduces another pathway for methane transport. It's important to understand all these pathways, since a loss of methane from an ecosystem represents a loss of both carbon and energy from that system.

Figure 7.—Methane distribution throughout the plant during summer growth.
Methane enters the roots and rhizome and escapes to the atmosphere through emergent leaves. The concentration of methane in the internal gas phase is highest in the roots and older leafless parts of the rhizome, decreasing towards the shoot apex.

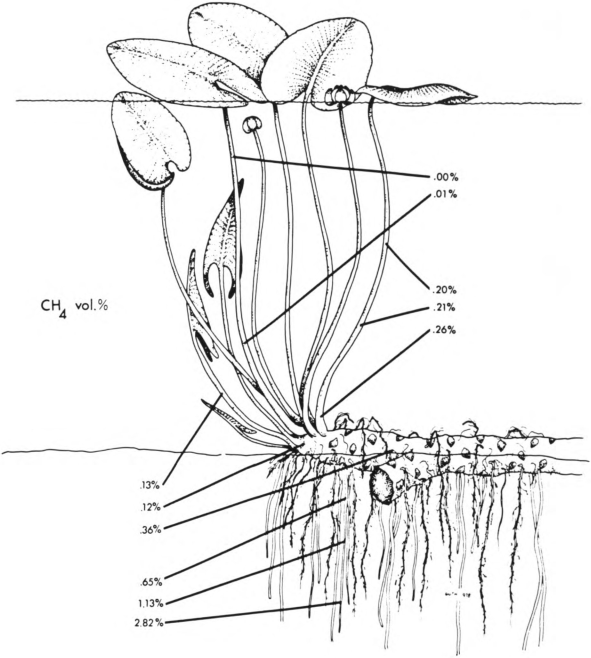
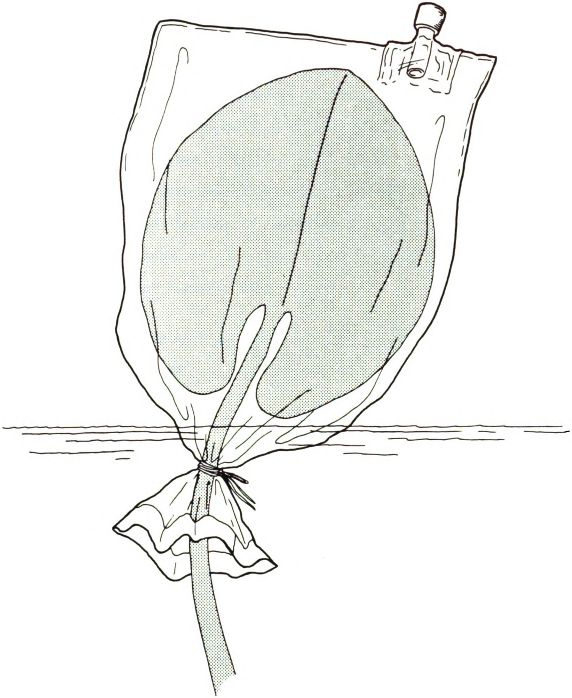

$CH_4$ vol.%
.00%
.01%
.20%
.21%
.26%
.13%
.12%
.36%
.65%
1.13%
2.82%

Figure 8.--Bag on emergent leaf.
A gas-impermeable Saran bag was placed over a single leaf and tied closed around the petiole. At the end of a run, the composition of the gas in the bag was determined by taking a sample by syringe through the stoppered port at the top of the bag. The volume of the bag was then determined by injecting an internal standard into the bag, gently squeezing the bag to mix the gas, and then re-sampling. In cases where the changes in bag volume over a time interval were monitored, the volume was also determined at the beginning of the run by the same internal standard method.



These experiments with bagged leaves yielded some other interesting results. Shading an individual leaf in a bag, or adding $CO_2$ (up to 3%) to the bag appeared to have no impact on the rate of methane efflux from the leaf. This corroborates current ideas that the function of these stomata is limited (Gessner, 1959).

It also became clear in the course of this study, that the rate of methane efflux was directly related to the age of the leaf. In Table 4, the rates of efflux from 6 leaves of a shoot apex are presented along with data on the length of the petioles (from rhizome to leaf) and the distance of insertion point of each leaf from the shoot apex. Since the leaves arise at the shoot apex, the oldest leaves are those farthest from the apex.

Table 4 Rate of $CH_4$ Efflux as a Function of Leaf Age

| Distance of petiole insertion from apex | Length of petiole | Rate of $CH_4$ efflux from the leaf |
|---|---|---|
| 8.0 cm | 61 cm | Not detectable |
| 9.0 cm | 85 cm | 1.0 ml/hr |
| 9.5 cm | 87 cm | 1.4 ml/hr |
| 10 cm | 90 cm | 1.6 ml/hr |
| 13 cm | 101 cm | 2.6 ml/hr |
| 15 cm | 112 cm | 3.0 ml/hr |

The most immediate explanation for the differential rate of methane efflux lies in an examination of the methane distribution data shown in Figure 7. The oldest leaves insert into the more basal region of the rhizome, and the concentrations of methane in the lacunae of these regions are higher than in the more apical regions. As will be shown in Chapter 3, there are other reasons for this pattern of efflux, and the explanation lies in the pattern of fluid flow of gases within the plant.

## Fluid Flow

It was found that by tearing a leaf, there was an immediate increase in the concentration of methane in the petiole of that leaf. The data shown in Table 5 are representative of a number of such experiments.

Table 5 Methane Increases in the Petiole of a Torn Leaf

| Distance along petiole from leaf | Time after leaf was torn | | | |
|---|---|---|---|---|
| | 0 min | 4 min | 14 min | 22 min |
| 3 cm | 0.04% $CH_4$ | .08% | .15% | .15% |
| 18 cm | .29% | .40% | .52% | .67% |
| 33 cm | .47% | .61% | .78% | .84% |

If the methane were moving from the rhizome up the petiole to the atmosphere simply by means of a diffusive flux, damaging the leaf should have exactly the opposite effect. It should decrease the methane concentration at any point along the length of the petiole. The fact that it increases means that the flux of methane is not simply diffusive; it has a fluid flow component. The total pressure of the gases in the petiole must have been slightly greater than barometric, and when the leaf is torn, the pressure is vented to the atmosphere by an accelerated fluid flow.

Other lines of evidence support this conception of fluid flow. It had been casually noted throughout much of the study that the volume of bags tended to increase during their period of attachment to leaves losing methane. Data on the rates of change of bag volume will be presented in Chapter 3.

The theoretical treatment of the lacunar "interphase" in Chapter 1 demonstrated that a fluid flow of gas might be expected simply on the basis of differential rates of exchange between the plant and the sediment water. In order to understand these flows, it was necessary to develop a method for studying them.

The method used most extensively involved the injection of a tracer gas (usually ethane, $C_2H_6$) at a point along the length of a petiole, followed by sampling of gas compositions at some distance from the point of injection (Figure 9). By this means it was possible to construct tracer concentration-time curves of the same form as the elution curves for chromatography (Figure 10). As the tracer gas moves along the petiole (carried by the fluid flow of the other gases there), it diffuses into those gases and may also be absorbed by the plant tissue. As a result, the initial spike of tracer gas at the injection point broadens into a slightly tailing peak typical of gas chromatograms as it is carried along in the petiole. The maximum in the curve (Figure 10) can be taken to represent the point where the tracer was injected into the flow.

The volume of gas in a given segment of petiole was estimated by measuring its upper and lower circumferences and the distance between the two.[1] The rate of volume flow was then calculated on the basis of

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[1] A regression was generated for the area predicted from the circumference (measured by a string pulled tight around the petiole) against the area measured of impressions of petiole sections pressed onto graph paper. The volume of the petiole was calculated by assuming

Figure 9.--Tracer movement through the petiole. Tracer injected into a petiole during daylight moved along the length of the petiole in a "plug". In the case of efflux leaves, the rate of gas flow up the petiole was determined by injecting tracer into the lower end, and sampling sequentially at the upper end. In the case of influx leaves (discussed in Chapter 3), the direction of flow is reversed, so tracer was injected into the upper petiole and sampled sequentially at the lower end.

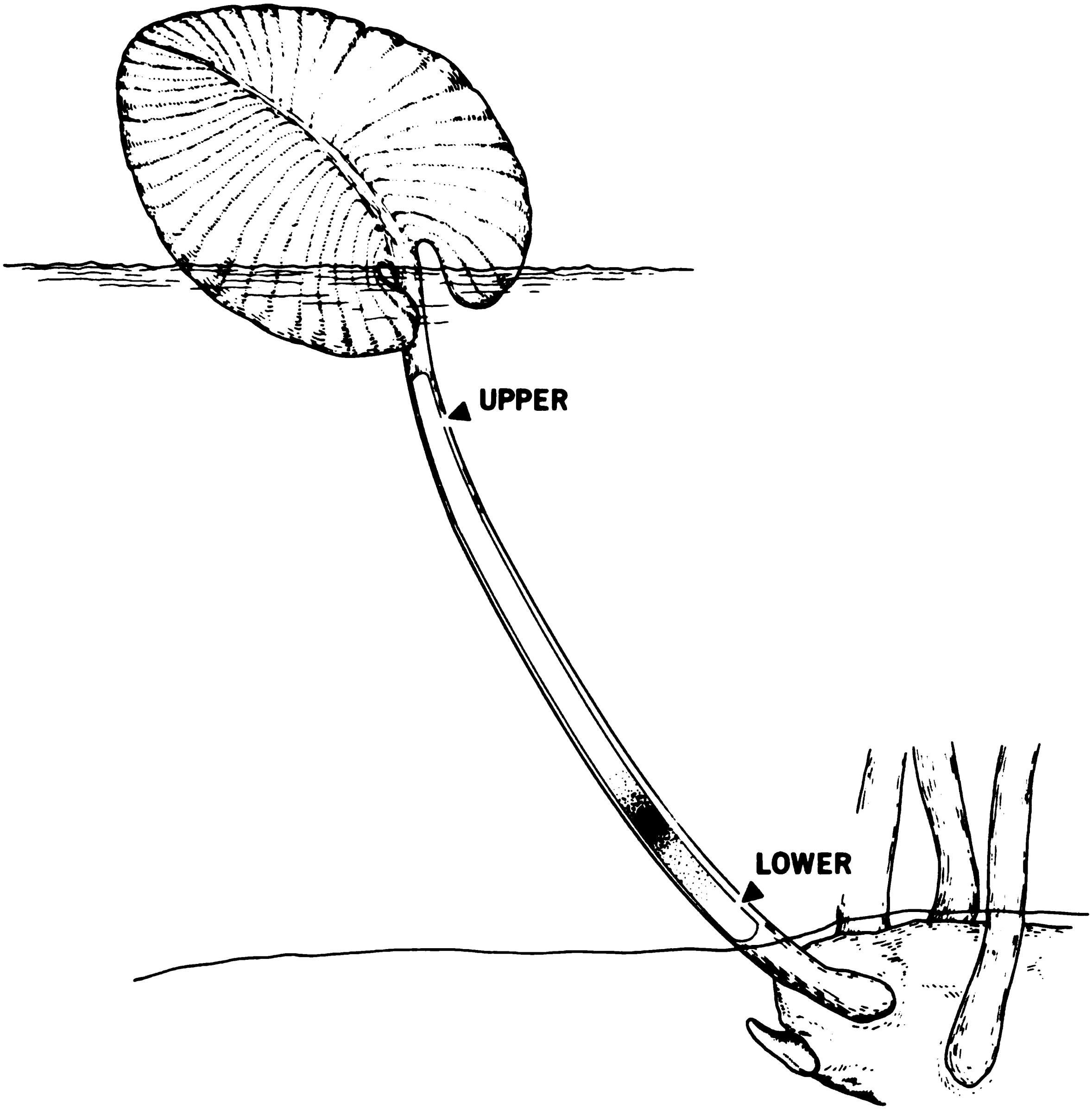
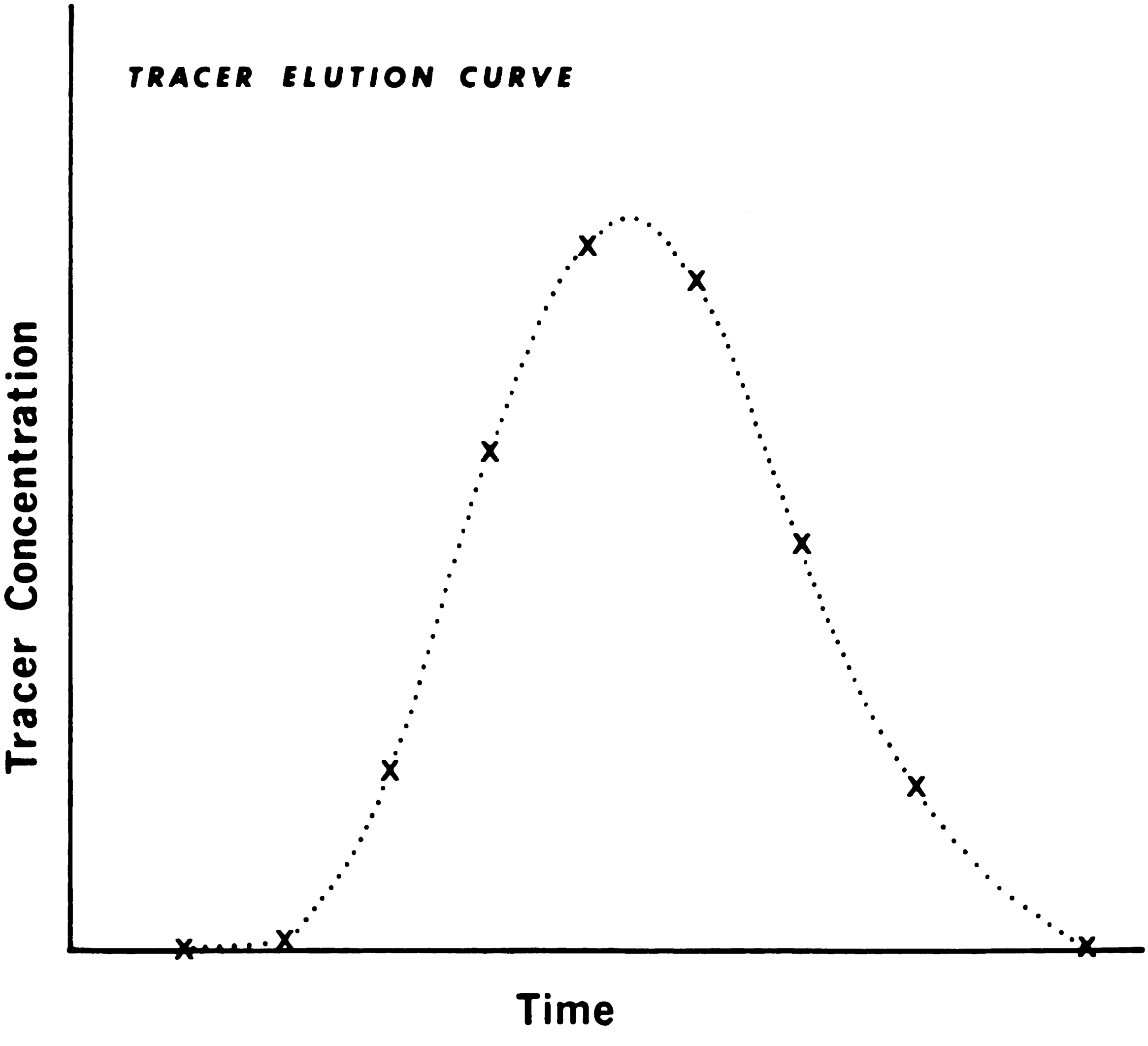

UPPER
LOWER

Figure 10.--Tracer elution curve.
This is the typical shape of the elution curve for the tracer "plug" when the tracer injection was made upstream of the sampling point. The time of the maximum was taken to represent the time required for the gas to be carried from the injection point to the sampling point.


TRACER ELUTION CURVE
Tracer Concentration
Time

the volume of the segment under consideration and the time elapsed between tracer injection and that time when the tracer maximum passed the sampling site.

The curve in Figure 10 can be integrated, so that on the basis of the rate of flow calculated for a particular experiment, the total amount of tracer passing the sampling point can be estimated. Typically 0.1 ml $C_2H_6$ was injected into the petiole, and it appeared that all (80% - 120%) of the tracer passes the sampling point whenever the sampling point was downstream of the injection point.[2] This proves unequivocally that the gas in the petiole moved in a fluid flow.

Early attempts in this study to measure the pressures that must be driving these flows were unsuccessful, so a simple lab experiment was set up to further investigate the flow. The rate of flow up a petiole was established for an intact plant growing in a tub in the lab. The petiole was cut off at the base, and the length of the petiole and its leaf were used to estimate the original pressure at the base of the petiole.

The basal end of the petiole was inserted into a large bore Tygon tube, and sealed with rubber (Rubbergel, L.D. Caulk Co.). The other end of the Tygon tubing was attached to a four liter erlenmeyer

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it takes the shape of a frustum of a right cone. Taking the gas spaces as 50% of the petiole volume, the volume of gas in any segment can be calculated without removing the segment from the plant.

[2] Rarely was tracer detected at a sampling point upstream from the injection point. This only occurred when flow rates were extremely slow, and the sampling point was near the injection point. Clearly, integrations of the type described here are meaningless under those circumstances.

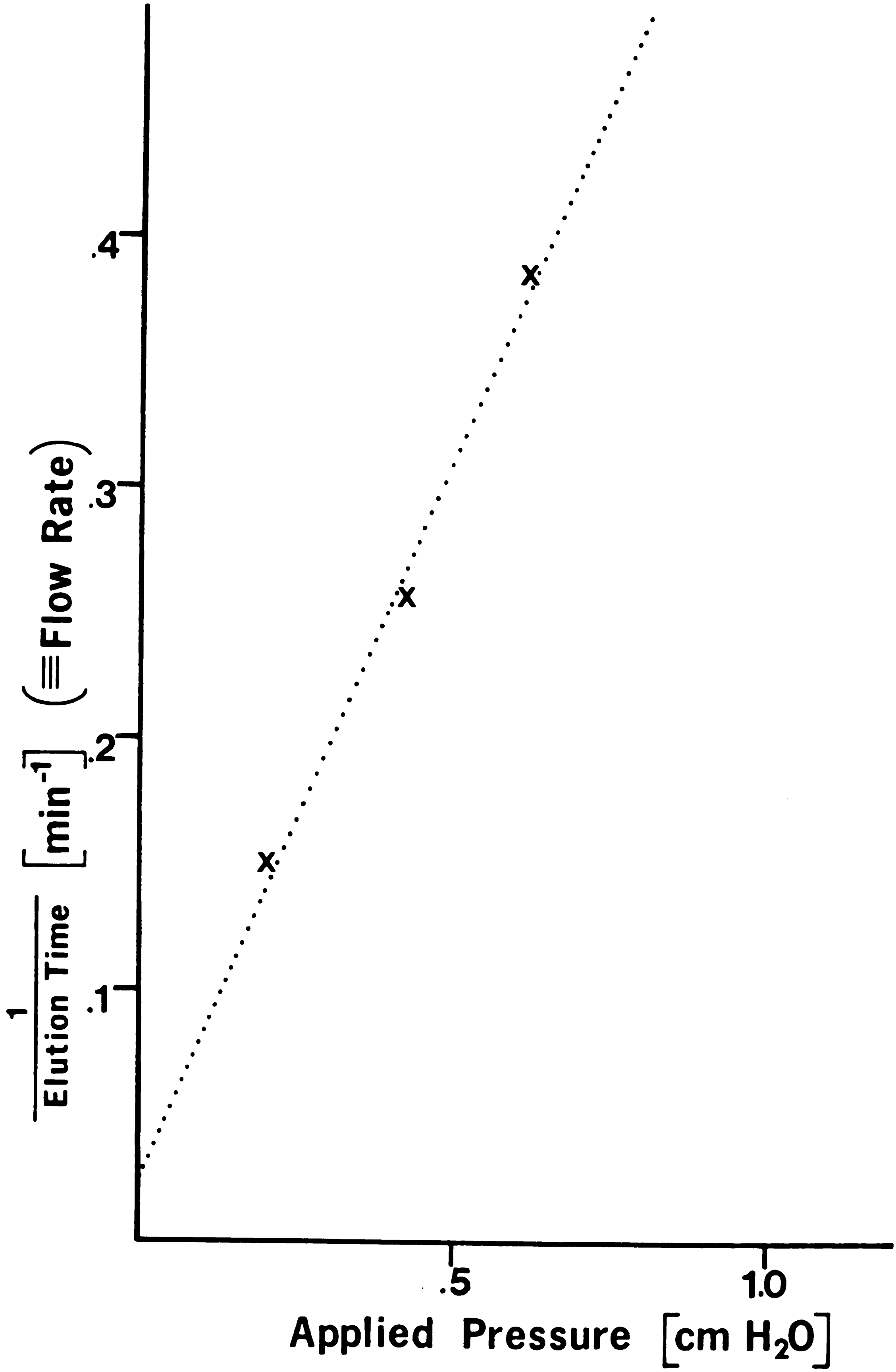
bearing a manometer, a high pressure gas inlet, and a gas outlet. The pressure in the flask was regulated by adjusting the flows of gas into and out of the flask; the pressure was monitored on the manometer. The tracer technique was applied in exactly the same way as it was for the intact plant, with tracer injected at the base of the petiole, followed by sampling at the upper end. The flow rates are shown as a function of pressure in Figure 11. Extrapolating these data to lower pressures, it appears that a pressure of 0.6 mm water forced the flow in the intact plant.

These experiments also demonstrate the applicability of Darcy's law for fluid flow through porous media (cf., Appendix C). The flows that occur in the petioles of these plants behave in the customary fashion — gas moves from a region of high pressure to a region of low pressure at a rate proportional to the pressure gradient.

Direct pressure measurements were obtained later for gases in the plants _in situ_, but only after certain improvements in the manometric system. These observations will be discussed in the next chapter.

It is impossible to conclude on the basis of the data presented thus far, that these flows are an example of net gas flow from the sediment to the atmosphere, as postulated in Chapter 1. In fact, there remains a major inconsistency in the data. The total volume flow of gas up individual petioles (as measured by tracer experiments) was found to be several orders greater than the volume of methane flow up the same petiole. For example, a leaf that loses several hundred milliliters of gas per hour may only lose several milliliters of methane during the same time interval. This means that the influx of methane

Figure 11.--Flow as a function of applied pressure. Since flow rate was determined by the tracer method, and the same segment of petiole was studied, the flow rates have been expressed as the inverse of the elution time (of the peak maximum). In this way, the linear rates of flow can be compared with that observed in the petiole before it was removed from the plant.


.1
.2
.3
.4
.5
1.0
1/Elution Time [min⁻¹] (≡Flow Rate)
Applied Pressure [cm H₂O]

from the interstitial water only contributes in a very small way to the observed flows. The same is true for the other significant gas in the interstitial water, $CO_2$. Clearly, there must be some other mechanism driving these flows; that mechanism is the subject of the next chapter.

# CHAPTER 3

# GAS CIRCULATION

## An Apparent Contradicition

Methane was found in emerging (not yet at the lake surface) leaves in concentrations approaching those in the rhizome (Figure 7) and exceeding the methane concentration in all but the oldest emergent leaves (Table 6).

Table 6 Methane in Submerged and Emergent Leaves[1]

| Leaf type | 0830 hrs | 2000 hrs |
|---|---|---|
| Submerged Leaf | 0.99% | 0.87% |
| Emergent Leaf (mean) | 0.38% | 0.32% |

Once such a leaf reached the surface, however, the methane concentration was usually undetectable (by thermal conductivity detector) during daylight, increasing dramatically at night (Table 7).

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[1] The concentrations shown are the volume percentage of methane in the petiole of an emerging (but still submersed) leaf and the mean volume percentage of methane in the remaining seven emergent petioles on the same rhizome. Some of the oldest emergent leaves had methane concentrations greater than that in the submersed leaf, reflecting their basal position on the rhizome (cf., Figure 7).

Table 7 Methane Concentrations in Newly-Emerged and Older Leaves[1]

| | Methane concentration | |
|---|---|---|
| Leaf type | 1530 - 1630 hrs | 0630 hrs |
| Newly-emerged | < 0.1% | 1.7% |
| Older, efflux | 0.3% | 1.7% |

The data in Table 7 strongly suggest that the drop in methane concentration in the youngest emergent leaves is the result of a gaseous exchange with the atmosphere. This idea is supported by other experiments where methane concentrations were found to increase in the petioles of young leaves after the upper surfaces of these leaves had been either coated with silicone grease or resubmerged (under water). During the investigation of methane efflux from leaves, it became evident that the young, newly-emerged leaves did not lose methane to the atmosphere (Table 4). This raises an important question about the distribution of methane throughout the plant. Hence the contradiction: there appears to be a significant exchange between the atmosphere and the lacunae of the young leaves, and yet there is no flux of methane from these leaves.

An investigation of the distribution of the other major gases in the lacunae corroborated this "anomaly" in methane distribution (Table 8). In Table 9, the average daytime distribution of $O_2$ and $CO_2$

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[1] The volume percentages of methane are condensed from the data in Table 8. Thus the estimates for the young leaves are the means of twelve determinations; those for the older leaves are the means of sixteen determinations. All samples were derived from a single shoot apex.

## Table 8 Gas Compositions throughout a Shoot Apex, August 19-21, 1978

The volume percentages of $CH_4$, $O_2$, $CO_2$, and $N_2$ are given on the next four pages. Each estimate is the result of a single analysis; an empty space means that the composition was not determined.

The petioles were numbered (youngest to oldest) according to apparent age at the beginning of the experiment. In these tables they are lumped according to their role in gas exchange: the three youngest leaves were influx leaves; the oldest four were efflux leaves. The lower sampling ports were all roughly the same distance above the sediment surface; the upper ports were just below the water line. The rhizome sampling port was basal to the oldest leaf, so it did not lie in the path of the flow-through system.

Table 8 **Methane** (vol %)

|  | Petiole rank by apparent age |  | Time<br>2030 hrs | 0030 hrs | 0615 hrs | 1630 hrs | 2115 hrs | 0630 hrs | 1530 hrs | 2030 hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| Influx | 1 | upper | < 0.1 | 0.1 | 0.3 | < 0.1 | < 0.1 | 0.4 | < 0.1 | < 0.1 |
|  |  | lower | 0.9 | 2.2 | 3.4 | < 0.1 | 1.1 | 3.1 | < 0.1 | 0.7 |
|  | 2 | upper |  | 0.2 | 0.5 | < 0.1 |  | 1.0 | < 0.1 | 0.1 |
|  |  | lower | < 0.1 | 1.6 | 2.8 | < 0.1 | 0.6 | 2.6 | < 0.1 | 0.6 |
|  | 3 | upper | < 0.1 | 0.2 | 0.4 | < 0.1 | 0.1 | 0.7 | < 0.1 | < 0.1 |
|  |  | lower | 1.1 | 1.8 |  | < 0.1 | 0.9 | 2.5 | < 0.1 | 0.3 |
| Efflux | 4 | upper | 0.6 | 0.6 | 0.9 | 0.1 | 0.2 | 0.5 | 0.1 | 0.1 |
|  |  | middle | 0.9 | 1.0 | 1.8 | 0.4 | 0.5 | 1.5 | 0.1 | 0.5 |
|  |  | lower | 1.2 | 1.9 | 2.8 | 0.1 | 0.9 | 2.7 | 0.1 | 1.0 |
|  | 5 | upper | 0.6 | 0.6 | 0.9 | 0.2 | 0.5 | 0.6 | 0.2 | 0.3 |
|  |  | lower | 1.2 | 1.9 | 2.8 | 0.3 | 0.7 | 1.1 | 0.2 | 0.6 |
|  | 6 | upper | 0.5 | 0.4 | 0.5 | 0.3 | 0.3 |  | 0.3 | 0.5 |
|  |  | lower | 1.4 | 2.0 | 3.0 | 0.4 | 1.1 | 2.6 | 0.3 | 1.1 |
|  | 7 | upper | 1.3 | 1.1 | 1.6 | 0.5 | 0.8 | 1.4 | 0.4 | 0.8 |
|  |  | lower | 1.4 | 1.8 | 2.7 | 0.5 | 1.1 | 2.3 | 0.7 | 1.0 |
|  | Rhizome |  | 7.7 | 8.4 | 9.6 | 7.9 | 8.0 | 7.1 | 7.9 | 7.5 |

**Table 8** (cont'd) Oxygen (vol %)

| | Petiole rank | by apparent age | Time | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2030 hrs | 0030 hrs | 0615 hrs | 1630 hrs | 2115 hrs | 0630 hrs | 1530 hrs | 2000 hrs |
| Influx | 1 | upper | 20.6 | | 17.3 | 20.9 | 19.3 | 16.8 | 20.9 | 20.3 |
| | | lower | 14.9 | 7.6 | 3.4 | 20.7 | 13.5 | 3.8 | 20.5 | 15.6 |
| | 2 | upper | | 19.4 | | 21.0 | | 14.8 | 20.7 | 20.2 |
| | | lower | 19.8 | 11.7 | 6.8 | 21.0 | 17.1 | 7.5 | 20.6 | 17.7 |
| | 3 | upper | 20.6 | 18.9 | | 20.0 | 20.0 | 15.6 | 20.8 | 20.5 |
| | | lower | 16.3 | 11.0 | | 20.8 | 14.5 | 5.9 | 20.7 | 18.5 |
| Efflux | 4 | upper | 19.0 | 15.7 | 13.2 | 21.2 | 19.3 | 16.4 | 21.0 | 19.9 |
| | | middle | 18.2 | 13.0 | 9.0 | 20.7 | 17.9 | 10.6 | 20.8 | 18.8 |
| | | lower | 15.6 | 9.9 | 5.7 | 20.4 | 15.5 | 6.3 | 20.1 | 15.5 |
| | 5 | upper | 20.0 | 17.3 | 15.5 | 20.6 | 18.6 | 16.7 | 20.9 | 19.7 |
| | | lower | 16.1 | 11.3 | 7.1 | 19.9 | 17.5 | 14.8 | 20.2 | 18.1 |
| | 6 | upper | 20.6 | 18.7 | 17.5 | 20.6 | 19.8 | | 20.1 | 18.8 |
| | | lower | 15.6 | 10.8 | 6.7 | 18.8 | 15.7 | 7.6 | 19.0 | 15.2 |
| | 7 | upper | 15.4 | 13.1 | 10.5 | 20.0 | 15.8 | 10.2 | 19.8 | 15.6 |
| | | lower | 14.3 | 10.6 | 6.8 | 18.9 | 14.8 | 7.0 | 18.2 | 15.3 |
| | Rhizome | | 8.3 | 6.2 | 3.2 | 8.8 | 8.3 | 7.4 | 8.7 | 9.1 |

Table 8 (cont'd) Carbon Dioxide (vol %)

| | Petiole rank by apparent age | | Time<br>2030 hrs | 0030 hrs | 0615 hrs | 1630 hrs | 2115 hrs | 0630 hrs | 1530 hrs | 2030 hrs |
|---|---|---|---|---|---|---|---|---|---|---|
| Influx | 1 | upper | 0.4 | 7.7 | 2.4 | 0.1 | 1.2 | 2.5 | 0.1 | 0.6 |
| | | lower | 3.7 | 7.7 | 14.6 | 0.2 | 3.7 | 7.9 | 0.2 | 2.9 |
| | 2 | upper | | 0.9 | 1.3 | 0.1 | | 2.4 | 0.0 | 0.5 |
| | | lower | 0.6 | 4.6 | 11.1 | 0.1 | 1.4 | 5.4 | 0.1 | 1.6 |
| | 3 | upper | 0.2 | 6.7 | 1.5 | 0.1 | 0.6 | 2.5 | 0.1 | 0.5 |
| | | lower | 3.2 | 9.5 | | 0.1 | 3.3 | 6.7 | 0.2 | 1.2 |
| Efflux | 4 | upper | 0.8 | 6.1 | 4.1 | 0.1 | 1.0 | 2.2 | 0.1 | 0.8 |
| | | middle | 1.0 | 6.0 | 5.7 | 0.3 | 1.3 | 4.0 | 0.2 | 0.8 |
| | | lower | 3.5 | 7.2 | 7.1 | 0.4 | 2.7 | 6.6 | 0.9 | 2.9 |
| | 5 | upper | 0.5 | 2.1 | 2.6 | 0.5 | 1.1 | 1.9 | 0.3 | 0.5 |
| | | lower | 3.5 | 5.4 | 11.9 | 0.9 | 1.7 | 2.0 | 0.9 | 1.4 |
| | 6 | upper | 0.1 | 1.2 | 3.0 | 0.7 | 0.5 | | 1.0 | 1.0 |
| | | lower | 4.3 | 5.6 | 6.5 | 2.2 | 3.1 | 5.6 | 2.0 | 3.5 |
| | 7 | upper | 4.1 | 10.4 | 5.1 | 1.3 | 2.9 | 4.4 | 1.4 | 3.2 |
| | | lower | 5.2 | 11.2 | 6.6 | 1.9 | 3.5 | 6.1 | 2.5 | 3.4 |
| | Rhizome | | 9.3 | 9.8 | 14.7 | 7.7 | 7.4 | 6.4 | 8.2 | 7.4 |

Table 8 (cont'd) Nitrogen (vol %)

| | Petiole rank by apparent age | | Time | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2030 hrs | 0030 hrs | 0615 hrs | 1630 hrs | 2115 hrs | 0630 hrs | 1530 hrs | 2030 hrs |
| Influx | 1 | upper | 78.6 | 78.9 | 79.1 | 77.7 | 78.6 | 79.3 | 78.3 | 78.1 |
| | | lower | 80.0 | 82.5 | 84.2 | 78.1 | 81.1 | 84.0 | 78.0 | 80.7 |
| | 2 | upper | | 78.9 | 79.4 | 78.0 | | 81.0 | 77.8 | 78.3 |
| | | lower | 78.9 | 81.5 | 83.7 | 78.0 | 79.4 | 83.4 | 77.9 | 80.0 |
| | 3 | upper | 78.6 | 78.8 | 79.3 | 78.0 | 78.4 | 80.0 | 78.0 | 78.6 |
| | | lower | 79.3 | 81.7 | | 77.7 | 78.1 | 83.6 | 77.7 | 79.8 |
| Efflux | 4 | upper | 79.2 | 80.0 | 81.1 | 77.6 | 78.7 | 79.4 | 77.4 | 78.5 |
| | | middle | 79.4 | 81.6 | 83.1 | 77.5 | 79.5 | 81.8 | 77.4 | 78.9 |
| | | lower | 79.3 | 82.0 | 84.4 | 77.2 | 80.2 | 83.1 | 77.9 | 79.3 |
| | 5 | upper | 78.7 | 79.5 | 80.3 | 77.4 | 78.9 | 79.1 | 77.5 | 79.1 |
| | | lower | 78.8 | 81.1 | 83.6 | 77.8 | 79.4 | 80.9 | 77.7 | 79.6 |
| | 6 | upper | 78.5 | 78.7 | 79.4 | 77.4 | 78.6 | | 77.3 | 79.2 |
| | | lower | 78.6 | 81.2 | 83.2 | 77.8 | 79.3 | 83.2 | 77.1 | 79.9 |
| | 7 | upper | 79.2 | 80.3 | 82.5 | 77.3 | 79.6 | 81.8 | 77.2 | 79.8 |
| | | lower | 78.7 | 81.3 | 84.1 | 77.7 | 79.9 | 84.0 | 76.7 | 80.2 |
| | Rhizome | | 76.1 | 76.9 | 77.4 | 76.1 | 76.4 | 76.1 | 75.4 | 76.2 |

are shown for the young and old petioles. Oxygen concentrations were essentially atmospheric (21%) along the entire length of the young petioles, while the levels were significantly lower in the older petioles. Carbon dioxide (like methane) was very low in the young petioles, higher in the older petioles.

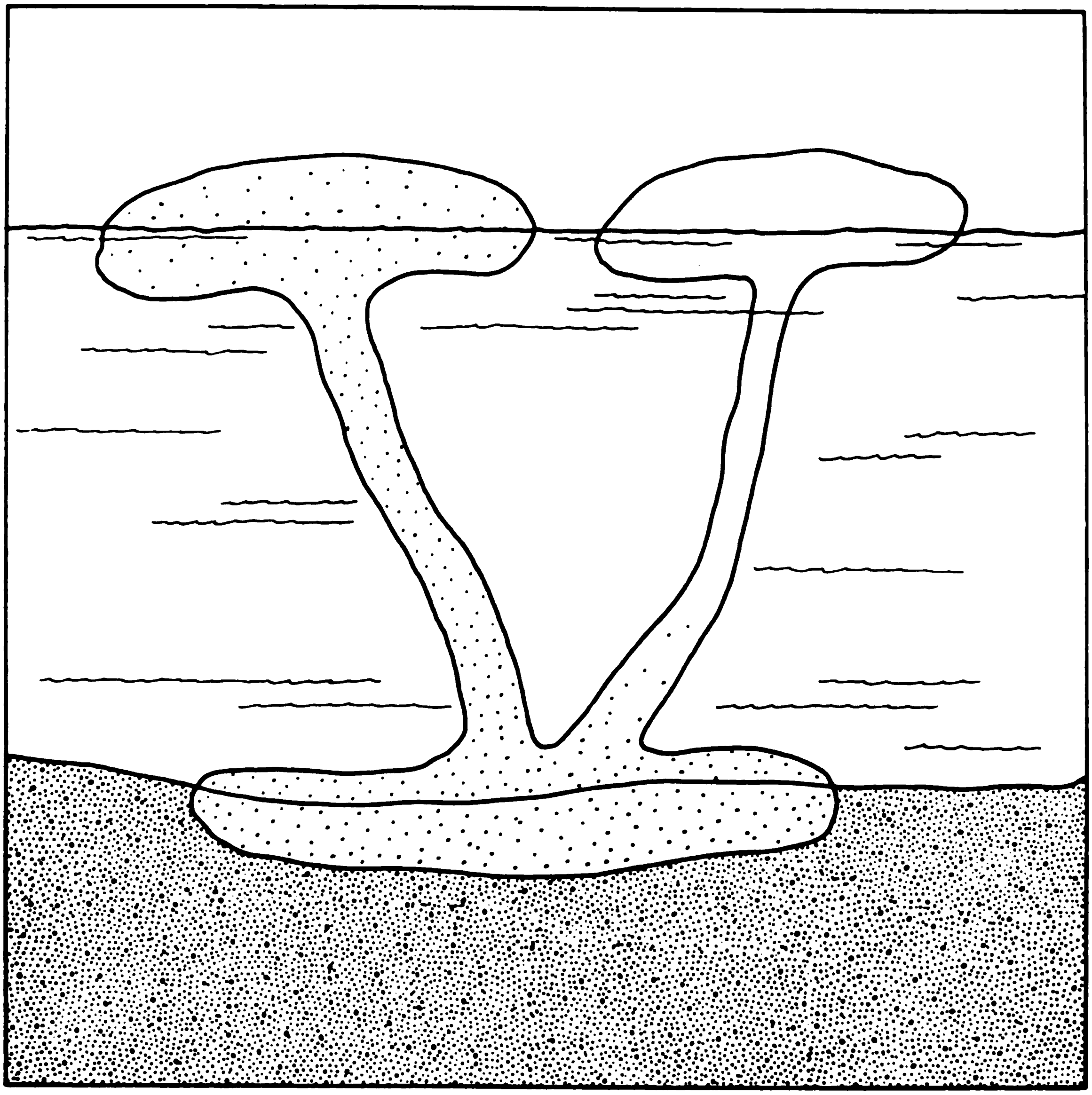
Table 9 $O_2$ and $CO_2$ in Newly-Emerged and Older Leaves (Midafternoon)
(st. dev. in parentheses)

| Leaf type | $O_2$ | $CO_2$ |
|---|---|---|
| Newly-emerged | 20.7 (.3) | 0.12 (.06) |
| Older, efflux | 20.0 (.9) | 1.05 (.75) |

Clearly, the behavior of the gases in the lacunar "interphase" is not as simple as the representation in Figure B2 (Appendix B). Considering the shading in Figure B2 to represent $CH_4$ and $CO_2$, the model must be adapted in the manner shown in Figure 12.

The representation in Figure 12 suggests that the lacunar gas spaces may constitute a flow-through system, with atmospheric gases entering the young leaves (influx leaves), while gas rich in $CO_2$ and $CH_4$ escapes to the atmosphere through the older, efflux leaves. This model resolves the apparent contradiction, suggesting that the methane escapes from the petioles of the young leaves by moving down to the rhizome, rather than by escaping out to the atmosphere through the young leaf. This model also solves the discrepancy noted at the end of Chapter 2, that the volume of gas moving up the efflux petioles far exceeds the volume of methane flux. It offers a mechanism for the large volume flows observed to pass out through the efflux leaves.

Figure 12.--Modified interphase.
The distribution of gases in the yellow waterlily suggests that a distinction must be drawn between the young emergent and the old emergent leaves. During daylight there is very little $CO_2$ or $CH_4$ in the young petioles, and a considerable amount of both in the older petioles. This idealized drawing is a modification of the hypothetical interphase in Figure B2.



## A Flow-Through System

Tracer experiments were undertaken to study the movement of gases throughout an isolated shoot apex. All the emergent leaves of a shoot apex were enclosed in Saran bags (cf., Figure 8), and 5 ml $C_2H_6$ was injected into the upper part of the petiole of the youngest emergent leaf (with a 30 gauge needle). The composition of the gas in the bags was sampled at intervals. Experiments with three plants at various depths in the lake (21 leaves total) showed that an average of 66% of the tracer had left the plant within several hours. As shown in Table 10, virtually none of the tracer had entered the bags on the youngest leaves.

Table 10 Tracer Efflux from a Shoot Apex

| Petiole rank by apparent age | Amount of $C_2H_6$ efflux from the leaf | $C_2H_6$ in petiole |
|---|---|---|
| 1<br>($C_2H_6$ injected here) | None detected | 83 ppm |
| 2 | 0.03 ml | 274 ppm |
| 3 | 0.79 ml | 422 ppm |
| 4 | 1.01 ml | 1154 ppm |
| 5 | 1.26 ml | 1238 ppm |
| | $\Sigma$ = 3.09 ml | |

The distribution of $C_2H_6$ throughout the plant confirms the pattern of leaf efflux. Even though the tracer (5 ml $C_2H_6$) was injected into the top of petiole #1, the concentration of ethane in that petiole is lower than in each of the others (Table 10). Furthermore, the concentration of ethane in each petiole correlates well with the amounts of ethane lost from that leaf (Table 10).

These data show unequivocally that there is a fluid flow of gas down the petiole of the youngest emergent leaves. The flow continues into and through the rhizome, up the petioles of the older emergent leaves to the atmosphere.

Experiments with bags on these newly-emerged leaves showed that the air moving down these petioles comes directly form the atmosphere. When a tracer was added to the bag on such a leaf, the gas composition in the petiole of the leaf quickly approached that of the gas in the bag (Table 11).

<u>Table 11</u> <u>Ethane Transport throughout a Shoot Apex</u>[1]

| Petiole rank | Ethane Concentration as a Function of Time | | | | |
|---|---|---|---|---|---|
| by apparent age | 5 min | 15 min | 30 min | 75 min | 140 min |
| 1 | —<br>(< 0.01%) | 0.16% | 0.18% | 0.18% | 0.18% |
| 2 | — | — | — | — | — |
| 3 | — | — | 0.01% | 0.04% | 0.02% |
| 4 | — | — | — | 0.03% | 0.03% |
| 5 | — | — | — | 0.01% | 0.01% |
| Bag gas | 0.19% | | | | 0.19% |

The absence of tracer in the gas of petiole #2 shows that it is also an influx leaf. Its presence on the shoot acts to dilute the tracer being forced into the rhizome by leaf #1, thus the concentrations of ethane remain low in the efflux petioles even after several hours.

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[1] A bag was tied onto leaf #1, and ethane was injected into the bag at time = 0. A dash means that the ethane concentration in the sample was less than 0.01%.

The fact that the flow-through system draws its air from the atmosphere is corroborated by another line of evidence. It was repeatedly observed that bags on the influx leaves tended to lose volume over their period of attachment. Using volume estimates based on internal standard injections, bags were monitored for several hours, during which time their volume frequently decreased by several liters. Better quantitative support for this influx will be presented below, but these observations with bags vividly illustrated the capacity for the leaves to withdraw air from the atmosphere. In some cases, bags containing nearly 6 liters of air when attached to the leaf were almost completely collapsed after several hours in sunlight (to volumes of a few hundred milliliters).

Clearly, the flow-through system is not a mechanical process of the sort exhibited by breathing animals. The waterlily is not forcing air through its lacunae by collapsing and expanding some section of its anatomy.

The most widely-known mechanism for such a flow-through system has been described by Vogel (1972, 1978). He showed, for example, that air is circulated from one end of a prairie dog tunnel system to the other. His model depends on an external air current in the atmosphere. According to the Bernoulli equation of fluid flow (Appendix C), the pressure applied by a moving fluid on the walls of its container decreases as the velocity of the flow increases. In the case of the tunnel system, the fluid is the atmosphere, and the ground is the surface along which it flows. By placing the openings of their burrows at different heights above the ground surface, the prairie dogs expose these openings to air moving at different velocities.

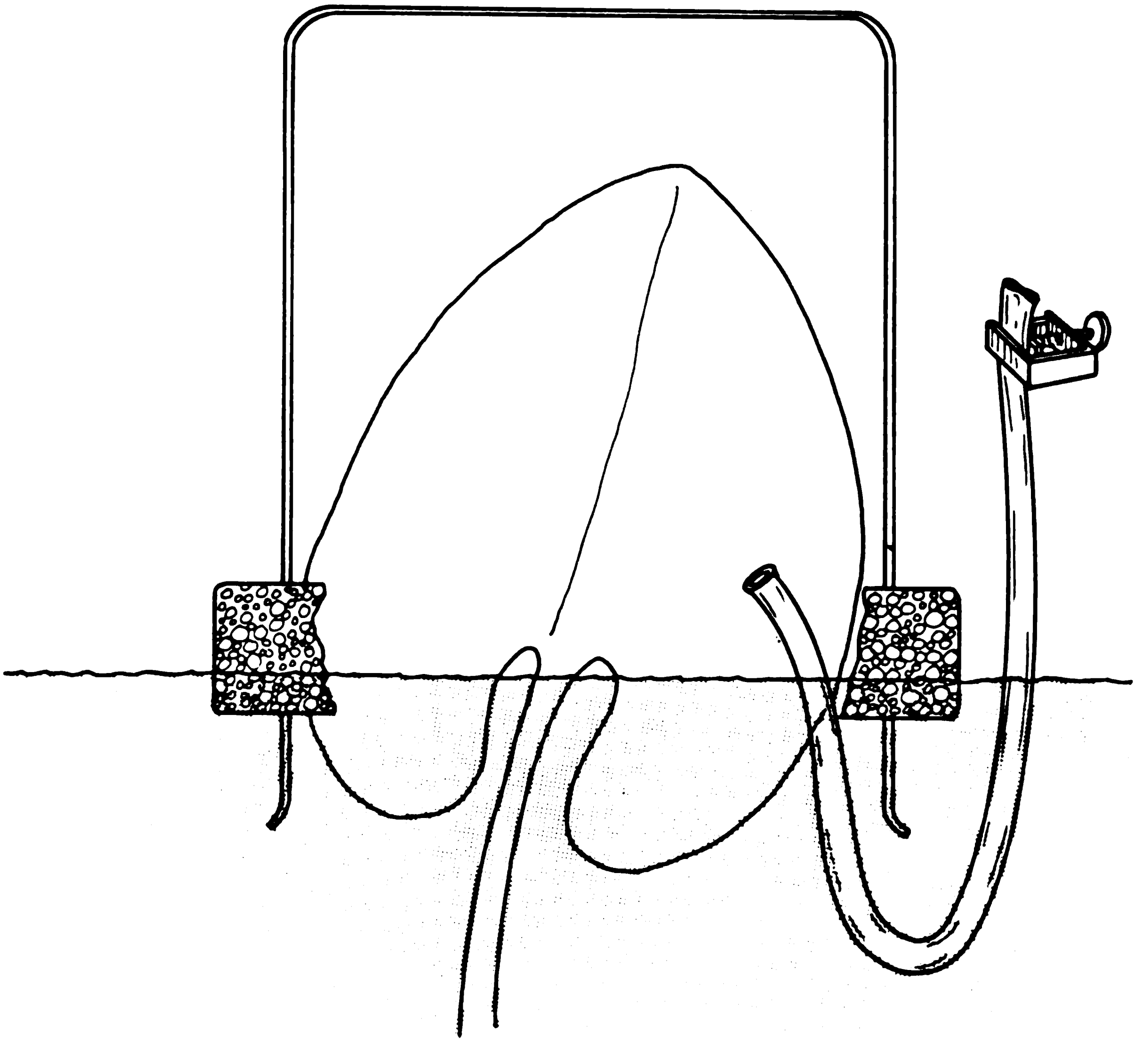
Since the pressure at an opening of the tunnel is a function of the wind velocity there, a pressure gradient is set up along the length of the tunnel system. Air is taken in through the lower holes and vented to the atmosphere at the more elevated holes. (For further discussion see Appendix C)

The similarities between the tunnel system and the waterlily system are obvious, but the mechanisms underlying the two are very different. For example, in the tracer experiment represented in Table 10, all the leaves of an isolated shoot apex were enclosed in bags. Thus, there could have been no velocity differential to support the kind of flow-through described by Vogel. Furthermore, the flows can be stopped by damaging the influx leaf. In a representative experiment, a leaf was torn inside its bag after it had been allowed to draw down the volume of the bag from 3.0 liters to 0.4 1 (in 80 minutes). Within 3 hours of tearing the leaf, the volume of the bag increased to more than 4.5 1.

The important point demonstrated by that experiment is the fact that the mechanism for the pump lies in the influx leaf. It has already been pointed out that stopping the exchange across the leaf surface (by silicone grease, for example) causes an increase in methane and carbon dioxide concentrations in the petiole. Tearing the leaf has exactly the same effect.

The capacity for these leaves to draw air into their lacunae was vividly demonstrated by inverting a large beaker over an influx leaf (Figure 13). The styrofoam collar around the beaker held it in position, and the large tube allowed the escape of air from the beaker as it settled into buoyant equilibrium over the leaf. Once the pressure

Figure 13.--Inverted beaker at $t = 0$.
A four-liter glass beaker was inverted over a young emergent leaf. Air was allowed to escape from under the beaker through a large rubber tube until the beaker reached buoyant equilibrium maintained by the styrofoam collar. The tube was then pinched shut, isolating the atmosphere inside the beaker.



inside the beaker had equilibrated with the atmosphere, the tube was pinched shut. With a healthy, undamaged leaf exposed to the sun's illumination inside the beaker, the water level in the beaker could be seen to rise up the inside wall of the beaker (Figure 14). This means that the leaf drew a vacuum inside the beaker in the process of transporting air into its lacunae. On a few occasions, the water level rose nearly 2 cm above the lake level (meaning that the leaf had drawn a vacuum 0.002 atm less than barometric).

## Pressures and Flows

Just as the young leaf tended to draw a vacuum under the beaker, it also tended to elevate the pressure in the lacunae of the leaf. Using a micromanometer (Gilmont) connected to the petiole in the manner shown in Figure 15, pressures were repeatedly measured in the lacunar gases that were significantly higher than barometric (the highest pressures being more than 2 cm water, or 0.002 atm greater than barometric).

These elevated pressures in these leaves drove the fluid flow of gas along a pressure gradient into the rhizome and subsequently up the petioles of the older emergent leaves to the atmosphere. The flow rates of gas down these petioles were measured by the same method used to study the rates of volume efflux described in Chapter 2. In this case, however, the tracer was injected into the upper end of the petiole, and the sampling was performed at the lower end. As in the case of efflux, integration of the tracer concentration-time curve showed that all the tracer passed the sampling point at the base of the petiole.

Figure 14.--Inverted beaker at t.
When a healthy young emergent leaf was exposed to the sun's illumination under the inverted beaker, it repeatedly drew a vacuum in the beaker (as manifested by the water level rising up the inside wall of the beaker). If the leaf were damaged, or old, it showed no such capacity to evacuate the beaker.

There was no measurable change in the relative proportions of the atmospheric gases in such an evacuated beaker (except for water vapor).

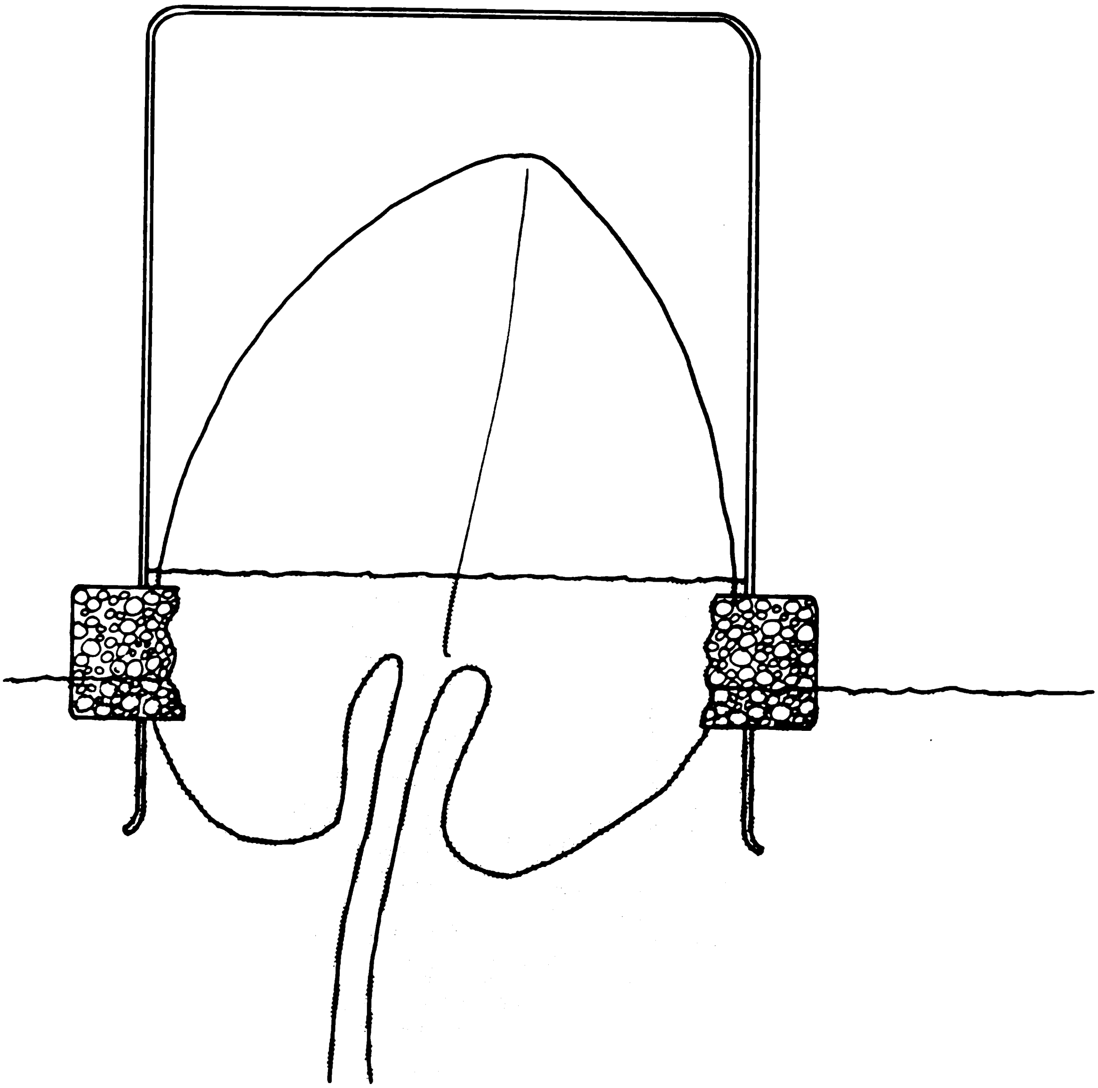


Figure 15.--Micromanometer
The manometer was connected to the leaf mid-rib or petiole by means of a 23 gauge needle and a large bore tubing (to facilitate gas flow to the manometer). Iso-octane (density 0.7 g/ml) was used as the manometric fluid.

In Figure 16, the volume rate of flow down the petiole of a young leaf is shown to be a linear function of the pressure in the upper petiole. This relationship proves that the gases inside the lacunae behaved according to Darcy's law for fluid flow through porous media. The rate of flow through the petioles of the waterlily depended on the pressure gradient along the petioles. Furthermore, the dominant process for pressurizing the internal gases during summer growth occurs in the young, newly-emerged leaves.

### A Pump

It certainly seems reasonable to call this uptake of atmospheric gas a "pump". Since the gas entered the leaf against a pressure gradient, the system has the capacity to do work: gas is forced out through the older emergent leaves. The mechanism must depend on an investment of energy.

The obvious source of energy for this pump is the sun. Shading an influx leaf had the immediate effect of decreasing the pressure of its internal gases as monitored by manometry. In Figure 17, the pattern of pressure exhibited by a single leaf during a day is drawn as a function of light intensity. Contradicting the "first criterion of a mediated-transport process" (Lehninger, 1970), the pump does not show any tendency to become light-saturated in these data, or in any of the other data collected during this study.

To further deny the possibility of the pump being a biological process, it was found that the pump's dependence on the sun was for its heat, not for its shorter wave radiation. Rapid changes in pressure in the lacunar gas of a young leaf were initiated by holding a hot electric

Figure 16.--Flow rate as a function of the mid-rib pressure in an influx leaf. The flow rates were determined *in situ* by the tracer technique throughout the day as the mid-rib pressure changed due to changing light conditions.

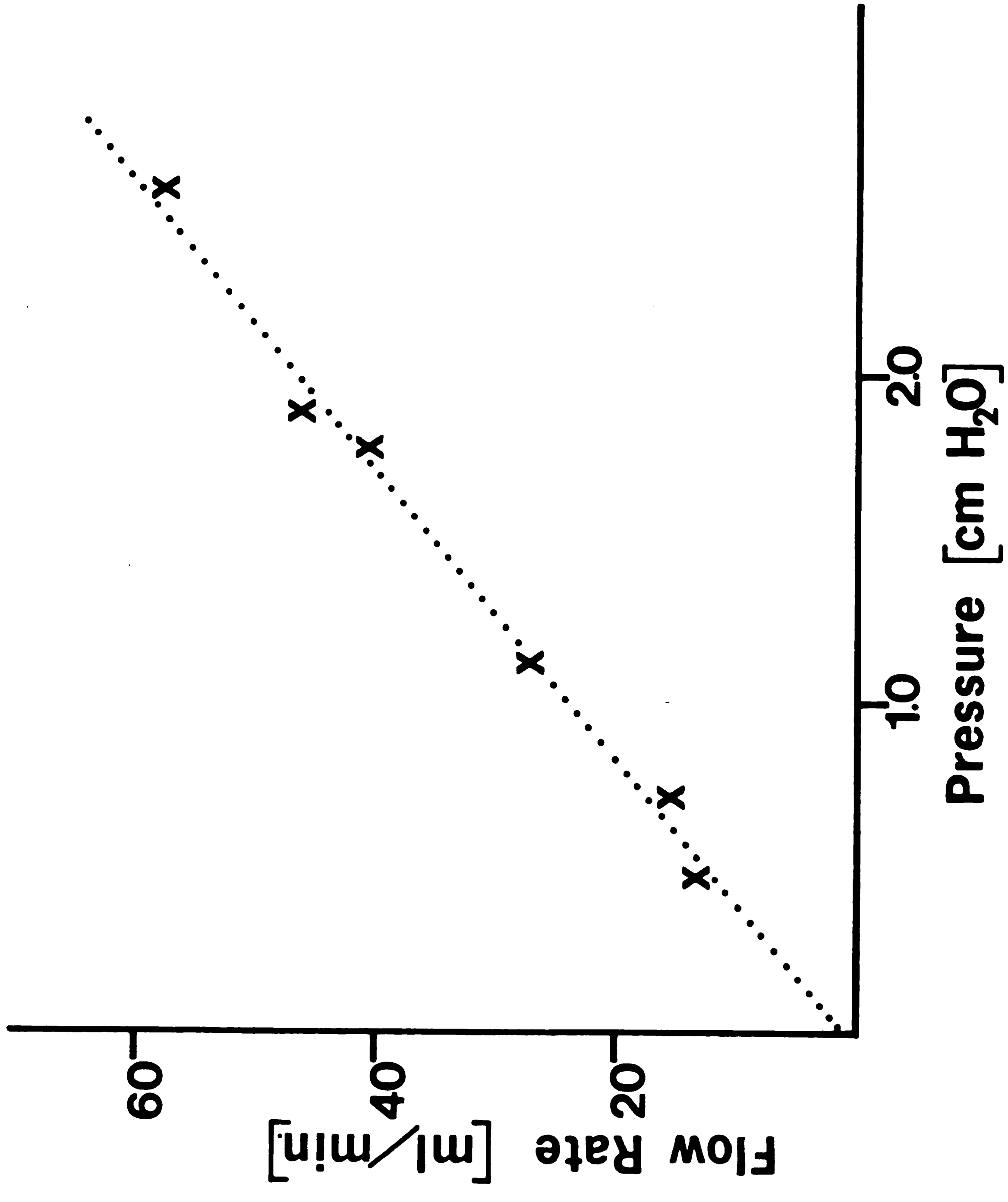
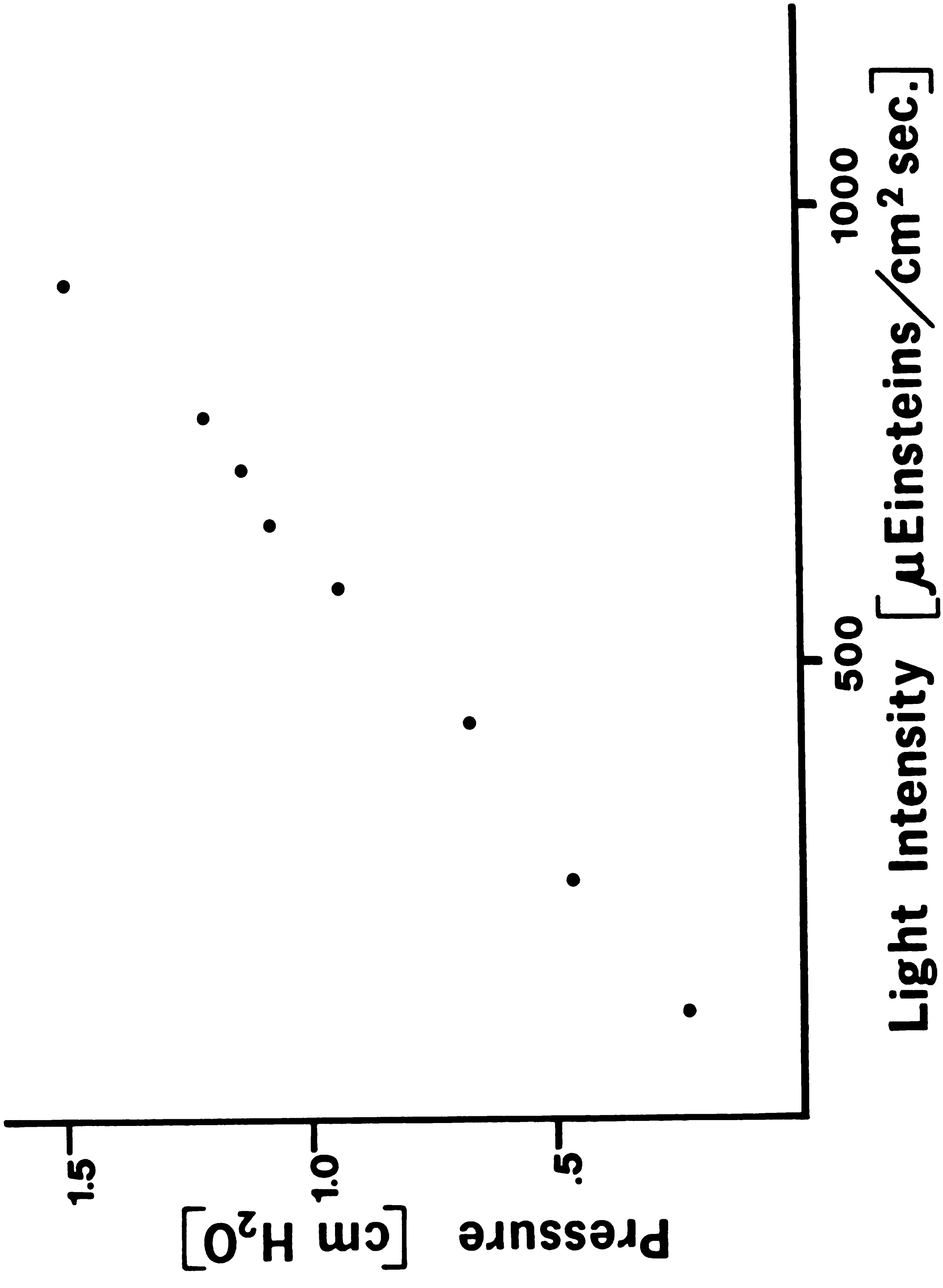

Flow Rate [ml/min]
20
40
60
Pressure [cm $H_2O$]
1.0
2.0

Figure 17.--Mid-rib pressure as a function of light intensity. The pressures and light intensities were measured *in situ*. Light intensities were taken in the direction normal to the surface of a young emergent leaf. In this case, the data were collected throughout a single day as the light intensity varied. The pressure in an influx leaf was similarly linear when the leaf was artificially shaded during periods of maximum insolation.


Pressure [cm $H_2O$]
1.5
1.0
.5
500
1000
Light Intensity [μEinsteins/cm² sec.]

frying pan at various distances from the leaf!

In a more detailed study, a young leaf was cut away from its rhizome and held with its petiole's cut surface below water (to restrict desiccation of the leaf tissues). A micromanometer was attached to the upper part of the petiole, and a 23 gauge thermistor probe was inserted into the mid-rib of the leaf. A heat lamp was directed towards the upper surface of the leaf, and the consequent fluctuations of pressure and temperature as irradiance was varied, are drawn in Figure 18.

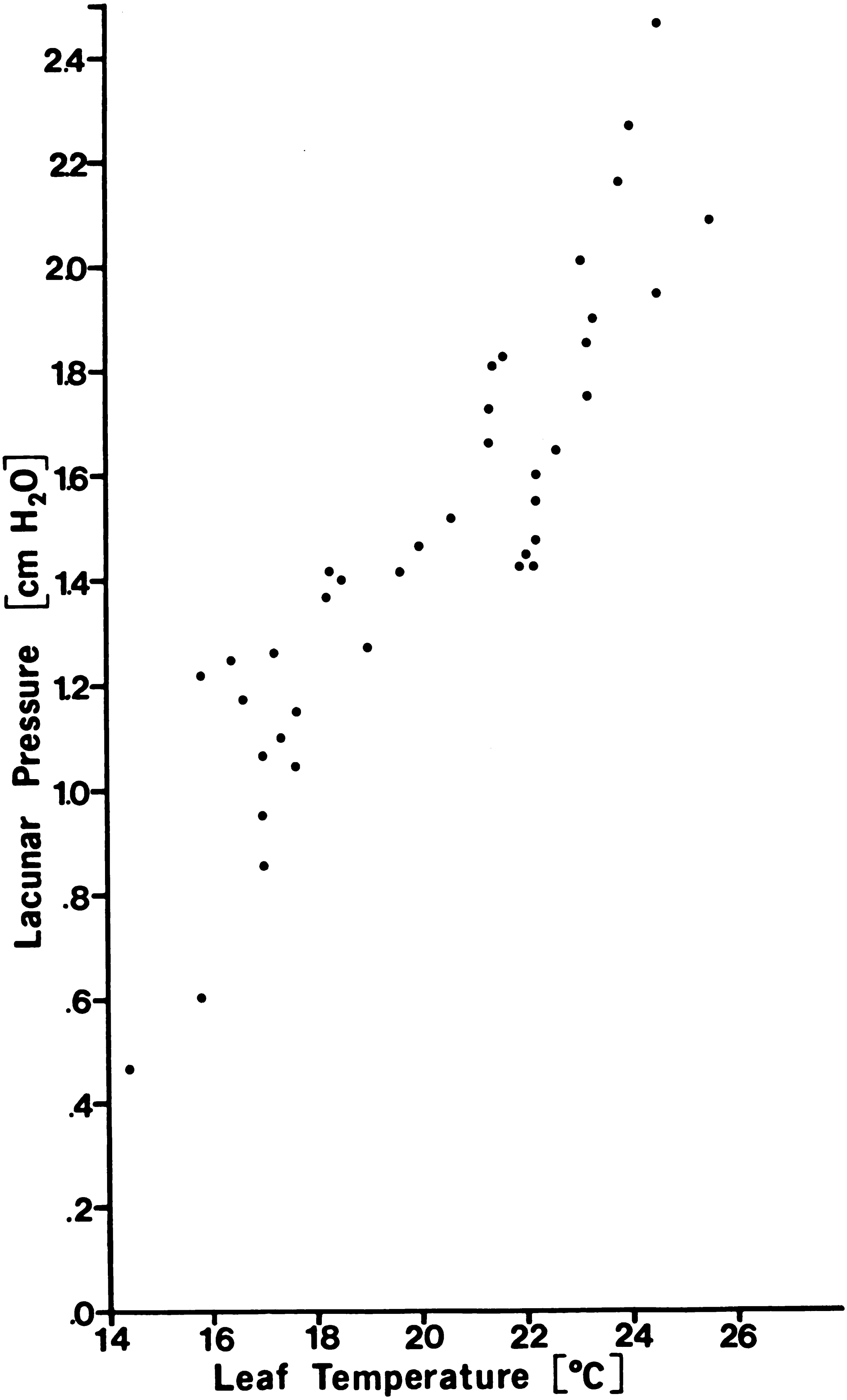
A third line of evidence supporting the notion that the pump is a purely physical process was supplied in a study of the gradients in gas composition between the atmosphere and the lacunae in the mid-rib of an influx leaf. In Table 12, these data are presented in terms of chromatogram integration units, since compositional gradients (not absolute quantities) are under investigation. Standard deviations for the samples are in parentheses.

Table 12 Comparison of Ambient and Lacunar Gas Compositions

| | $CO_2$ | $O_2$ | $N_2$ | $O_2/N_2$ |
|---|---|---|---|---|
| Ambient Atmosphere (Lake) n=7 | 272 (41) | 92749 (629) | 343765 (2629) | .2698 (.0003) |
| Lacunar Atmosphere n=13 | 233(126) | 91930 (612) | 339592 (2124) | .2700 (.0008) |

The volume-percentages of $O_2$ and $N_2$ in the lacunar gas are significantly (5% confidence) less than their respective concentrations in the atmosphere. The concentration of $CO_2$ is much more variable, and does not appear to be different in the two phases. These data show that there is no strong selection for one gas over another; $O_2/N_2$

Figure 18.—Lacunar pressure as a function of leaf temperature. The leaf was removed from the plant, and the cut surface of the petiole was held underwater (to prevent gas loss from the petiole and restrict leaf wilting). The leaf temperature was monitored with a 23 gauge YSI thermistor probe inserted into the mid-rib. The temperature of the leaf was changed by holding a heat lamp at varying distances from the leaf. The temperature of the room air (about 17°C) was measured constantly; room air was blown past the leaf surface by a fan in order to maintain a steep temperature gradient between the leaf and the air.


Lacunar Pressure [cm $H_2O$]
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
.0
14
16
18
20
22
24
26
Leaf Temperature [°C]

ratios are not significantly different in the two phases. If the pump were biologically mediated, it would most likely be highly specific for the gas transported; such selectivity should lead to accumulation of that gas in the lacunae.

The decrease in the volume-percentages of oxygen and nitrogen can be explained by increased humidity (water vapor) in the samples taken from the lacunae of the leaf. The depletion is about 1% (0.01 atm), which is well within the range of humidity gradients observed between the leaf and the atmosphere outside the leaf. Estimates of the water vapor pressure in lacunar gases were highly variable, but were consistently higher than those for the atmosphere outside the leaf. For example, in a representative case the total pressure in the lacunae of the leaf was 0.002 atm greater than barometric and the water vapor pressure in the leaf was 0.010 atm greater than barometric. This means, therefore, that there was a 0.008 atm gradient in the partial pressures of the constituents of dry air ($N_2$, $O_2$, etc.) from the atmosphere into the leaf. This gradient supports the diffusion of these gases into the lacunae, while water vapor tends to diffuse from the leaf to the atmosphere (along the 0.010 atm gradient). This process is described more fully below.

Interestingly, some of the strongest evidence for the physical basis of this pump was found in a body of literature more than a hundred years old. This early work has been entirely ignored by modern researchers; in fact, it has received very little attention since 1912. Perhaps the lack of recognition afforded this work results from the timing of the original research. The literature is reviewed here in detail, in an effort to put the work into the context of its time.

During the time span discussed below, two relevant conceptual frameworks were evolving; presumably the ideas presented here "fell through the cracks".

The kinetic theory of gases, which forms the basis of our modern understanding of gases and their behavior, was only developing during the 19th century, and was only consolidated during the early part of this century (cf., Loeb, 1934). Simultaneously, the fundamental principles of plant physiology were being developed. Much of the relevant discussion throughout this time period was directed at the role of stomata in gas exchange (cf., Haberlandt, 1914). It is worth noting that Haberlandt makes no mention of this body of literature in his monumental review (1914) "Physiological Plant Anatomy", even though he himself had investigated the stomata of waterlilies (1887).

## „Seerosenphänomen"

By the middle of the 19th century, it was widely recognized that stomata are the principal opening to the atmosphere for the internal gas system in plants, although it wasn't until the end of the century that their precise role in gas exchange was established. Dutrochet (1837) reported that gases streamed from the base of a cut petiole of Nymphaea when the leaf was exposed to light. He observed that this only occurred when the leaf was submerged, and concluded that the stomata on the upper surface of the leaves allowed the escape of oxygen produced during photosynthesis ("normal mode of respiration in leaves"). When a leaf is submerged, this avenue for gas escape is cut off, and the gas is forced down the petiole and out the cut end in a stream of bubbles. He also reports in the same discussion that the leaves could be asphyxiated when deprived of the oxygen produced in photosynthesis, since $O_2$ is needed for respiration ("the auxiliary mode of respiration"). He does not comment on the possibility that oxygen might also be available to the respiring plants from the atmosphere by way of the stomata.

Raffineau-Delile (1841) reported a similar bubbling process from the leaves of Nelumbo (a related waterlily). Her claim was fundamentally different than Dutrochet's, however. She found that the bubbling stopped (rather than began) when the leaves were submerged. This discrepancy precipitated a series of sharp exchanges between her and Dutrochet; these remarks are published in the pages following Raffineau-Delile's original report.

The discussion of plant gases continued, with Lechartier (1867) and Barthelemy (1874) emphasizing the role of gases entering the plant from the sediment water in determining the pressure relations of the plant gas. Barthelemy concluded that: "the stomata, always in contact with (the air canals), have the purpose of allowing the internal gases to pass to the outside; whereas they are generally disposed so as to oppose their re-entry." Furthermore, "gas movements are caused by evaporation, variable temperature distribution, atmospheric variability, etc."

The first discussion of a mechanism for the pressurization of gases in the leaf observed by Raffineau-Delile (and again in this study) was put forth by Merget (1873). He explained the process in terms of "thermodiffusion", a term borrowed from a physicist, Fedderson (1873).

With the work of Maxwell outlining the velocity distribution of gas molecules (ca. 1867), it became apparent that gases should segregate between the two sides of a porous membrane that supports a temperature gradient. Fedderson was apparently the first to investigate these thermal effects, but according to Violle (1875) and Reynolds (1879), his results were confounded by the presence of water vapor in his experimental apparatus.

The phenomenon studied by Reynolds, and termed "thermal transpiration" by him, is discussed in Appendix D. In short, the thermal transpiration of gases through small pores leads to an equilibrium condition wherein the pressures on either side of the porous partition are proportional to the square roots of their respective temperatures. Accordingly,

$$\frac{p_1}{p_2} = \frac{T_1^{\frac{1}{2}}}{T_2^{\frac{1}{2}}}$$

where $p_i$ and $T_i$ are the pressures and absolute temperatures of the respective gas mixtures at equilibrium.

The pressure differentials across such a porous partition can be further accentuated when the gas on one side of the partition is in contact with a volatile liquid. Dufour (1872, 1874) studied this effect of differential "humidity" on opposed sides of a porous partition, and found that air tends to move from the drier side toward the wetter side (cf., Appendix E). Such a flow of gas supported a sustained stream of bubbles when a hose from the wet side was held under water. This effect can be achieved without a temperature gradient (as required by the related process of thermal transpiration).

Merget (1873) applied the principles of Dufour to explain the movement of gas into the leaves of Nelumbo. He verified that the composition of the external gas was not important in the process; it worked with oxygen, nitrogen, hydrogen, carbon dioxide, carbon monoxide, and nitrous oxide.

Merget (1874) further demonstrated the purely physical nature of the gas transport system in experiments with dead leaves. Leaves of Nelumbo were dried to brittleness, stored for five months, and remoistened by holding them between damp cloths. Once remoistened, these leaves again exhibited their capacity to support "thermo-diffusion".

Ohno (1910) repeated much of that work and also showed that cooling the air outside the leaf had the effect of increasing the pressure inside the leaf (cf., Appendix E).

The literature is well reviewed by Ursprung (1912). The only original English text that refers to the work is Arber's (1922). Her treatment is very superficial, and suggest that she failed to understand the mechanisms proposed in the earlier literature.

This phenomenon is again discussed by Gessner (1959), with no new data added. Gessner accepted the purely physical nature of the pump, and stated that its mechanism lies in some unspecified thermal effect. He rejected the model proposed by Kundt (1877) which is similar to the hygrometric diffusion component supported in this thesis (Appendix E). Gessner went on to speculate briefly that the effect must somehow be derived from the small size of the pores and the changing character of flows through pores as their size decreases. In his discussion of this process, Gessner made some brief comments about the possible consequences of such pressures on plant metabolism, and, in what appears to be the last published word on this topic to date, he suggested that the question should be studied further.

## Mechanism

It is evident from the work of Merget, Ohno, and this study that the pump is the result of purely physical processes. As suggested above and in Appendices D and E, there are two parallel mechanisms that contribute to the generation of pressure in these leaves — thermal transpiration and hygrometric diffusion. Thermal transpiration (Appendix D) leads to pressure gradients simply of the basis of temperature gradients across a nanoporous partition. "Hygometric diffusion" leads to the same result wherever there are vapor gradients across such a partition. Both these processes would lead to a pressurization of the lacunar gas when exposed to sunlight. Thermal transpiration requires a continuous input of heat to maintain the thermal gradient. Hygrometric diffusion requires a continuous input of heat to vaporize water in the leaf. It also requires a steady supply of water so the leaf won't dry out; this water is presumably supplied by the plant's conductive tissue.

By the very nature of the system, it is impossible to distinguish between these effects in practice. Both processes should operate simultaneously and independently. Together, they should lead to a steady state where the total pressure gradient is the sum of the two independent gradients. Thus, under ideal conditions, the steady state pressure is the sum of the thermal transpiration pressure $p_2(T_1/T_2)^{\frac{1}{2}}$ and the hydrometric gradient $w_1 - w_2$ ($\Delta$ water vapor pressure).

For example, when the leaf in Figure 18 was 22°C, the air being blown across its surface (by fan) was 17°C. Ideally, the equilibrium thermal transpiration gradient would be 0.008 atm. Assuming the room air was saturated with water vapor, the hygrometric pressure would be 0.007 atm. Therefore, ideally, the total pressure gradient would be 0.015 atm, or 15 cm $H_2O$. The relative humidity in the room was certainly considerably below 100% saturation, adding still more to the expected pressure gradient. For example, at 50% saturation for the same data, the ideal total pressure gradient would be 24 cm $H_2O$. However, as shown in Figure 18, the actual pressure measured in the petiole of the leaf was 1.4 cm. Clearly, the leaf is far from being an "ideal" diffusive exchange partition.

In their normal state, these leaves are attached to a petiole that allows the escape of gas down to the rhizome. Therefore, the pressures measured in such leaves represent a steady state balance between the net rate of diffusive entry into the leaf and the loss from the leaf in fluid flow down the petiole. The leaf in Figure 18 had been cut from the rhizome and its cut petiole was sealed so gas could not escape through the petiole.

There are, however, a number of other explanations for the continued divergence of observed pressure from ideal behavior. For example, temperature gradients across the surface of the leaf deny the homogeneity of temperature that the ideal calculation assumes.

These calculations also require that the exchange between the atmosphere and the lacunar gases be diffusive. These conditions are only met when the average pore size through the exchange surface is small relative to the free mean path of the molecules in the gas. For a gas mixture at 1.0 atm, the free mean path is about $10^{-5}$cm, so the pores in the leaf must be smaller than 0.01μm.[1]

The fact that the leaf pressurizes at all means that the influx system is predominantly a diffusive system. Over the surface of the leaf there is presumably a range of pore sizes, and the pressure in the petiole represents a net effect of the exchange processes over the entire leaf.

As the leaves age, and become more porous (presumably by increased stomatal apertures and increased porosity in the pallisade parenchyma below the stomata), the leaves lose their ability to resist fluid flow. The diffusive processes of gas uptake give way to the fluid processes of gas efflux.[2]

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[1] Direct measurement of pore sizes by surface impression methods and scanning electron microscopy have been unsuccessful. It is likely that the pores lie below the leaf surface in the unexpanded parenchyma of the young leaves (cf., Esau (1953) for discussion of leaf expansion). An estimate of pore dimensions can be made on the basis of a few assumptions. Although these are likely several pores (in the parenchyma) below each stoma, a calculation assuming one pore/stoma will give a maximum pore size.

Since air molecules strike a surface at a rate of roughly $10^4$ml/cm$^2$ sec (Danckwerts, 1971), they can be said to enter the pores in the leaf surface at the same rate. Since there is roughly a .01 atm gradient in the partial pressures of (dry) air molecules, the net flux into the leaf (e.g., 30 ml/min) represents 1% of the total flux (or 100 ml/cm$^2$ sec). This means the total pore area per leaf is about $5 \times 10^{-3}$cm$^2$. With $1.5 \times 10^7$ stomata/leaf (on a leaf grown in the lab), the average pore area (one pore/stoma) is therefore $3.3 \times 10^{-10}$cm$^2$. Assuming these pores are circular in cross-section, the diameter of a pore is $2 \times 10^{-5}$cm. This is certainly an overestimate of the average pore size, but it serves to illustrate that the pores are very small.

[2] There is another relevant change that accompanies leaf aging. The youngest leaves are commonly pigmented with anthocyanins, so they appear red or reddish-green. This pigmentation presumably has the

## Physiological Significance

It is actually this aging process of the leaves that makes the pressurization phenomenon a physiologically significant process: it gives the system its flow-through character. The newly-emerged leaves are responsible for the increased pressure in the lacunar gas; the older emergent leaves allow the escape of the pressurized gas to the atmosphere (Figure 19). The flow-through system that results has two physiologically significant effects: oxygen is transported to the rhizome from the influx leaves, and carbon dioxide is carried to the efflux leaves.

The transport of oxygen into the plant is reflected in the strong diurnal pattern of oxygen concentration throughout the plant (Table 8). During daylight, $O_2$ at atmospheric concentration was found in the young leaves and petioles; its concentration is significantly diminished as gas enters the base of the efflux petioles (Table 13).

Pressure data were collected throughout the day for the leaf used in the flow rate/pressure regression (Figure 16). Integration of the pressure throughout the day showed that more tha 16 liters of air passed down that petiole during the day. That translates into several liters of oxygen entering the rhizome from this one petiole. During the summer growth period there are usually several of these influx leaves on each shoot apex, so the rhizome may be receiving considerably more oxygen.

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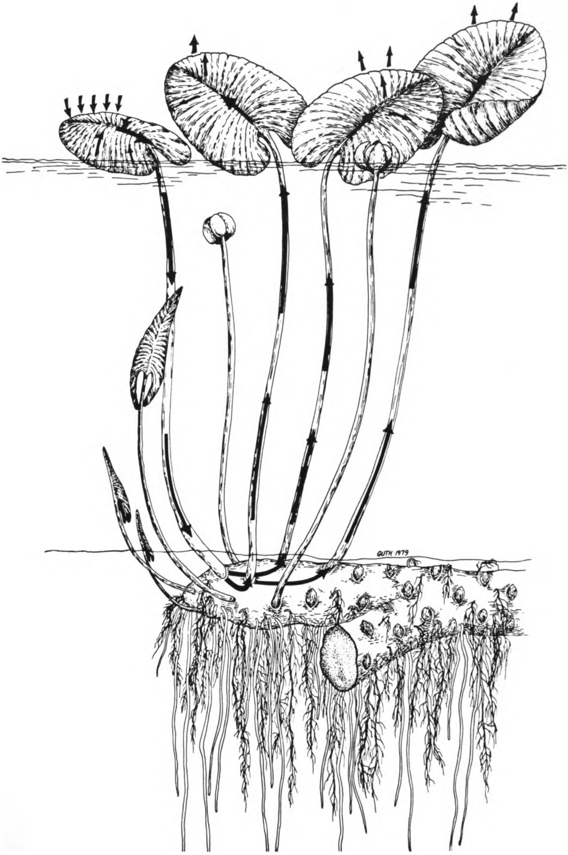
effect of increasing leaf temperature during daylight, thereby facilitating the pressurization phenomena. The leaves gradually lose their red color (turning green), reflecting the decreased importance of elevated temperature in these older (efflux) leaves.

Figure 19.— The pressurized gas flow-through system. Air enters the youngest emergent leaves against a pressure gradient. This elevated pressure forces a fluid flow of air down their petioles to the rhizome, and back out to the atmosphere through the older emergent leaves.



GUTH 1979

Table 13 Gas Compositions in Efflux Petioles during Daytime and Nighttime (st. dev. in parentheses)

| Position on petiole | Daytime | | | | Nighttime | | | |
|---|---|---|---|---|---|---|---|---|
| | $CO_2$ | $O_2$ | $CH_4$ | $N_2$ | $CO_2$ | $O_2$ | $CH_4$ | $N_2$ |
| Upper | 0.7%(.5) | 20.5%(.5) | 0.3%(.1) | 77.4%(.1) | 3.9%(2.6) | 15.0%(2.8) | 0.8%(.4) | 80.2%(1.2) |
| Lower | 1.4%(.8) | 19.4%(.8) | 0.3%(.2) | 77.5%(.4) | 6.8%(2.6) | 8.6%(2.7) | 2.4%(.7) | 82.7%(1.3) |

The pattern of $CO_2$ flowing through the plant is also evident in Table 8. A gas mixture with several percent $CO_2$ is carried from the rhizome toward the efflux leaves; the concentration depends on the time of day, and presumably, the flow rate.

In Table 13, it is evident that the $CO_2$ level drops dramatically as the gas moves up the petiole. All the evidence suggests that the $CO_2$ decline is due to photosynthetic fixation, in the leaf and possibly in the upper petiole. Its unlikely that the $CO_2$ is diffusing from the petiole into the surrounding water, since its residence time in the petiole is only a few minutes. At night, however, large concentrations of $CO_2$ persist without diffusing away to the water.

The $CO_2$ gradient (0.7%) compares well with the opposite oxygen gradient (1.1%) during daytime, suggesting photosynthetic fixation as the gas mixture flows up the efflux petioles. Furthermore, there is no equivalent gradient in methane, which is a conservative gas within the plant, and like $CO_2$ it enters the petiole from the rhizome.

These gradients in gas composition are further documented in Table 14. Using the ethane tracer method, the flow rate up the petiole was found to be 8.7 ml/min. Again, $CO_2$ and $O_2$ exhibit the strongest concentration gradients along the length of the petiole, suggesting photosynthesis in the leaf and petiole. These data show that for a plant *in situ*, three hundred microliters of $CO_2$ (equivalent to all the $CO_2$ in 1 liter) were transported through the petiole each minute.

Table 14 Gas Composition Gradients along an Efflux Petiole (8.7 ml/min)

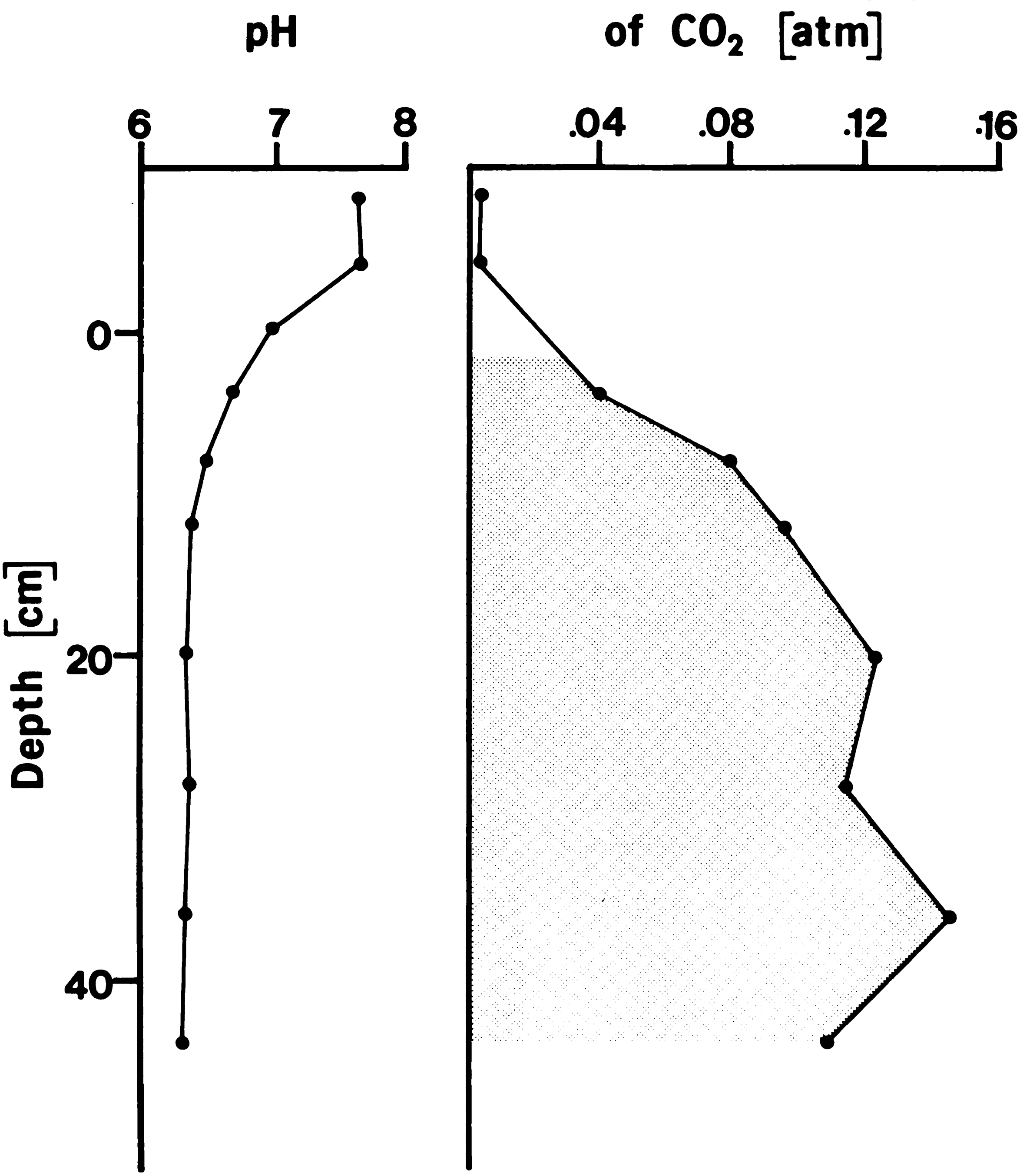
| | $CO_2$ | $O_2$ | $CH_4$ | $N_2$ |
|---|---|---|---|---|
| Leaf mid-rib | 0.6% | 19.6% | 0.7% | 77.7% |
| Upper petiole | 1.6% | 17.9% | 1.4% | 78.2% |
| Lower petiole | 3.6% | 16.3% | 1.5% | 78.7% |

The relative contributions of the processes that produce the $CO_2$ concentrations in the rhizome were not established. The $CO_2$ levels found there must result form both plant respiration and exchange with the sediment water. Laing (1940b) has established that the rate of respiration of the rhizome can be considerable, even under anaerobic conditions.

As suggested in Chapter 1, there is also considerable potential for the diffusion of $CO_2$ into the roots and rhizome from the interstitial water. A profile of the partial pressure of $CO_2$ (Figure 20) shows its range to be from 0.03 to 0.14 atm in the upper 50 cm of the sediment. Since the depth profile of the roots and rhizome surfaces for gas exchange are unknown, it's impossible to estimate the steady state $CO_2$ concentration to be expected. The average partial pressure of $CO_2$ over the upper 50 cm was 0.11 atm. On this basis, the partial pressure in an equilibrated gas phase would be 11% (at 1 atm). As shown in Table 8, the $CO_2$ concentration only exceeded this amount once in 8 samples. This suggests that there is usually a diffusion gradient for $CO_2$ into the buried lacunar spaces, but the kinetics remain uncertain.

It's important to realize that this flux of $CO_2$ up the petioles from the rhizome is not the only source of $CO_2$ available to

Figure 20.—pH and $P_{CO_2}$ profile in Duck Lake sediments. The values were determined by the methods described in Chapter 2 for dialysis-equilibrated samples. The shaded area in the $P_{CO_2}$ profile represents the area integrated to give the 0.11 atm estimate used in the text. These data were obtained from samples taken from the lake during early October. Seasonal pH data for the interstitial water of nearby Wintergreen Lake suggest that the $P_{CO_2}$ profile does not change greatly through the summer. These values may be slightly lower than the summer average, since the pH tends to rise during winter (lowering $P_{CO_2}$).



*DUCK LAKE SEDIMENTS*

the plant. There is great potential for rapid exchange of $CO_2$ between the atmosphere and the emergent leaves; its importance remains uncertain.

## Nitrogen

In a sense, $N_2$ acts as a carrier for the three gases of biological interest ($CO_2$, $O_2$, and $CH_4$) — it is a passive gas, and the dominant gas in the lacunae and the atmosphere. Even so, its dynamics cannot be interpreted without understanding the dynamics of the other gases. Contrary to a strictly diffusive model, there are considerable increases in the concentration of $N_2$ in the rhizome at night (Table 8). These presumably result from the exchange (during respiration) of relatively insoluble $O_2$ for the more soluble (in plant tissue and sediment water) $CO_2$. This apparently leads to a slight decrease in the gas pressure in the lacunae. It's unlikely this phenomenon has any significant physiological effect, but it serves to make the point that the dynamics of individual gases cannot be understood without knowing a good deal about the other gases in the mixture.

## Conclusions

By means of a purely diffusive exchange with the atmosphere, the pressure of the gas phase in the lacunae of the youngest emergent leaves increases over barometric. This elevated pressure drives a fluid flow of gas down their petioles to the rhizome, and from the rhizome up the petioles of the older emergent leaves to the atmosphere. This pressurized flow-through system serves to deliver large volumes of oxygen to the rhizome buried in anaerobic sediment. It also serves to carry carbon dioxide from the rhizome to the photosynthesizing tissue of the older emergent leaves.[1]

In current theory, the transport of gases in the lacunae of plants proceeds along diffusion gradients which are thought to be primarily the product of gas metabolism in the plant — the varying uptake and release of $CO_2$ and $O_2$, and the release of water vapor during transpiration. These gases diffuse independently within the gas mixture according to the gradients in their partial pressures. That is the model of gas transport proposed by Laing (1940) for the yellow waterlily. Except where external air currents may influence the transport of gases internally (cf., Vogel, 1978), this diffusion of gases in a static gas phase is the model for gas flux in all plants.

Clearly, the generality of that model must be reexamined. The gas flows in the waterlily do not depend on external air currents; they are the product of diffusive exchange with the atmosphere. This process is purely physical, but its significance lies in its biological implications.

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[1] Larvae of the moth Bellura sp. (cf., Levine and Chandler, 1974) were found frequently during this study. The larva burrows through the surface of the emergent leaves of Nuphar and down into their petioles. Such an attack on a young leaf effectively stops its flow of gas down its petiole by allowing the pressurized gas to escape to the atmosphere through the burrow opening. This efflux was confirmed by covering the opening of a burrow with a thin soap film — it quickly expanded into a convex bubble, and burst. Such termination of the flow-through system must have short-term effects on plant metabolism, but it's not clear what effect long term infestations might have on the waterlily population. Clearly, the impact of the larva may depend more on the location of its attack (i.e., young emergent leaves) than on the actual amount of plant biomass consumed.

# SUMMARY

The underlying principle in this study can be stated consisely: although the gas mixture in the lacunae of plants manifests biological processes, it obeys physical laws. Its behavior cannot be adequately described in biological terms alone. The dynamics of the gases can be completely understood only within a physical framework.

In the case of the waterlily, this approach has uncovered a little-known physical process that has a profound impact on the physiology of the plant. By means of what is nearly a "biological steam engine", large volumes of oxygen are transported to the plant parts that lie buried in the anaerobic sediment. Simultaneously, carbon dioxide and methane are carried from these sediments toward the atmosphere. Much of the carbon dioxide is subsequently fixed by plant photosynthesis, and the methane escapes to the atmosphere at rates that are significant in terms of the carbon and energy budget of the lake ecosystem. The fact that heat supplies the energy behind this gas circulation system is a striking reminder of the economy of nature.

# APPENDICES

# APPENDIX A

# GAS SOLUBILITY

Ecologists find the concept of gas solubility easy to understand intuitively, but rather harder to deal with in practice. Without an understanding of the principles involved, a literature has arisen which misapplies terms, and subsequently confounds the interpretation of physiological and environmental conditions. Since the work in this study is directed towards understanding the relations of gases (in gas phase) in plants, this discussion will focus on the principles that apply in such a study.

First consider the gas phase. Dalton, a pioneer of atomic theory, observed that the total pressure exerted by a mixture of gases is equal to the sum of the pressures which the several gases would exert if each were to occupy the vessel alone (Dalton's law). Avagadro's rule can be restated to say that: "under the same conditions of temperature and pressure, equal volumes of different gases contain the same number of molecules" (Loeb, 1934). Combining these two statements, it becomes clear that the volume-percentage of a gas in a mixture of known total pressure can be directly related to the partial pressure of that gas. This simple assertion is important because it allows a direct relation between the volume estimate achieved by gas chromatography and the partial pressure of the original sample.

Henry's law states that the solubility of a gas in a liquid phase is proportional to the partial pressure of that gas in the equilibrated gas phase. A gas dissolves in a liquid until the rate of escape of gas molecules from the surface of the liquid equals the rate at which gas molecules enter the liquid. The converse also holds true, as in the case of bubble generation. Gases leave the liquid until equilibrium is achieved with the adjacent gas phase. At equilibrium, the partial pressure of each gas in the bubble equals the partial pressure of that gas in the aqueous phase.

Consider the case of a volume of water in equilibrium with the atmosphere (i.e., 21% $O_2$ and 78% $N_2$ at 1.0 atm). A small bubble at the surface of the water will contain 21% $O_2$ and 78% $N_2$ (or 0.21 atm $O_2$ and 0.78 atm $N_2$, respectively). If this bubble were moved down into the depths of the water, it will lose volume due to the hydrostatic pressure. As the pressure in the bubble increases, so do the partial pressures of the constituent gases. Since the partial pressures of these gases exceed their partial pressures in the water column, the bubble dissolves.

It is clear that in order for a bubble to remain at depth, the partial pressures of the gases dissolved in the water must equal, or exceed, the partial pressures of the gases in the bubble. This fact has immediate application in the phenomenon of bubble formation in submerged sediments. A bubble will only be produced in this environment when the sum of the partial pressures of the dissolved gases in the interstitial water equals or exceeds[1] the hydrostatic pressure at bubble depth.[2]

The absolute solubility of gases in a function of temperature; their behavior in solution is treated in thermodynamic theory as one of liquid mixture (a gaseous liquid mixing with a liquid, cf., Glasstone, 1947). The solubility of the gas is then related to the vapor pressure of the gas at the temperature of interest. This explains the changes in gas solubility as a function of temperature. Since the heat of vaporization is positive, the ideal solubility of gases decreases with increasing temperature.

The use of partial pressures to express the solubility of gases avoids the issue of changing solubility with temperature. Most ecologists use terms of absolute solubility, such as wt.gas/vol.water, vol.gas/vol.water, and moles gas/vol.water. These terms cannot be used to discuss the condition of the dissolved gases relative to an adjacent gas phase without a simultaneous estimate of temperature. Since this investigation involves the interaction of a gas phase in the lacunae (and in the sediment bubbles) with gases dissolved in the adjacent

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[1] The pressure required for the initiation of a bubble under hydrostatic pressure should exceed the latter pressure because of surface tension effects. The actual pressure inside a small bubble at depth is not only a function of its depth, but the surface tension of the water and the diameter of the bubble. As the bubble grows in size, the pressure increment decreases.

This effect can be very large when nucleation sites are absent. However, the particles of sediment constitute such nucleation sites, and the effect is presumably quite small.

[2] According to Trevelyan (1975), it was William Henry (Henry's law) who first identified the combustible gas in lake sediments as methane.

water, the concentrations of the latter are reported in terms of equilibrium partial pressures (thus incorporating quantities and temperature).

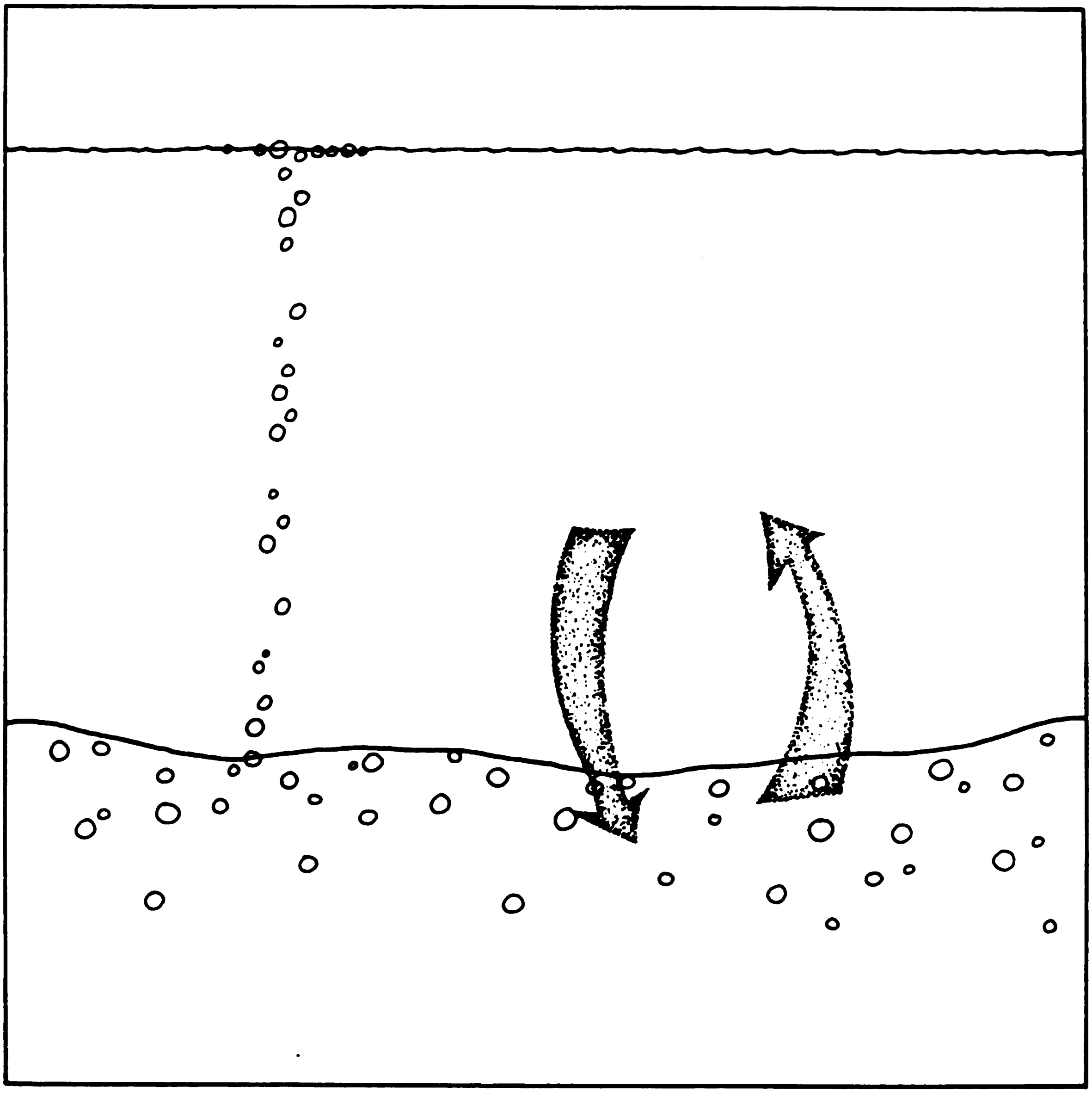
# APPENDIX B

# THE LACUNAR INTERPHASE

Consider the simple model presented in Figure B1. As described in Chapter 1, the water column and the atmosphere tend to remain near equilibrium; the interstitial water of the sediment diverges from atmospheric because the particulate matter of the sediment restricts turbulent and diffusive exchange with the overlying water. Exchanges do, of course, exist; carbon dioxide and methane diffuse from the sediment water, and oxygen moves into the sediment water. The flux of gas from the sediment is augmented by ebullition, which occurs whenever the sum of the partial pressures of the gases dissolved in the sediment water exceeds the hydrostatic pressure (+ surface tension; cf., footnote 1, Appendix A). The kinetics of the various transports are a function of a great many variables (e.g., sediment porosity, concentration gradients, depth, turbulence, bioturbation, etc.). These are the transport processes that have received the most attention in the literature of sediment-water exchanges.

This research has shown that the gas in the lacunae of emergent vascular plants can be important in the exchange processes between the atmosphere and the interstitial water. The impact of this interphase in the case of methane efflux has been demonstrated

Figure B1.—Sediment-water exchanges.
This is a representation of the standard conception of the gas exchange mechanisms between the sediment and the overlying water. Gases diffuse, or are turbulently mixed into and out of the sediment. Gases also escape from the sediment by ebullition.



elsewhere (Dacey and Klug, 1979). The purpose of this appendix is to develop a purely physical model of the consequences of this extension of a gas phase into the sediment interstices.

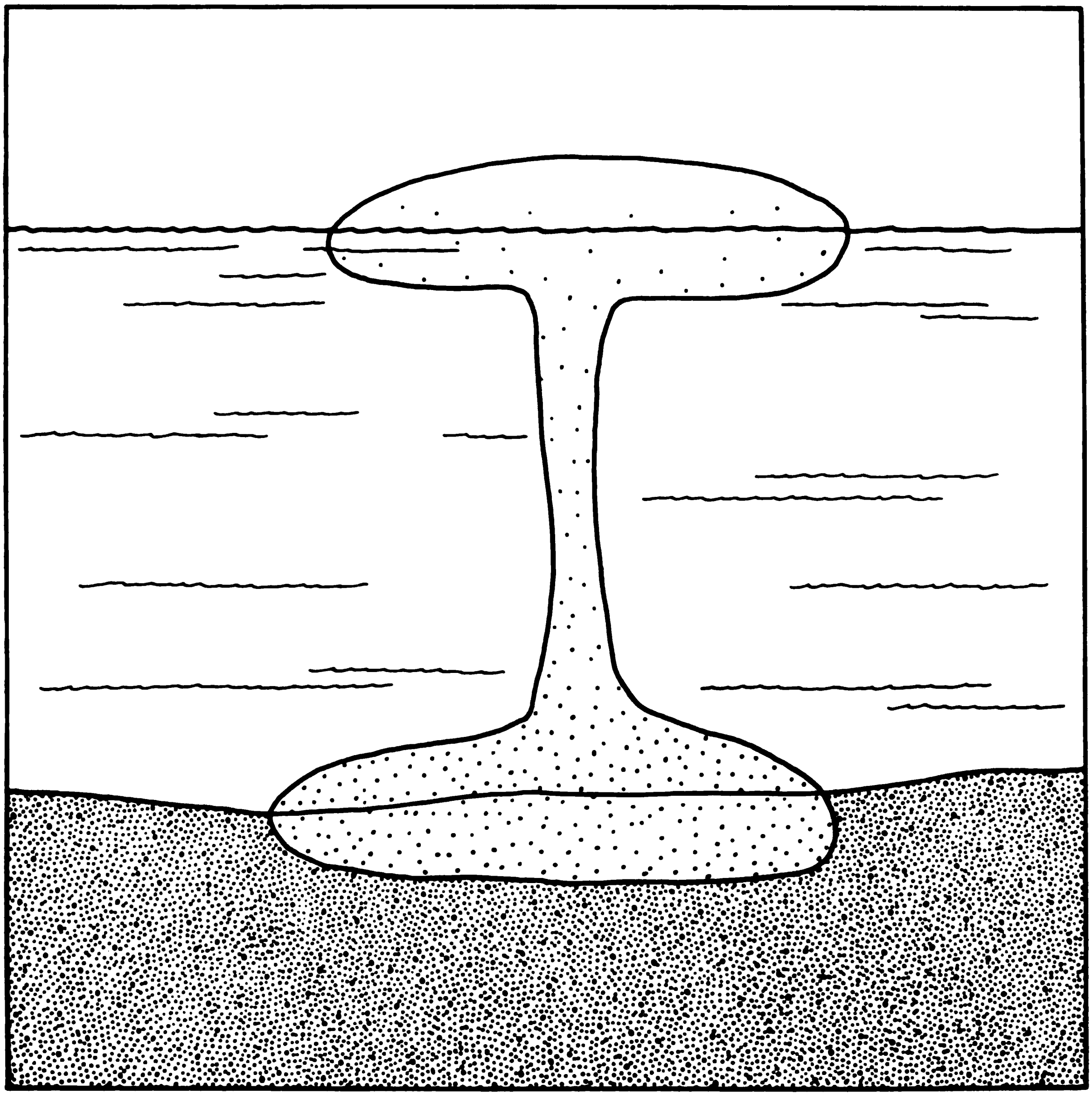
During summer growth, the lacunae of the waterlilies are essentially a continuous gas phase extending from the atmosphere into the anaerobic sediment water. The lacunae are represented in Figure B2 by a tube of variable porosity. Clearly, at the top of the tube the exchange processes will bring about a gas composition very hear atmospheric. The total pressure will be 1 atm.

The consequences of the exchange at the lower end of the tube are not so obvious, and must depend somewhat on the porosity of the tube leading to the surface. For example, as the porosity is decreased, the rate of diffusion of atmospheric gases (most notably $O_2$) to the bottom of the tube is limited. Similarly, the rate of diffusion of the sediment gases away from the base of the tube is also restricted. Under these conditions, the accumulation of sediment gases would be expected in the buried portion of the lacunar gas phase. If the porosity were so restricted so as to inhibit the fluid flow of gases (Appendix C), the total pressure of the gases in these lacunae might also increase above atmospheric. This happens in the case of the waterlily when the rhizome is isolated from the atmosphere during the winter months.

Such extreme conditions are absent during the summer growth of the waterlilies. Their lacunae are definitely pervious, since $O_2$ is present in significant quantities throughout the plant.

As the porosity increases, the influence of the internal structure of the lacunae on diffusion decreases. The focus of attention

Figure B2.—The lacunar interphase.
The passive transport of gases through such an interphase should reflect the gradients at its exchange surfaces (the atmosphere and the interstitial water). The stippling in the interphase is intended to represent gases of sediment origin diffusing towards the atmosphere.



moves from the lacunae to the interface of the lacunae with the sediment water. This exchange might dominate the character of the gas dynamics in the lacunae.

The simplest case to consider is one wherein the gases on either side of the interface are identical with respect to solubility in water and diffusivity in water and air. Under these circumstances, there would be a net pressure gradient into the plant, since the sum of the partial pressures is 1.1 atm in sediment water (submerged 1 m) and the pressure is 1 atm in the lacunae. The resulting partial pressure gradients would lead to a differential in the diffusive exchanges resulting in a net flux of gas into the plant. This net transport of gas could be observed as a fluid flow of gas up through the plant to the atmosphere; the rate of flow would depend on the differential in the exchange rates between the lacunae and the sediment water.

In lake sediments, however, all the gases are not alike. As a result, the character of the exchange between the two phases is unpredictable. There are four main gases of interest: nitrogen, oxygen, methane, and carbon dioxide. The former two are abundant in the atmosphere; the latter two, in the sediment water. The resulting fluxes of gases between the phases depend in part on the solubilities and diffusivities of the gases in water. In actualility, there are other processes that may have even greater significance — the biological production and consumption of gases near the interface. These processes tend to steepen the diffusion gradients between the lacunae and the water.

The oxygen gradient is certainly steep due to its consumption by the plant surface tissue, and possibly by bacteria in the rhizosphere. The methane gradient is also very likely steep due to methanogenesis in the vicinity of the plant. The $CO_2$ gradient is also steep due to the strong buffering capacity of the sediment water. Nitrogen, being a conservative gas (assuming denitrification has run its course in the sediment water), is most likely to have a small diffusion gradient into the sediment.

The net effect of all these simultaneous processes is clearly difficult to predict. This treatment is intended to make the point that this interface submerged in the sediment is bound to be a source and a sink for different gases. The exchanges that occur here may lead to a net flux of gas through the plant; such a fluid flow upwards from the sediment would tend to inhibit the availability of $O_2$ in the buried plant parts. Whether or not such fluxes occur was not established in this study. It was found that, in the case of the waterlily, the other exchange processes between the plant and the atmosphere have influences that dominate the dynamics of the lacunar gases.

# APPENDIX C

# FLUID FLOWS

The flows discussed in this appendix are described by the body of physical theory known as fluid dynamics (hydrodynamics, aerodynamics). The fluid flow of gas involves the mass movement of gas from a region of high pressure to a region of lower pressure, like a wind. The mass flux at any point along a tube carrying such a flow is described by:

$$\frac{dm}{dt} = \rho A v \qquad \text{eq. C1}$$

where $\rho$ is the gas density, A is the cross-sectional area of the pipe, and v is the speed of the flow through the cross-section. This equation reveals the well-known relationship that the speed of flow is inversely proportional to the cross-sectional area of the tube (or inversely proportional to the square of the tube diameter).

The concepts of fluid flows are applied in this study to the mass movement of gases along the length of the petioles. Since the pressure gradients are very small, the density of the gas can be considered to remain constant. It is also apparent from this study that there is very little exchange of gases with the water outside the petiole, so at any given time, dm/dt can be said to remain constant along the length of the petiole. Under these conditions, equation C1 is called the equation of continuity.

The pressures that generate the flows in the waterlilies result from diffusion processes through the upper surface of young, newly-emerged leaves (cf., Appendices D and E). Once such a pressure develops in these leaves, there results a flow of gas down their petioles to the rhizome, and subsequently from the rhizome up the petioles of the older emergent leaves. These flows operate in accordance with Darcy's law for fluid flow through a porous medium:

$$u = \frac{B\Delta p}{L} \qquad \text{eq. C2}$$

where $\Delta p$ is the pressure drop along length L, and B is the permeability coefficient of the petiole interior (cf., Carman, 1956).

Since the pressure gradients are extremely small, the flow rates are also small in comparison with the rates of gas flow usually measured by physicists. The standard means of measuring gas flow (e.g., Pitot tube, Venturi meter) are not applicable in this study because of the slow flow rates and the inaccessibility of the flow channel. The flow rate was studied by injecting a tracer gas (such as $C_2H_6$) into the petiole. A fluid flow along the length of the petiole carries the tracer gas much like the carrier gas in a chromatograph carries the sample. As the tracer is carried along, it mixes somewhat with the air as a result of diffusion and absorption processes. As a result, when the tracer gas passes a point further along the petiole, the original spike of tracer has broadened into a slightly tailing peak (as is usually the case in chromatography). Such a peak is detected by sampling the gas at intervals (of time) at a predetermined distance from the injection point. The time when the maximum of the tracer "elution" curve passes the sampling point is taken to be the time taken for the whole gas mixture to move from the injection point to the sampling point. Linear and volume flow rates are easily calculable by methods in the text of Chapter 2. As described in that chapter, the elution curve can be integrated, and knowing the volume rate of flow, the total amount of tracer passing the sampling point can be estimated. Comparison of this amount with the amount actually shows that all (80-120%) of the tracer passed the sampling point. Since an insignificant amount is lost to the surrounding water through diffusion, the equation of continuity can be said to hold. Furthermore, this observation supports the claim in Chapter 1 that the water column gases play no significant role in the internal gas dynamics in the waterlilies.

Continuity is assumed in the treatment of fluid flow that gives rise to Bernoulli's equation, a fundamental assertion of fluid dynamics:

$$p + \tfrac{1}{2}\rho v^2 + \rho gh = \text{constant} \qquad \text{eq. C3}$$

It can be seen that the dimensions of the components are all energy/volume. The Bernoulli equation is thus essentially a statement of the energy-work theorem of fluid dynamics (Halliday and Resnick, 1960). The last term, $\rho gh$, represents hydrostatic potential energy, and therefore disappears from the equation under the conditions of interest in this study. Thus,

$$p + \tfrac{1}{2}\rho v^2 = \text{constant} \qquad \text{eq. C4}$$

This equation describes the relationship between the pressure on the inside walls of the tube and the velocity of the gas in the tube. This relationship has no direct application in this study, but it is of theoretical interest for two reasons.

This equation has been successfully applied in studies of other fluid circulation systems of biological interest. Vogel (1972, 1978) has applied these principles to show how a number of animals, and possibly plants, use velocity gradients in their environment to circulate the fluid environment to great benefit. A brief consideration of the Venturi meter in Figure C1 shows that there is a pressure gradient within a tube connecting a region of low velocity to a region of high velocity. Therefore, when the mercury column is removed from the Venturi meter, as in Figure C2, there is a bulk flow of gas from high to low pressure (or from low to high velocity). Vogel has shown that flows of this character circulate air through the burrows of prairie dogs, and water through sponges. He even speculates on the possibility of flows within leaves arising from velocity (and therefore pressure) gradients across the surface of the leaves.

There are many lines of evidence that prove the flows in waterlilies are not derived in this way. The pressures in the waterlilies exceed barometric. Vogel's model requires that the pressures within the circulating system are less than barometric.

The second aspect of equation 4 that must be considered involves the mode of pressure measurement. In this study, these measurements were made within the gas spaces of the plant where gases typically were moving, so that the manometric system represents half of a Venturi meter (see Figure C1), and as such measures an aggregate of the internal static pressure and the flow rate. Measurements of both pressure and flow rate establish that the velocity component in the Bernoulli equation is small relative to the pressure component, and the pressures measured by manometer are essentially static pressures (and therefore

Figure C1.—Venturi meter.

Since the linear rate of gas flow is greater above the right arm of the manometer, the pressure exerted by the gas on the wall is less at the mouth of the right arm than at the mouth of the left arm. This pressure differential is manifested by the displacement of manometric fluid toward the right side.

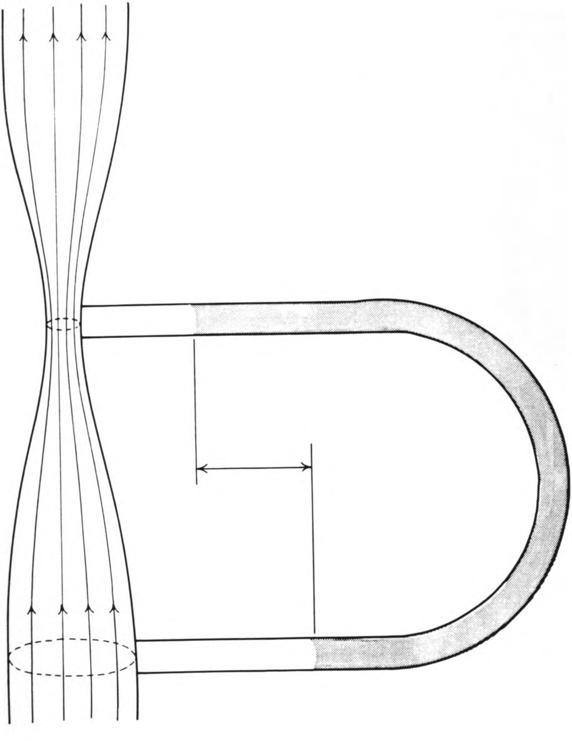
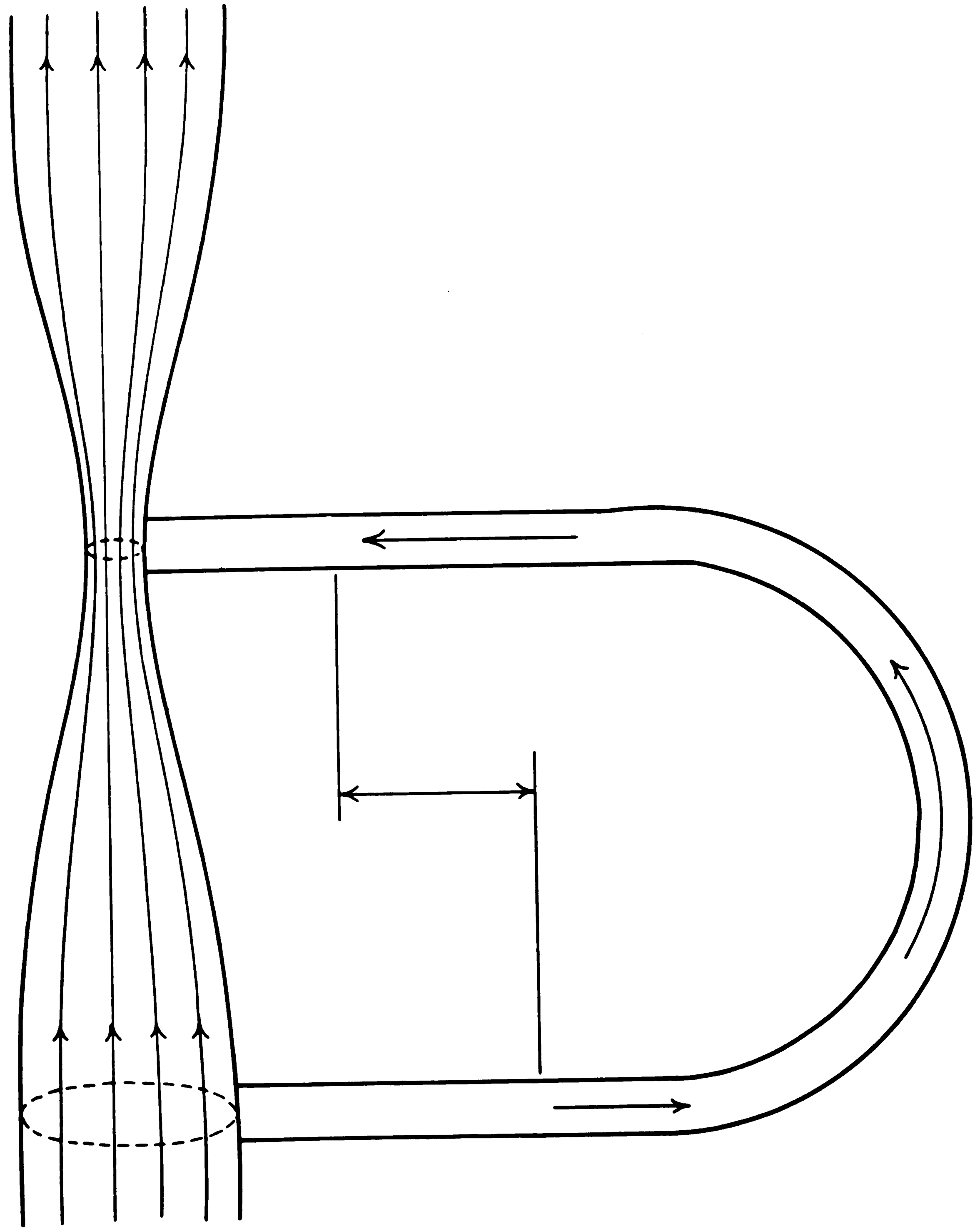


Figure C2.--Venturi flow.
Removal of the manometric fluid from Figure C1 allows a flow of gas from the region of higher pressure (lower velocity) to the region of lower pressure.



directly relevant to processes in kinetic theory (Appendix D and E).

# APPENDIX D

# THERMAL TRANSPIRATION

The character of the flow of gases through an opening depends on the size of the opening. If the opening is large, the flow accords with the conception of flows held by most readers: that is, a wind from a region of high pressure towards a region of lower pressure. Under these circumstances, the motion of the gas is described by the theory of fluid dynamics (cf., Appendix C).

When the pore size is small relative to the free mean path of the gas molecules, the flow of gas through the pore is called molecular effusion, and is described by another body of physical theory: the kinetic theory of gases.

The assumptions underlying the kinetic theory can be listed as follows:

1) The volume of the gas under consideration is made up of a large number of molecules, and in a given type of gas these molecules are all alike.

2) The molecules are small relative to the distances between them, and their motion as described by Newtonian mechanics.

3) The molecules are spherical, and undergo elastic collisions.

4) There are no appreciable forces of attraction or repulsion

between the molecules themselves, or between the molecules and the walls of their container.

On the basis of these assumptions, a system has been developed that, in the words of Loeb (1934), "is as well-established a theory as any theory accepted by science."

The phenomenon of thermal transpiration was first investigated and named by Reynolds (1879):

> "I have now ascertained that a difference of temperature may be a very potent cause of transpiration through porous plates. So much that with hydrogen on both sides of a porous plate, the pressure on one side being that of the atmosphere, a difference of 160°F (from 52° to 212°) in the temperature on the two sides of the plate secured a permanent difference in the pressure on the two sides equal to an inch of mercury; the higher pressure being on the hotter side. With different gases and different plates, various results were obtained which are, however, connected by definite laws."

To understand the theory predicting thermal transpiration, the reader should imagine a small plane of area A drawn anywhere in a gas space. It is possible to show (given the assumptions listed above) that the rate at which molecules strike one side of A is equal to $N\bar{c}A/4$, where N is the number of molecules per unit volume and $\bar{c}$ is their average velocity.

Now imagine that the surface A is a pore in a thin diaphragm. When the linear dimensions of A are smaller than the free mean path of the gas ($L = 10^{-5}$cm at 1 atm), all molecules striking either side of A will pass through it. In the case of such a plane within a gas at equilibrium, the number of molecules moving through the plane (pore) is equal in either direction. Therefore, at steady state:

$$N_1\bar{c}_1 = N_2\bar{c}_2 \qquad \text{eq. D1}$$

Taking $N_1\bar{c}_1/N_2\bar{c}_2 = 1$, and multiplying both sides by $\bar{c}_1/\bar{c}_2$ yields:

$$\frac{N_1\bar{c}_1^2}{N_2\bar{c}_2^2} = \frac{\bar{c}_1}{\bar{c}_2} \qquad \text{eq. D2}$$

Pressure is an expression of the rate of change of molecular momentum on a wall, and can be shown to be:

$$p = \frac{1}{3} Nm\bar{c}^2 \qquad \text{eq. D3}$$

where m is the mass of a molecule. Since p is therefore proportional to $N\bar{c}^2$, $p_1/p_2$ can be substituted into the left side of eq. D2.

For n moles of gas contained in volume V, the empirical equation of state $pV = nRT$ can be combined with eq. D3, yielding:

$$nRT = \frac{1}{3} NVm\bar{c}^2 \qquad \text{eq. D4}$$

Eq. D4 shows that $\bar{c}$ varies with the square root of T, so $T_1^{1/2}/T_2^{1/2}$ can be substituted into the right side of eq. D2. The substitution of eq. D3 and eq. D4 into eq. D2 yields:

$$\frac{p_1}{p_2} = \frac{T_1^{1/2}}{T_2^{1/2}} \qquad \text{eq. D5}$$

This equation describes a steady state system, where the flows of molecules through the pore in both directions are identical. It shows that if there is a temperature gradient between the two sides, there will arise a pressure difference between the two sides. When there is no temperature gradient, $p_1/p_2 = 1$; then the pressures are equal. As soon as a temperature gradient is set up, there is a net flow of gas from the cooler side to the warmer until the conditions in eq. D5 are met.

The fact that the gas flows from cooler to warmer can be demonstrated with the following mechanistic model (cf., Figures D1 and D2). Recalling that the rate at which molecules enter the pore from each side is equal to $N\bar{c}A/4$, it is instructive to study the differential effects of temperature on the temperature-sensitive parameters of that formula, N and $\bar{c}$. Since N (the number of molecules per volume) varies directly with density, it varies inversely with temperature in an open system. On the other hand, $\bar{c}$ varies with the square root of temperature (as shown in eq. D4). These opposing effects cause the rate of entry of molecules into the pore to be proportional to the inverse square root of the absolute temperature. Therefore, if $T_2$ is greater than $T_1$, then $N_1\bar{c}_1A/4$ (the rate at which molecules enter from side 1) is $(T_2/T_1)^{\frac{1}{2}}$ times greater than the rate from side $T_2$. This means that there is a net flow of gas from the cooler side to the warmer side. This flow continues until the pressure differential in eq. D5 has been achieved, or until the temperature gradient is removed.

Reynolds (1879) found that these laws apply, not only for small pores, but also for membranes such as porous porcelain, stucco, and meerschaum. The same principles apply; when a temperature gradient is set up, a pressure gradient ensues.

Thermal transpiration is treated more thoroughly in texts on kinetic theory, such as Loeb (1934), Kennard (1938), and Jeans (1940). Interestingly, each author comments on the fact that if the chambers were connected by a large tube, there would be a steady flow of gas from the warmer to the cooler along this pipe as long as the temperature gradient across the porous partition is maintained (see Figure D3). Such a system can do work, and it superficially resembles a perpetual

Figure D1.—Thermal transpiration (steady state). Two gas phases are separated by a porous partition and a temperature gradient is maintained across the partition. Since the stippled molecules are of higher temperature, their density is less and their velocity is greater than the cooler gas. The equilibrium pressure of the hotter gas is also greater than the cooler.

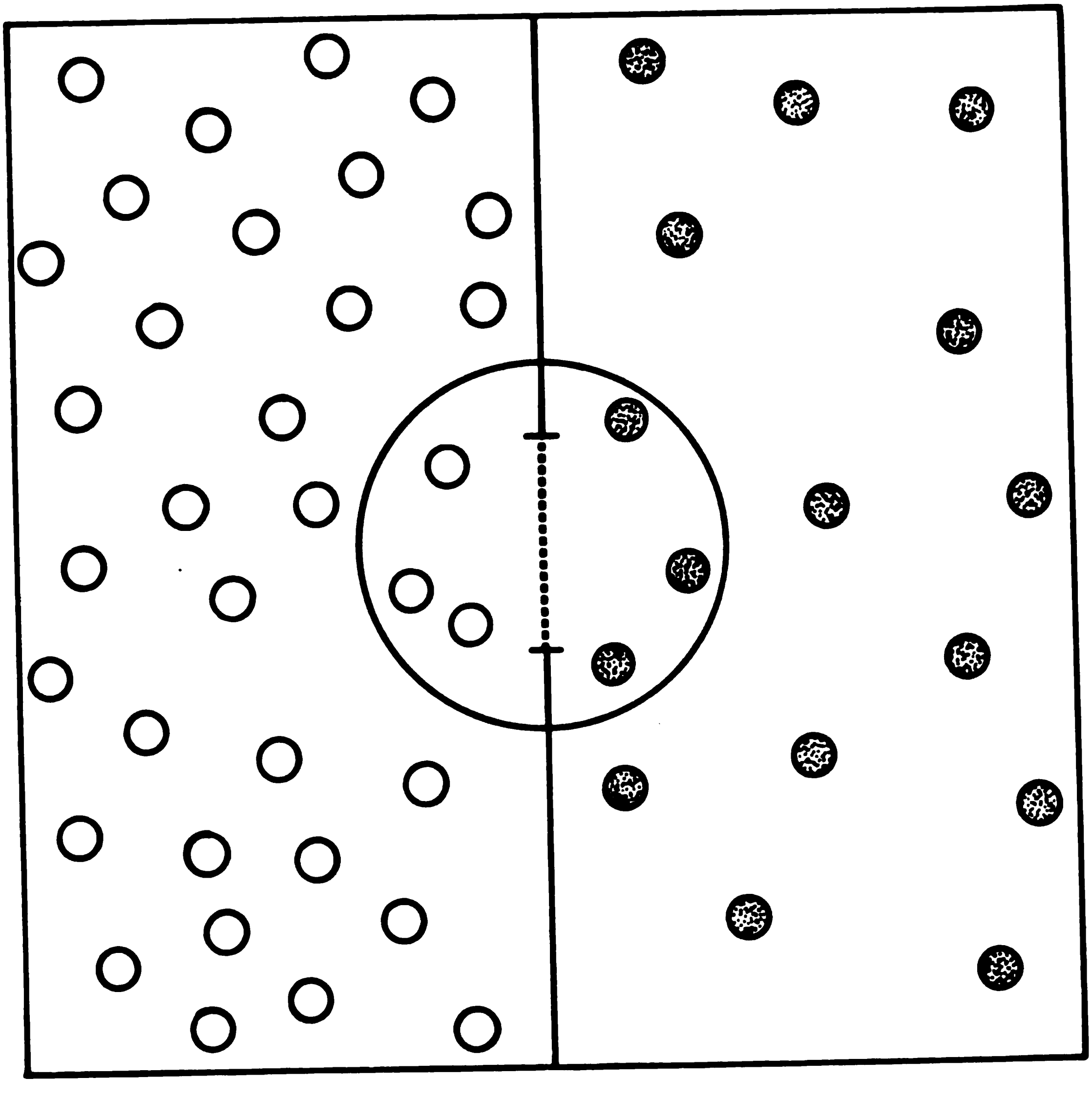
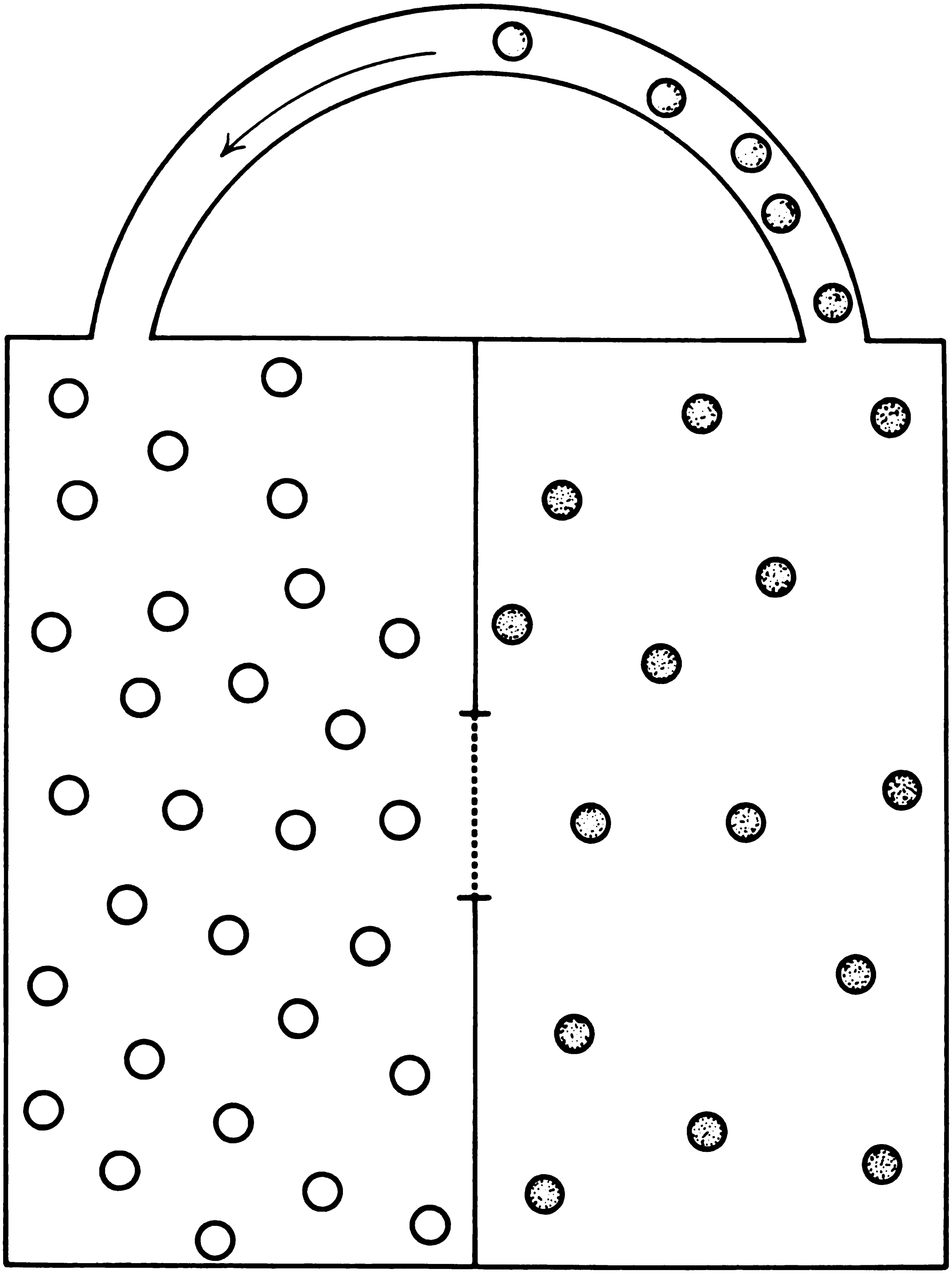


Figure D2.—Porous partition.
This is an enlargement of the circled area in Figure D1. When the total pressures on each side are the same, but the temperatures are different, there results a net movement of gas through the partition. Although the cooler molecules have lower velocities, in terms of diffusive exchange there are disproportionately more of them per unit volume. Therefore, they tend to enter the hotter side faster than the hotter molecules leave.

Figure D3.—Thermal transpiration (flow).
The thermal transpiration pressure derived from diffusive exchange can be allowed to drive a fluid flow of gas if a tube of moderate diameter (greater than 0.1μm) is connected between the two phases. This flow will continue as long as the temperature gradient is maintained.



motion machine. However, Bridgman (1932) points out that the tendency for the temperature gradient to break down fulfills the requirements of the second law of thermodynamics.

Figure D3 is an accurate representation of the gas system in Nuphar. The cooler side can be considered to represent the atmosphere. The warmer side represents the gas spaces in the young, newly-emerged leaves; the porous plug is the upper surface of these leaves. The remainder of the plant — petioles, rhizome, and older emergent leaves, are represented by the external pipe connecting the two sides of the porous plug.[1]

It's interesting to note that Reynolds (1879, p. 842) anticipated some significance for the dimensional properties of gas, of which thermal transpiration is one:

> "Although the results of the dimensional properties of gases are so minute that it has required our utmost powers to detect them, it does not follow that the actions which they reveal are of philosphical importance only. The actions only become considerable within extremely small spaces, but then the work of construction in the animal and vegetable world, and the work of destruction in the mineral world, are carried on within such spaces. The varying action of the sun must be to cause alternate inspiration and expiration of air, promoting continual change of air within the interstices of the soil as well as within

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[1] It might be argued that since the gases in the lacunae are confined in volume by the size of the plant, the conditions for such a system are not met. The warming of the gases in the leaves would tend to increase the pressure there as a simple result of thermal expansion.

However, since the leaves are not closed systems, the gases are free to escape down the petiole and out the other leaves. Certainly, then, there would be an initial (and very small) flow caused by the thermal expansion of the gases as one side is warmed. Such a flow continues until $N_2$ has varied inversely with $T_2$. The flow continues down the petiole for the rest of the day as a result of kinetic processes in the upper surface of the leaf.

the tissue of plants. What may be the effects of such changes we do not know, but the changes go on; and we may fairly assume that in the processes of nature the dimensional properties of gas play no unimportant part."

# APPENDIX E

## HYGROMETRIC PRESSURE

Hygrometric pressure was first studied by Dufour (1873) who found that: "when a porous wall separates two air masses of differing humidity, there results two opposed and unequal diffusion flows across the wall. The more abundant flow moves from the drier air to the damper air. The difference in these currents depends principally on the difference of vapor pressures from one side to the other."

The explanation for this phenomenon was given by Kundt (1877). In many ways the process is analogous to thermal transpiration, except that the gradient across the porous partition is not thermal, but a gradient in the vapor pressure of a volatile liquid. As in the case of thermal transpiration (cf., Appendix D) the exchange of molecules through the porous partition is a diffusive process, and the pressures on either side of the wall can diverge in the process of seeking a diffusive steady state.

As long as there is no temperature gradient and both sides of the partition are dry (i.e., there is no volatile liquid on either side), the gas pressures on both sides are equal. Calling the pressure of the dry atmosphere on one side of the partition $P_a$, these conditions will lead to a pressure $P_1$ of the "dry" constituents on the other side so that $P_1=P_a$. As long as there is no temperature gradient between the two sides, $P_l$ will tend to equal $P_a$, since the "dry" constituents of the gas phase on both sides will tend towards diffusive equilibrium.

When a volatile liquid is added to one side of the partition (Figure E1), the liquid will evaporate and tend to add its vapor pressure $P_v$ to the pressure of the gas on that side. If the gas on this side occupies a closed volume, the total pressure of the gas mixture will also tend to increase. According to Henry's law (cf., Appendix A), the addition of the vapor does not affect the pressure of the "dry" constituents, so $P_l$ is not affected. The pressure on the wet side of the partition therefore approaches the sum of the pressures of the two components there, $P_t = P_l + P_v$.

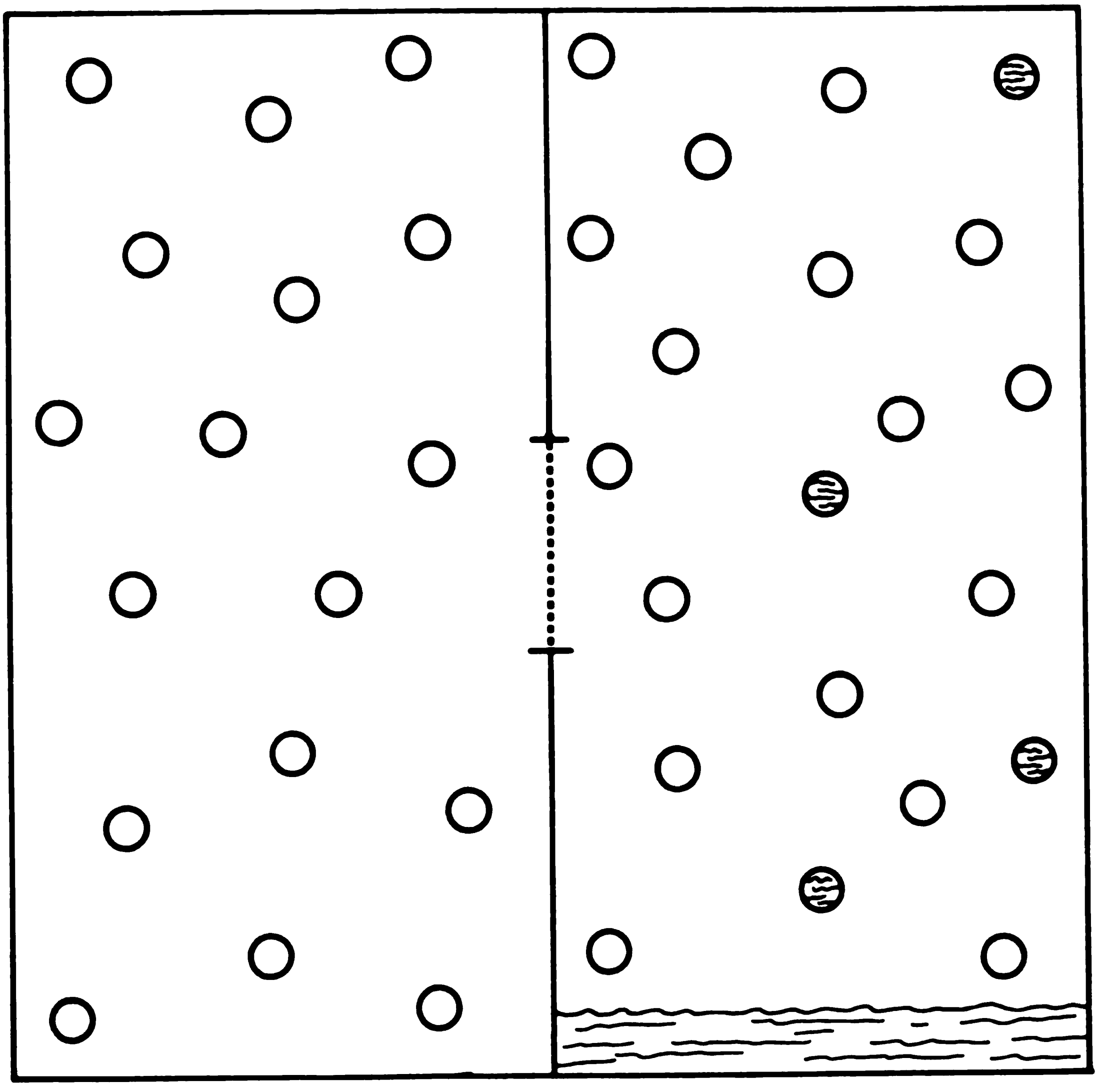
As $P_v$ increases, the vapor tends to diffuse through the partition to the dry side. If this vapor is removed from the gas phase on the dry side (for example, by absorbing it, or by diluting it in a large volume of dry gas), it won't contribute significantly to the gas pressure on the "dry" side. As it diffuses away from the "wet" side, however, it tends to diminish $P_v$ on that side. The liquid on that side continues to evaporate in order to maintain saturation of the vapor in the gas phase on that side. Once all the liquid has evaporated, the vapor will eventually diffuse through the partition to the dry side, $P_v$ approaches zero, and the total pressure $P_{tot}$ on the previously "wet" side approaches $P_l$ ($=P_a$).

Clearly, a liquid phase is required to support the increased total pressure on the "wet" side. The evaporation of that liquid replaces the vapor lost by diffusion through the porous partition. The vaporization of liquid consumes energy and tends to cool the atmosphere

Figure E1.—Hygrometric pressure.

If no temperature gradient exists across the porous partition, the dominant gas mixture (open circles) equilibrates at equal density on either side of the partition. If a volatile liquid is present on one side of the partition, it tends to add its vapor pressure to the total pressure on that side.

The vapor will also tend to diffuse through the partition, but as it does, more liquid evaporates in order to maintain its vapor pressure on the "wet" side.



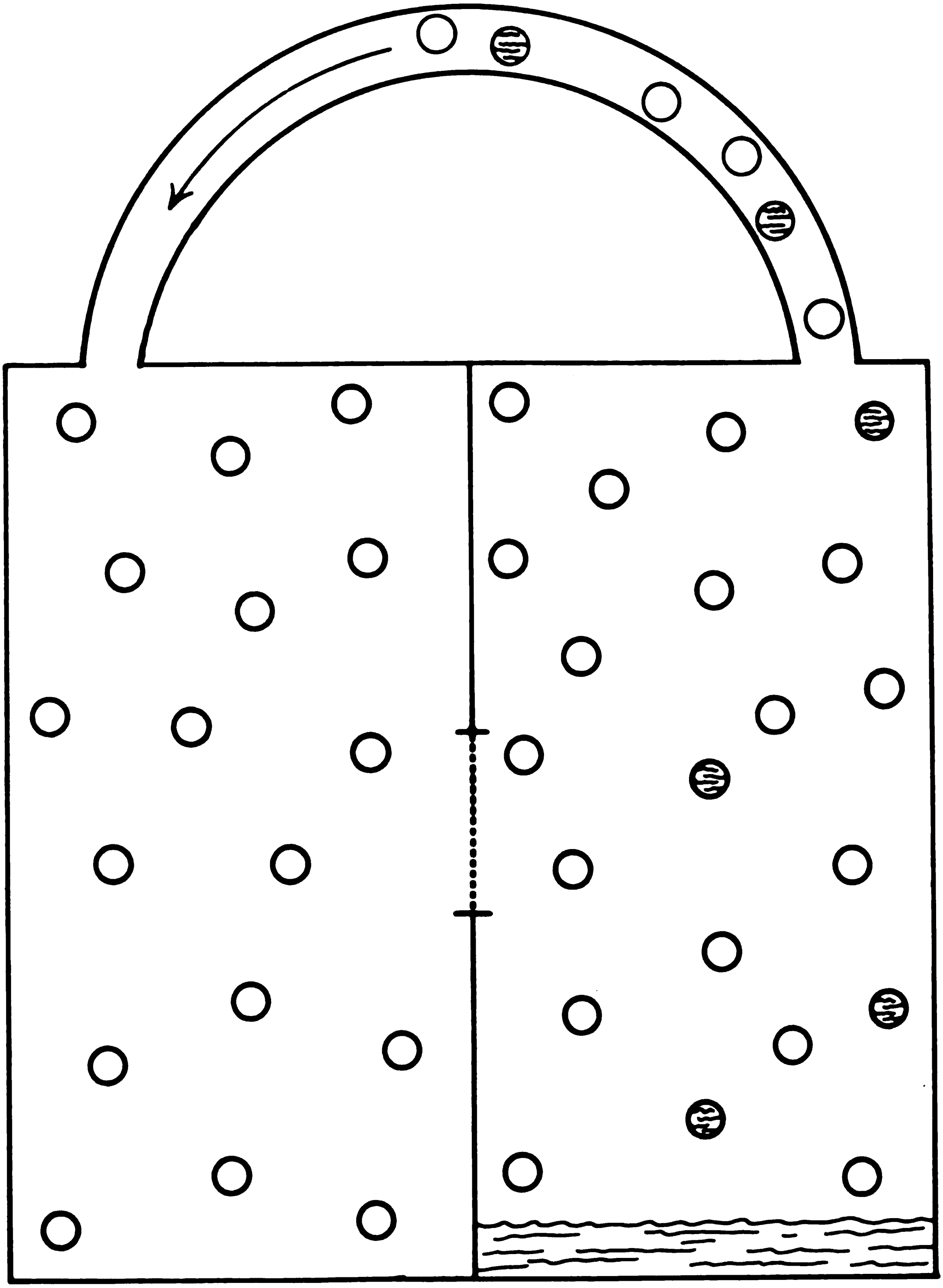
on that side of the partition, so if the system is to remain isothermal, heat must be transported to that side. This process depends, therefore, on the continual input of liquid and heat. This is analogous to the continual input of heat required to maintain the thermal gradient that drives thermal transpiration.

If a large tube is attached to this system so as to allow a fluid flow of gas away from the region of high pressure (Figure E2), the dynamics of the system are changed. The fluid flow of the gas mixture ("dry" components + vapor) away from the "wet" side decreases the total pressure of the gas phase on that side. Since this flow removes the various gas components in proportion to their abundance in gas phase, both $P_1$ and $P_v$ decrease. Since $P_1$ is now smaller than $P_a$, there is a net influx of the "dry" constituents of the gas into the "wet" side through the porous partition. This net influx of dry gas is accompanied by evaporation of the liquid on the wet side, so the pressure $P_{tot} = P_1 + P_v$ on the wet side tends to return towards the earlier steady state value. This tendency to increase the pressure forces a continual flow along the large tube away from the region of high pressure. As long as liquid remains on the wet side, such a steady flow of gas (+ vapor) is generated.

In the foregoing discussion, it was assumed that the vapor pressure of the liquid was negligible on the "dry" side. In fact, for such a system to operate, the vapor pressure of the liquid need only be less than its vapor pressure on the other ("wet") side. In the case of the waterlily, the upper surface of the leaf is the porous partition. The volatile liquid is water. The vapor pressure gradient depends very much on the ambient relative humidity, since the air inside the

Figure E2.--Hygrometric pressure (flow).
As in the case of thermal transpiration, a tube can allow a continuous fluid flow of gas mixture (including vapor) from high pressure to low pressure. As the pressure drops in the "wet" chamber, there is a net rate of diffusion into that side from the "dry" side, tending to maintain pressure equilibrium. Simultaneously, liquid evaporates.

Such a system can support the flow as long as liquid remains on the wet side, and there is a humidity gradient across the partition.



leaf is presumably saturated at all times.

As is discussed in the text of this thesis, the system is very rarely at thermal equilibrium. During daylight, the leaves are usually warmer than the ambient air, so the vapor pressure of water inside the leaf can be considerably higher than that of the environment. (These temperature differentials also contribute to thermal transpiration.)

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