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**Principal Investigator:** Robert G. Wetzel, Bishop Professor  
Department of Biological Sciences  
University of Alabama  
Tuscaloosa, AL 35487-0206



Robert G. Wetzel, PI/PD

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## WETLAND INTERFACE ECOSYSTEM

The land-water interface region consists of two major components: the wetland, and the down-gradient adjacent littoral floating-leaved and submersed, macrophyte communities. These components form a gradual down-slope gradient system. The interface components have in common: (a) extremely high productivity and growth capacities which generate large amounts of particulate organic matter, much of which is deposited in the interface region; (b) high rates of decomposition with the release of massive amounts of dissolved organic matter (DOM); and (c) a selective decomposition of the DOM that is dependent upon many biotic and environmental conditions, but in particular the time available for metabolic "processing". Because of the importance of very high production and nutrient turnover of attached microbiota (epipelic attached to detritus and sediments and epiphytic microbiota sessile on macrophyte parts), a major emphasis of this investigation was placed upon these biota and their metabolic capacities for assimilation and release of organic compounds and nutrient retention and cycling. Examination of the capacities of wetland and littoral communities to regulate fluxes of nutrients and organic compounds often has been limited to input-output analyses (e.g., excellent review of Bowden 1987 for nitrogen). These input-output data are an integral part of these investigations, but most of the research effort concentrated on the biotic and metabolic mechanisms that control fluxes and retention capacities (Fig. 1) and their effects upon biota in the down-gradient waters. The important regulatory capacities of dissolved organic compounds on enzyme reactivity was examined experimentally and coupled to the wetland-littoral organic carbon flux budgets.

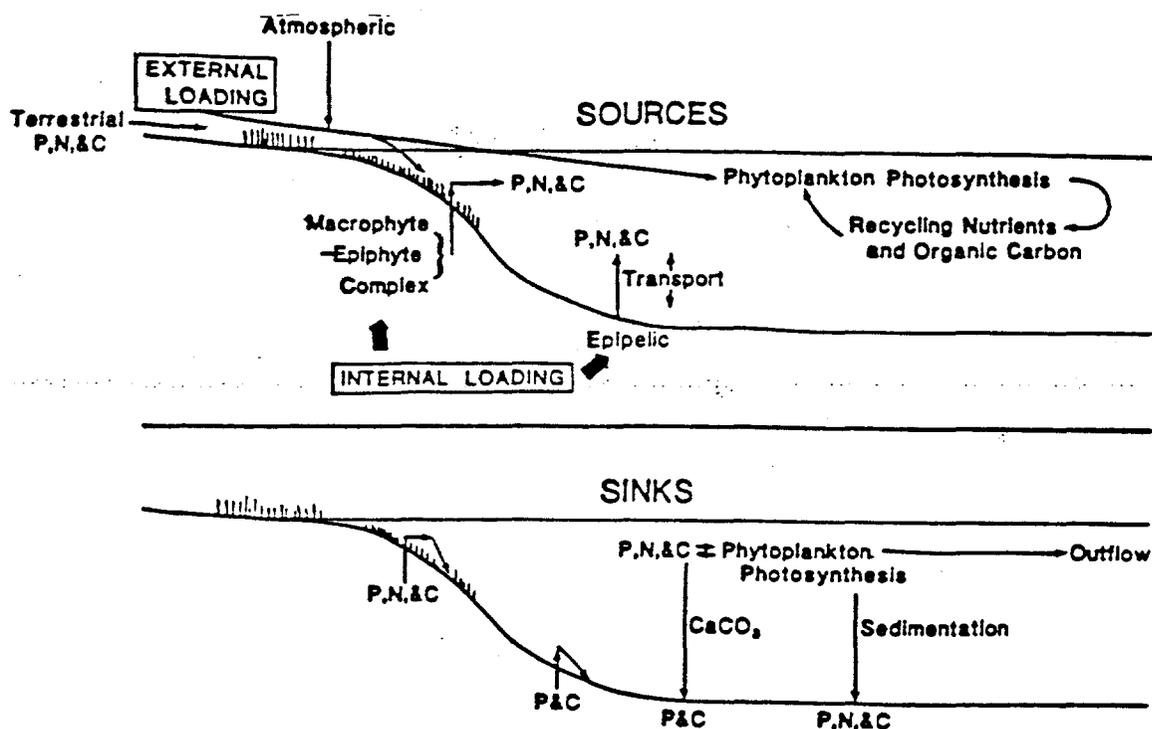


Figure 1. Sources and fates of nutrients or carbon as influenced by metabolism within wetlands, littoral regions, the sediments at all steps along the gradients, and within the river or lake water.

## Data Integration, Analyses, and Modelling

### 1. Organic Carbon Fluxes and Budget

The Lawrence Lake Ecosystem was used as a model system for a representative small lake. However, many aspects of these quantitative evaluations are applicable to other lake and river floodplain ecosystems. The organic production, loadings, and losses were evaluated as follows.

#### A. Allochthonous loadings of particulate (POC) and dissolved organic carbon (DOC)

Data demonstrated that:

- (a) POC loading to lakes is very low and is retained largely in wetlands and littoral reaches
- (b) Conversion of allochthonous POC is largely to CO<sub>2</sub> which evades and to DOC which is subsequently transformed enroute to the lake per se;
- (c) The major DOC loading from allochthonous sources is from plant polyphenolic compounds, which augment those produced by wetland and littoral macrophytes;
- (d) Allochthonous loading of DOC is only moderately variable seasonally. High loadings of DOC during major precipitation events are sharply modulated by the adsorptive and biotic reactivity of wetland and littoral microflora;
- (e) The macrophytes do not modify allochthonous DOC loadings greatly, but rather served as a major interface source of DOC.

**B. The emergent wetland macrophytes constitute a major source of particulate and dissolved organic matter loaded to the wetland and littoral zones**

Data were used to (a) estimate bulk synthesis and loading of organic matter to the system, (b) separate riverine/allochthonous inputs from wetland/littoral sources, and measure insite spatial heterogeneity in biomass, productivity, and detrital loadings.

Methods for analyzing the population dynamics and productivity of emergent macrophytes followed those of Dickerman and Wetzel (1985; cf. also Dickerman et al. 1986). Growth analyses of the belowground tissue of emergent plants were analyzed by the methods of Grace and Wetzel (1981a, 1981b), in which pulse labelling with <sup>14</sup>CO<sub>2</sub> was used in addition to biomass-change analyses of rooting tissue.

Many growth responses of emergent plants to controlled perturbations must be determined over long periods of time by measures of changes in organic carbon biomass. In certain cases, however *in situ* rates of photosynthesis were measured directly (assimilation rates of

CO<sub>2</sub>, dark CO<sub>2</sub> evolution rates, CO<sub>2</sub> compensation points, transpiration rates, and stomatal conductance; LiCor 6200/6250 portable photosynthetic system).

The results demonstrated that:

- (a) The wetland vegetation is one to eight times more productive in synthesis of organic matter than the littoral or pelagic zones;
- (b) Expression of the productivity in biomass is influenced by the availability of nitrogen and phosphorus in the hydrosols. Under limiting nutrient conditions, much greater resource allocations are made to below-ground biomass than to above-ground biomass;
- (c) The below vs. above-ground biomass allocation patterns markedly influence
  - i. the extent of decomposition of the plant biomass produced,
  - ii. the amount of plant biomass permanently buried in the wetlands, and
  - iii. most importantly, the amounts and chemical composition of the dissolved organic compounds released to the water flowing through the wetlands to the receiving water.

The organic acids and other polyphenolic organic compounds that are released from decomposing emergent macrophytes were a major focus of on-going research of this contract (see below). These organic compounds can radically decrease the efficacy of exoenzyme activities, including phosphatase activities.

**C. Decomposition of dissolved organic matter of macrophytes: Control parameters, rates, and organic matter sinks**

*In situ* analyses have addressed the fates of organic matter synthesized in place (largely macrophytes and periphyton) and "imported" organic matter from upstream and allochthonous sources. The latter "imported" organic matter is primarily DOM and refractory materials from the selective metabolic and physical removal processes occurring up-gradient. Both *in situ* and

experimental examinations have coupled directly to the products exported from the riverine component of the total riverine-wetland gradient. Loadings have been coupled to the different sources of dissolved and particulate organic matter, including upgradient production of plants and entrapped particulate organic matter (POM) of different types.

During senescence of macrophytes or phased cohorts during the growing season, most of the particulate organic matter remains largely in the vicinity of its production (detrital deposits). These studies demonstrated selective modification of these compounds by microbial degradation and major transport of these carbon products as dissolved organic matter to down-gradient ecosystem components. Past studies (Technical Progress Reports 1986, 1987, 1988, and 1989) permit us to evaluate both the total loadings of DOM from allochthonous, wetland, and littoral sources as well as qualitative changes in DOM composition (by ultrafiltration, ion chromatography, and mass spectrometry). In the experimental portion of the present studies the effects of these compounds were demonstrated upon exoenzymatic activities and nutrient cycling. These interactions demonstrate a major functional coupling between the land-water boundary biota and the physiology of organisms of pelagic regions.

#### **D. Senescence and overwintering among emergent and submersed angiosperms:**

##### **Physiological adaptations and effects on organic carbon budgets**

Senescence in both emergent and particularly submersed aquatic plants is a continuous process and exceedingly difficult to evaluate accurately. Among emergent plants (e.g., cattail *Typha latifolia*), we successfully demonstrated three annual cohorts and the major enhancement that this production yielded (Dickerman and Wetzel 1985; Grace and Wetzel 1981a, 1981b). The phased production also resulted in phased detrital loadings of POC and particularly DOC during periods of high rates of both aerobic and anaerobic decomposition.

Senescence among submersed macrophytes is even more difficult to quantify. Current studies utilized the changing rates of carbon fixation and concentrations and activities of carboxylation enzymes (RuBP carboxylase oxygenase; PEP carboxylase) as one means of accurately evaluating the photosynthetic capacities of the plants at different life stages. Because the methodology is very laborious, enzymatic assays are limited to specific experimental manipulations (see below) of nutrient and organic loadings.

Boylen and Sheldon (1976) provided the first evidence that certain submersed macrophytes remain photosynthetically active beneath ice in temperate lakes. The gross photosynthetic rates of plants during the winter were only 20% of the rates measured during the summer, when both were measured at natural growth temperatures. However, when the photosynthesis of both winter and summer plants were measured at 2°C, the rate of the winter collected plants was almost 50% greater than that for the summer plants. Furthermore, the photosynthetic rate measured at 23°C was only 16% greater in the summer compared to the winter collected plants. Therefore, it appears that winter collected plants possessed a degree of acclimation to photosynthesis under ice.

Since these studies, little has been accomplished concerning the physiological mechanisms underlying the acclimation of photosynthesis and growth under ice. Both the physical conditions and resources change considerably beneath an ice cover in temperate lakes. Low temperatures (2-5°C) reduce the rate of most enzymatic reactions, and the fluidity of thylakoid (chloroplast) membranes (Oquist and Martin 1986). The result is a potential reduction in both the dark and light reactions of photosynthesis, as well as the enzymatic steps in other plant metabolic pathways including Krebs cycle (dark respiration), and photorespiratory pathway.

*Ceratophyllum demersum* is a common SAM in many temperate lakes and survives the winter in a physiologically active state as apical tips with short internodes. Since *C. demersum* is rootless, all of its nutrient requirements must be obtained via stem and leaf tissue. During spring and summer growth, nutrient availability is extremely reduced resulting from the growth of other autotrophic and heterotrophic organisms. Unlike the rooted SAM which can obtain nutrients from the hydrosol while maintaining photosynthetic tissue in a favorable irradiance, *C. demersum* growth is restricted to the surface where it is disconnected from the supply of nutrients in the hydrosol.

Net carbon gain of winter acclimated *C. demersum* occurred at 5, 10, and 15°C. Net photosynthesis was greatest at 5°C and decreased with increasing temperature; no net carbon gain occurred at or above 20°C. The optimum temperature for photosynthesis is certainly between 5 and 10°C, and may be below 5°C, which represents a value lower than most winter-acclimated terrestrial plants possess.

The decline in net photosynthesis with increasing temperature is likely related to the almost linear increase in dark respiration with temperature. Dark respiration increased from a low value of 0.8  $\mu\text{mol CO}_2/\text{mg Chl/h}$  at 5°C, to a high value of 8.8 at 25°C. Apparently dark respiration is highly regulated in winter acclimated *C. demersum*. Comparison was made of these results with those obtained from summer acclimated plants.

The  $\text{CO}_2$  exchange balance between respiratory  $\text{CO}_2$  loss and uptake may be evaluated by the photosynthetic  $\text{CO}_2$  compensation point (CCP). The CCP for the winter acclimated plants had a low value of 175  $\mu\text{L CO}_2/\text{L}$  at 5°C, and a high value over 500 at 25°C. The high values are substantially higher than those reported for other SAM and represent a significant loss of carbon via respiration (Spencer and Bowes 1989). The increased dark respiration rates and CCP may

represent a response to a seasonal increase in temperature which would increase the turnover rate of ATP and phosphorylated intermediates required for the biosynthesis of Calvin cycle enzymes involved in photosynthesis.

The irradiance at which net photosynthesis is saturated increased with increasing temperature up to 20°C. Apparently a greater irradiance was required to saturate photosynthesis at the higher temperatures. These results indicate the  $V_{\max}$  of the photosynthetic light reactions are limited by temperatures below 20°C in these plants. Results obtained indicated (i) that significant submersed angiosperm metabolism and net growth can occur in winter, and (ii) more importantly that species with this capability possess a competitive advantage for accelerated growth in spring.

The photosynthetic light compensation point increased in an exponential fashion from 15  $\mu\text{mol quanta/m}^2/\text{s}$  at 5 to over 500 at 20°C. These results indicate that at higher temperatures the irradiance required to maintain a zero  $\text{CO}_2$  exchange rate greatly increased. This observation is most likely the result of increased dark respiration associated with the elevated temperatures. The  $K_{1/2}$  (quantum flux) changed relatively little across this temperature range. These findings suggest that the efficiency (amount of light required to fix one mole of carbon) of the light reactions remained relatively constant across the temperature range.

#### **LITTORAL ZONE: MACROPHYTE-PERIPHYTON COMPLEX**

Quantitative analyses of nutrient fluxes, uptake mechanisms, and recycling in microcommunities on the plants and within the plants continued as a major functional site of nutrient regulation among freshwater interface zones. Limiting nutrient factors were dissolved inorganic carbon, phosphorus, and in some cases, nitrogen.

The importance of nutrient fluxes within wetland regions of fresh waters is totally different than in the pelagic, and planktonic concepts are not applicable in many cases. The macrophyte-epiphyte complex exists in a viscous medium where regions of greatly reduced flow and no turbulence occur at surfaces. As a result of the greatly reduced flow, lack of turbulence, and extremely slow rate of diffusion in water, diffusional processes predominate within the boundary layer. During periods of high metabolic activity, i.e., during photosynthesis, nutrients are likely to become depleted within the boundary layer, constraining production and placing the macrophyte and associated microflora in direct nutrient competition. However, once nutrients have entered the complex via diffusion or sedimentation from the bulk phase, or uptake through the macrophyte rhizosphere, exit across the boundary layer is retarded. The close juxtaposition of the biota results in a rapid cycling and, ultimately, in concentration of nutrients. This occurs until the end of the macrophyte vegetative life span when the supporting macrophyte loses its integrity and release of dissolved matter exceeds the retentive capacity of the microbiota. A quantity of this material released by the macrophyte is retained in the epiphyton when the macrophyte-epiphyte complex sinks to the sediment (Moeller, Burkholder, and Wetzel 1988).

These studies quantified both nutrient fluxes as well as productivity dynamics of the macrophytes and the sessile microbial communities. Analyses have demonstrated the tight seasonal shifts in algal and bacterial productivity and phosphorus limitations among these microbes (Burkholder and Wetzel 1989a). The macrophytes provide a distinct habitat for epiphytic algae and bacteria. Artificial substrata do not simulate natural plant substrata; under natural conditions, the macrophytes contribute both inorganic nutrients (P, CO<sub>2</sub>) and organic compounds to the epiphytic microflora (Burkholder and Wetzel 1989b, 1989c; Moeller et al.

1988). Phosphorus and carbon movements occur from sediments, through the submersed macrophytes, and are released in variable quantities to the microflora.

**Diffusive transport across the water column interface with the submersed macrophyte-epiphyte complex as affected by microscale hydrodynamics.** The submersed macrophyte-epiphyte community possesses a large and metabolically active surface area. These sites are where dissolved or suspended constituents of water passing through the community are altered. Suspended material may settle out of the water column as a result of lost momentum or be removed by impaction upon vegetative surfaces. The inorganic and organic dissolved content of the water is also greatly affected, particularly in calcareous waters where these constituents may be co-precipitated with carbonate or decomposed (cf. Otsuki and Wetzel 1972, 1974; Mickle and Wetzel 1978a, 1978b, 1979). The impact of the submersed macrophyte-epiphyte community on water is a function of community structure (species composition, biomass, and density) mediated by the hydrodynamics of the system (microscale flow regime around leaves, and exchange of water between the open water and within the littoral plant communities).

Submersed macrophytes and epiphytic periphyton exist in intimate chemical and physical juxtaposition, surrounded by a layer of water where flow is laminar. As a consequence of this laminar flow boundary layer, transport of dissolved substances between the water column and the macrophyte-epiphyte complex surface must be via diffusion, a process  $10^4$  times slower in water than in air. Transport of dissolved substances within the complex must also be via diffusion. Diffusive transport of dissolved substances across the water column/macrophyte-epiphyte complex interface may be described by Fick's first law of diffusion:  $J = -D_s(dC/dx)$  where,  $J$  is flux rate,  $D_s$  is diffusion coefficient, and  $dC/dx$  is concentration gradient over the surface (Crank 1975). Since the rate of diffusive flux is inversely proportional to the diffusive path length

(distance  $x$  above the surface where flow is equal to the free-stream flow rate), boundary layer development is critical. From measurements of *in situ* flow rates and boundary layer theory, we predict boundary layers will range from several micrometers to greater than 1 mm in thickness and represent a significant barrier to the transport of dissolved substances across the water column (Losee and Wetzel 1988). These conclusions agree with others who have investigated the uptake of inorganic carbon by submersed macrophytes (cf. Raven 1970, Smith and Walker 1980), based upon physical models.

The tightness of the chemical relationship between the organisms of the macrophyte-epiphyte complex is influenced by the hydrodynamics of the system. Whether laminar or turbulent conditions occur at submersed surfaces depends on the ratio of inertial to viscous forces (i.e., laminar conditions prevail at low Reynolds number,  $Re = (\rho l U) / \mu$  where,  $\rho$  = density,  $l$  = characteristic linear dimension such as, diameter or distance from leading edge of a leaf,  $U$  = velocity of flow, and  $\mu$  = dynamic viscosity). Water movement in submersed macrophyte beds is very slow (Losee and Wetzel 1988); therefore, laminar conditions nearly always predominate.

Boundary layer thickness ( $\delta$ ) can be estimated for laminar flow from theoretical considerations as:

$$\delta_l = F l (Re_l)^{-1/2}$$

where,  $l$  is the distance from the leading edge,  $Re$  is Reynolds number, and  $F$  is a factor specified to define the thickness of the boundary layer demarcated by flow as some percentage of free-stream flow rate.  $\delta$  is proportional to the inverse of the square root of  $Re$ . However,  $\delta$  and  $Re$  are both directly proportional to length from the leading edge ( $l$ ). Therefore, as length from the leading edge increases, boundary layer thickness increases (cf. also Riber and Wetzel 1987).

Slow non-turbulent flow will result in a thick boundary layer and a large diffusive resistance to transport across the water column/submersed macrophyte-epiphyte complex interface. This large diffusive resistance may result in the depletion of nutrients within the complex and competition for resources. As an example, during periods of intense photosynthesis the fixation of CO<sub>2</sub> often can exceed the supply rate of dissolved inorganic carbon (DIC) from the water column, and result in depletion of DIC in the boundary layer, thus placing the epiphytic algae and macrophyte in direct competition for this resource. Under conditions of thick boundary layer development, fortuitous and beneficial mutualist associations must exist as well, where nutrients are tightly cycled and accumulate within the complex. Under rapid flow and turbulent conditions boundary layers will be thinner and the supply of nutrients from the water column will be of relatively greater importance.

A five channel warm bead thermistor flowmeter, modified from LaBarbara and Vogel (1976) was constructed with each channel temperature ranged and compensated. The output from each flowmeter channel was read by a Campbell CR10 Datalogger with a sampling frequency dependent on experimental requirements. The thermistor probes were arrayed vertically and horizontally through the *S. subterminalis* and *P. praelongus* plant beds, from the interior to above the canopy or towards the open water of the lake. The spatial pattern of water movement is determined on a small scale within the plant bed, at two depths, and on the windward and leeward shore. The temporal pattern of water movement is determined on a long (seasonal), medium (complete wind event cycle), and short (on order of a second) term time scales.

Since plant orientation behavior is critical to boundary layer development, a flume (90 cm x 90 cm x 12 cm) was constructed to closely study orientation behavior of submersed plants.

Flow was varied over the range of flows the plants experience in nature. Wave oscillations were superimposed over flow to test the effect of wave induced surge. The flume was used to investigate the effects of increasing structural complexity on water movement patterns.

The prevailing characteristic of all flow measurement series was that flow rates within the vegetation were always less than  $1 \text{ cm s}^{-1}$  and usually were in the range of  $0.0$  to  $0.3 \text{ cm s}^{-1}$ . This observation includes a measurement series made during fall turnover, when the ratio of momentum to frictional resistance of water movement was at a maximum and currents were greater than  $20 \text{ cm s}^{-1}$  above the vegetative canopy.

Flow rates above the vegetation usually were only a few  $\text{cm s}^{-1}$  and therefore, whether the submersed macrophyte bed is on the windward or leeward shore has little consequence for flow rates within the canopy. In a lake with a very large fetch wave energy may be great enough to significantly affect flow within vegetation. However, under these conditions submersed macrophytes are sparse or nonexistent. Flow differences between depths seem to be minimal.

Characteristic of all flow measurements is a rapid fluctuation of flow rates. This fluctuation is indicative of small scale eddies present within the plant bed on the order of millimeter to centimeters in diameter.

Three stages in the orientation behavior of *S. subterminalis* with respect to the direction and rate of water movement have been identified. At low flow rates ( $<1 \text{ cm s}^{-1}$ ) the leaves remain erect against the water movement, held still by their buoyancy. At slightly greater flow rates the plants begin to oscillate or sway with the water movement. At high flow rates the plants bend in the current and undulate. At this high flow rate the broad surface of the leaves is presented to the current.

Boundary layer development may be directly assessed by measuring microscale flow rate profiles in three dimensions over the leaf surface. The timelapse photographic technique of Ackerman (1986) was adapted for determining boundary layer development. Briefly, neutral density particles, of small diameter, released in a flowing flume, are photographed with time-lapse, as the particles float past the leaf surface. The leaf was photographed in cross-section with a narrow depth of field; therefore, particle movement recorded on the film was in a single plane. The leaf and exposed particles, which define the flow path, were digitized and analyzed for flow rate as the change in particle position per strobe flash interval. Many exposures were made, for each free-stream flow rate, along the length of the leaf to reconstruct the three-dimensional micro-flow profile. *S.-subterminalis* and *P. praelongus* leaves were positioned with three orientations: the leaf upright and (1) perpendicular, (2) broadside, and (3) reclining and parallel to the direction of flow.

Whether turbulence occurs at the submersed macrophyte-epiphyte complex surface was evaluated by video taping dye streamlines as they traversed the vegetation. The video tape was analyzed by slow and stop-motion video. Turbulence only occurred with flow rates greater than 1 cm s<sup>-1</sup> and with wave surge superimposed over the directional flow. Under these conditions, turbulence occurred when the oscillating leaf reversed direction and moved against the current, when the relative flow rate was maximal. Increased structure resulted in a decrease of flow rate but had no effect on turbulence at leaf surfaces downstream on the structural elements (leaves).

Notable in this time-lapse photograph of neutral-density particles was the smoothness of flow paths and lack of turbulence. A composite of the flow field for leaf cross-sections was constructed from several time-lapse photographs of a single flow condition. The resultant flow rate isoclines revealed the complex nature of the flow pattern around a submersed leaf. Water

piles up on the upstream side of the leaf resulting in the reduced flow in this region. Flow was accelerated as water was forced around the sides of the leaf, and a large wake was formed downstream. Shear rate (the change in flow rate with distance away from the surface or slope of the flow profiles) was inversely proportional to boundary layer thickness. Flow rate profile axes were scaled such that the apparent slopes are equivalent. Shear rate was much greater to the sides of the leaf as compared to upstream and downstream.

### **Carbon Metabolism in Submersed Macrophytes (SAM): Biochemical Adaptations to Carbon Limitations and Species Competition**

**SAM "weeds" were superior competitors because their growth was less sensitive to stress conditions (low DIC and light availability) than non-weed SAM.** SAM weeds possess physiological characteristics, reviewed in Spencer and Bowes (1989), which suggest these plants are capable of continued growth under stressful (low resource conditions). For example, the light compensation point (LCP) of several SAM weed species has been shown to be as low as 0.5% of full sun values, whereas light compensation points for non-weed SAM, which the SAM weeds are known to displace, have light compensation points as high as 3.0% full sun. The ecological significance of this physiological characteristic may be related to the chronological variation of irradiance in water. Irradiance increases exponentially after sunrise, but for the first hour or two a submerged community is exposed to an irradiance below the compensation point of some of its members. During the day, when light is adequate, DIC can become limiting because of photosynthetic depletion. A weed species with a low light compensation point could begin net carbon accumulation earlier in the day than the non-weeds with higher light compensation points and therefore utilize the DIC replenished from nocturnal community respiration and diffusion.

Repetition of this scenario over several days could result in greater growth of the species with the lowest LCP. If the SAM weed were able to continue net photosynthesis at an irradiance below which non-weed SAM cannot, then unlike SAM non-weeds the SAM weeds may continue growth. Furthermore, if the same species which possess the lowest sensitivity of growth to light stress were also superior in the depletion ability of DIC, then they would quickly dominate the system. The above scenario was empirically demonstrated and may partially explain the competitive dominance of SAM weeds, which combines both the "competitive" and "stress-tolerant" dominance theories of Grime into a single growth strategy.

Although LCP of apical stem sections of *H. verticillata* have been shown to acclimatize to growth irradiances in the lab and continued growth at low irradiances, growth sensitivity to irradiance by SAM weeds (at different LCP) and non-weeds have not been experimentally determined.

It has been demonstrated that the SAM weeds including *M. spicatum* and *H. verticillata* are plastic in terms of their photosynthetic gas exchange characteristics. Individuals of both species possess CO<sub>2</sub> compensation points (CCP) ranging from C<sub>3</sub> to C<sub>4</sub>-types of metabolism. No other group of photosynthetic organisms possess this capability. SAM weed individuals which have C<sub>4</sub> gas exchange characteristics possess low CCP, low photorespiratory CO<sub>2</sub> loss, and reduced inhibition of photosynthesis by oxygen, when compared to individuals with C<sub>3</sub> gas exchange characteristics.

The gas exchange characteristics typical of terrestrial C<sub>4</sub> plants result from two coadjutant phenomena which depend upon the activity of the phosphoenol-pyruvate carboxylase (PEPcase) and unique leaf anatomy. Ribulose 1,5 bisphosphate carboxylase-oxygenase (Rubisco) is the only enzyme by which significant amounts of inorganic carbon enters plant metabolism and organic

components of the biosphere in general. The enzyme is bifunctional and not only carboxylates the substrate ribulose 1,5 biphosphate (RuBP), but in the presence of  $O_2$  may also oxygenate RuBP. Carboxylation results in the net fixation of one molecule of  $CO_2$  via further reactions of Calvin Cycle.

However, oxygenation of Rubisco results in the loss of one molecule of  $CO_2$  via the reactions of the photorespiratory cycle. Furthermore,  $O_2$  behaves as a competitive inhibitor of  $CO_2$  such that for any given molecular of Rubisco whenever oxygenation occurs carboxylation is prevented. Therefore,  $O_2$  has two deleterious effects upon carbon fixation by Rubisco, (1) a net loss of one  $CO_2$  molecule and (2) competition with  $CO_2$  for the enzyme activated substrate RuBP. Photorespiration is an inescapable characteristic of normal  $C_3$  photosynthesis and represents a significant loss of carbon from  $C_3$  plants. It has been suggested that the elimination of photorespiratory  $CO_2$  loss in  $C_3$  crop plants could result in a 40-50% increase in biomass production without the application of additional agricultural chemicals. Therefore, any inducible (as in certain SAM weeds) physiological/biochemical mechanism that reduces photorespiration in nature potentially represents a method by which  $C_3$  crop plants can be genetically engineered to reduce photorespiration.

Both PEPcase and leaf anatomy function to reduce the deleterious effects of  $O_2$  in terrestrial  $C_4$  plants. The leaves of  $C_4$  plants possess Kranz (ringlike) anatomy and, unlike  $C_3$  plants, the bundle sheath cells surrounding the vascular bundle possess large numbers of chloroplasts. Enclosing the bundle sheath cells are several layers of spongy mesophyll which also possess chloroplasts. The oxygen sensitive Rubisco is sequestered in the bundle sheath cells, while the surrounding spongy mesophyll contains the oxygen insensitive PEPcase. Carbon dioxide (really from bicarbonate via the activity of carbonic anhydrase) is initially fixed into four

carbon acids by PEPcase in terrestrial C<sub>4</sub> plants. The resulting organic acids are transported out of the mesophyll cells and into the bundle sheath cells where decarboxylation provides CO<sub>2</sub> for Rubisco.

The relationship between the variable photorespiratory rates of SAM and relative growth rates was evaluated. It was demonstrated that individuals with low photorespiratory rates generally have increased net photosynthetic rates, reduced CO<sub>2</sub> compensation points, and concomitant increases in growth rate.

Pivotal to the importance of induced PEPcase activity in certain SAM is the possibility of C<sub>4</sub>-like photosynthesis occurring in the absence of Kranz anatomy. With a view towards improvement of net photosynthesis of crops and a subsequent increase in yield, by the introduction of genes which allow the production of PEPcase within the cytoplasm, it is necessary that the cytoplasmic PEPcase be able to effectively scavenge and refix both photo- and dark respired CO<sub>2</sub>, as well as increase the concentration of CO<sub>2</sub> around Rubisco. The production of cytoplasmic PEPcase within the cytoplasm without anatomical changes would be much easier than an attempt to effect both a biochemical and anatomical change. It was unequivocally demonstrated that C<sub>4</sub> metabolism was responsible for the C<sub>4</sub>-like gas exchange characteristics associated with *H. verticillata*. Then Kranz anatomy was not necessary for C<sub>4</sub> photosynthesis.

Although elevated PEPcase activity has been demonstrated in certain SAM, and some exogenously applied <sup>14</sup>CO<sub>2</sub> was fixed into C<sub>4</sub> acids, it was not known to what degree PEPcase contributes to the low photorespiratory state. Strong evidence existed that the carbonic anhydrase mediated DIC concentrating mechanism found in low photorespiratory microalgae and in the SAM *M. spicatum* (which do not contain elevated PEPcase) was also operating in PEPcase containing *H. verticillata*. When the carbonic anhydrase inhibitor ethoxzolamide was applied to

both low photorespiratory *H. verticillata* and to *M. spicatum*,  $V_{\max}$  of photosynthesis decreased, and the CCP and oxygen sensitivity of net photosynthesis increased.

The PEPcase inhibitor 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate (DCDP) was shown to reduce photosynthesis of excised leaves of  $C_4$  plants up to 98% (Jenkins 1988). Indubitably, it will be shown that growth and crop yield will be similarly reduced by the activity of this inhibitor. The large reduction in net photosynthesis of  $C_4$  plants demonstrates that Rubisco alone is unable to support the net photosynthesis of  $C_4$  plants. This finding was anticipated since Rubisco in  $C_4$  plants is sequestered within the bundle sheath cell surrounded by several layers of spongy mesophyll cells. Diffusion of atmospheric  $CO_2$  into the bundle sheath cells is apparently insufficient to support carbon fixation via Rubisco. Therefore,  $C_4$  plants are obligately dependent upon the activity of PEPcase to maintain maximum net photosynthetic rates. The cytoplasmic PEPcase within SAM was responsible for increased net photosynthesis, and the inactivation of this enzyme by the use of the PEPcase inhibitors DCDP or malonate substantially reduced net photosynthesis and increased the  $CO_2$  compensation point of these species.

Low photorespiratory *H. verticillata* and *M. spicatum* were exposed to the PEPcase inhibitors malonate and DCDP. Malonate is readily available (Sigma) and has been shown to be an effective inhibitor of PEPcase in certain cyanophytes. Since malonate also inhibits succinate dehydrogenase (TCA cycle), we substituted DCDP as the preferred inhibitor.

Efficacy of inhibition on SAM PEPcase was determined in vitro by assaying PEPcase activity from *H. verticillata* in the absence and presence of the inhibitors. Extraction and the spectrophotometric assay of PEPcase followed the procedures of Jenkins (1988).

Gas exchange measurements, utilizing infrared gas analysis, and polarographic oxygen measurements including CCP, net photosynthesis, photorespiratory  $CO_2$  release, dark respiration,

and the inhibition by  $O_2$  on net photosynthesis were determined prior to, and following exposure of each plant to the inhibitors. Verification of in vivo PEPcase inhibition was determined by rapid assay of PEPcase activity extracted from plants exposed to the inhibitors.

A Hansatech polarographic oxygen electrode apparatus was used to determine the effect of the inhibitors upon photosynthetic oxygen evolution at several DIC concentrations. From these data we determined the kinetic parameters of photosynthetic DIC uptake,  $V_{max}$  and the apparent  $K_m$  for both  $CO_2$ , and  $HCO_3^-$ .

**An increased flux of carbon fixation through PEPcase results during nitrogen assimilation by nitrogen-starved plants.** Elevated PEPcase activity is prevalent in nitrogen starved plants. During  $NH_4^+$  assimilation carbon fixation via PEPcase provides a source of 4-carbon organic acids to the TCA cycle to replenish alpha-ketoglutaric acid utilized during  $NH_4^+$  assimilation. Growth under nitrogen-limited conditions greatly increased the capacity for  $NH_4^+$  assimilation by the increase in PEPcase activity. In the absence of a nitrogen pulse approximately 10% of the total carbon fixed was through PEPcase, the remainder through Rubisco. However, immediately after the N pulse, almost 90% of the carbon flux was through PEPcase.

If elevated PEPcase of low photorespiratory *H. verticillata* participates in nitrogen assimilation, the anaplerotic function of PEPcase prevailed in low photorespiratory *H. verticillata*. Although 50% of the  $^{14}CO_2$  label was incorporated into  $C_4$  acids in low photorespiratory plants (with elevated PEPcase activity) after a 20 s pulse, no initial increase in label occurred in 3-phosphoglycerate or triose phosphates (Salvucci and Bowes 1983) even though the label in malate steadily decreased. Although considerable label was initially fixed into  $C_4$  acids and subsequently turned over, little of the label was passed to Calvin cycle intermediates. A reasonable fate of the missing label may be its incorporation into amino acids integral to nitrogen assimilation.

When low photorespiratory *H. verticillata* was exposed to the carbonic anhydrase inhibitor ethoxzolamide, net photosynthesis decreased, oxygen sensitivity of photosynthesis increased, and the CCP increased to C<sub>3</sub> values from C<sub>4</sub>. The effects were similar to those in low photorespiratory *M. spicatum* (which does not possess high activity of PEPcase) and unicellular algae.

Given that (1) the frequent occurrence of PEPcase operating in an anaplerotic fashion in plant biochemistry, (2) a carbonic anhydrase DIC concentration system may be operating in *H. verticillata*, (3) a malate induced CO<sub>2</sub> efflux via activity of the TCA cycle is present in low photorespiratory *H. verticillata*, and (4) the conditions used to induce the low photorespiratory state in *H. verticillata* are characterized by low inorganic nitrogen, our results indicated that PEPcase has an anaplerotic role in supplying TCA cycle substrates to support NH<sub>4</sub><sup>+</sup> assimilation in SAM.

**If low CCP and LCP increase the ability of SAM weeds to tolerate low levels of DIC and irradiance, then SAM weeds that experience low levels of these resources *in situ* should exhibit low CCP and LCP.** It has been suggested that the plastic physiological parameters including the CCP and LCP may be important components of the competitive success of SAM weeds (Spencer and Bowes 1989). However, studies which demonstrate the occurrence of a range of values for CCP and LCP, *in situ*, across gradients of light and DIC availability in dense populations of SAM are lacking.

Laboratory studies have shown that the LCP of SAM varies with the irradiance received during growth. LCP for *H. verticillata* of 7, 10, 15, and 20  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  were measured for plants grown for three weeks at 6, 30, 120, and 300  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  respectively. Furthermore, the K<sub>1/2</sub> (irradiance) for photosynthesis was reduced by growth at lower irradiance,

which indicated that the photosynthetic apparatus was able to maintain higher net photosynthetic rates at the lower light levels than the non-acclimatized plants.

At locations both in Florida and Michigan, prolific monocultures of SAM weeds (including *H. verticillata* and *M. spicatum*) were used. The physical and chemical characteristics of the site including pH, concentration and components of DIC, and irradiance as photosynthetically active radiation (PAR) were measured at each micro-site for both SAM weed and non-weed locations. Components of DIC and pH were determined in the field utilizing a portable pH meter and gran titrations. Irradiance at each site was determined utilizing a submersible LiCor quantum probe. Dissolved O<sub>2</sub> concentrations were determined using a Beckman field O<sub>2</sub> electrode. The CCP and LCP of individuals from each micro-site were determined using the procedures mentioned above.

Inducible C<sub>4</sub>-like photosynthesis metabolism in *Hydrilla verticillata* leaf tissue elicits variability in photosynthetic phenotype, expressed as CO<sub>2</sub> compensation point ( $\Gamma$ ). We conducted a field and laboratory study to investigate the ecological and adaptive significance of this physiological phenomenon. Spatial horizontal environmental heterogeneity was observed within clonal populations of *H. verticillata* in Florida. Measured at midday, the edge habitat at the expanding periphery of the clone exhibited a dissolved inorganic carbon (DIC) concentration of 0.7 mmol/L, pH 7.1, a dissolved oxygen (DO) level of 0.13 mmol/L, and biomass of 0.2 kg/m<sup>2</sup>. The mat habitat, located 200 cm towards the interior of the surface mat, exhibited DIC 0.1 mmol/L, pH 10.2, DO 0.48 mmol/L, and biomass 0.8 kg/m<sup>2</sup>. DIC depletion and DO supersaturation characterized the mat habitat for most of the day and much of the growing season. Furthermore, net photosynthesis, daily carbon gain, and relative growth rate (RGR) of *H. verticillata* were reduced 80% by mat conditions compared to edge conditions.  $\Gamma$ s of *H. verticillata* were positively correlated with CO<sub>2</sub> and bicarbonate concentration, and negatively

correlated with pH, DO, and biomass. Low and high  $\Gamma$  photosynthetic phenotypes were associated with the mat and edge habitats, respectively. Photosynthetic phenotype of *H. verticillata* appears to acclimate to environmental heterogeneity within a clone in the field.

Net photosynthesis and daily carbon gain of low  $\Gamma$  phenotype *H. verticillata* was 128% and 40% greater than the high  $\Gamma$  phenotype when measured in the mat habitat, but was 21% lower than the high  $\Gamma$  photosynthetic phenotype when measured in the edge habitat under low quantum flux. Laboratory experiments showed a negative curvilinear relationship between the  $\Gamma$  of *H. verticillata* and plant density. The data demonstrate that plasticity in photosynthetic phenotype of *H. verticillata* is a density-dependent, physiological response that optimizes carbon gain within a stressful heterogeneous environment. Evolution of facultative C<sub>4</sub>-like photosynthetic metabolism in *H. verticillata* may have been an adaptation to the constraints imposed upon carbon gain by DIC and quantum flux limitation in the mat habitat.

**Dissolved Organic Phosphorus: Competition Bacteria and Algae for DOP and the Metabolic Coupling to Littoral and Wetland Sources.** Several different strategies can be employed by a planktonic organism that may increase its competitive ability for nutrients that are in limiting supply. Since growth rates are a function both of uptake and internal utilization of the limiting nutrient (Kilham 1978), adaptations in either capacity can lead to increased competitive ability. One way of increasing the efficiency of nutrient utilization is through storage internally during periods when nutrients are in excess of cell demands. Another mechanism is to evolve uptake enzymes (permeases) that have a very high affinity for the limiting nutrient. Finally, organisms can increase the effective size of the nutrient pool by developing enzymes or enzyme systems that mineralize organic compounds into inorganic constituents.

Alkaline phosphatase hydrolyzes DOP to an organic alcohol and orthophosphate and therefore could mobilize a significant amount of phosphorus into the phosphate pool. Chrost and Overbeck (1987) suggested from *in situ* studies that bacterial alkaline phosphatase is inhibited less by phosphate than algal alkaline phosphatase, suggesting a less tightly coupled regulation of DOP utilization in bacteria relative to algae. The mechanisms of regulation of this enzyme are important in that it gives us an idea of the conditions under which it evolved. In this portion of our study, we are evaluating the ability of bacterioplankton to mineralize orthophosphate from phosphomonoesters via alkaline phosphatase and the inhibition of this reaction by orthophosphate.

**Bacterioplankton obtain the largest proportion of the dissolved organic phosphorus (DOP) pool in the oligotrophic lakes and phytoplankton obtain the largest proportion of DOP in eutrophic lakes.** Planktonic bacteria and algae are the primary pelagic organisms mineralizing and assimilating nutrients in aquatic ecosystems. Bacteria, and to a lesser extent fungi, are the primary organisms responsible for decompositional processes in aquatic ecosystems (Wetzel 1983). Phytoplankton synthesize organic compounds photosynthetically and release a portion of these compounds to the water as dissolved organic matter either through autolysis or extracellular release (Wetzel et al. 1972).

Because of the central role of bacterioplankton and phytoplankton in the turnover of nutrients, recent studies have examined the interactions of algae and bacteria for phosphorus. Globally phosphorus is the nutrient most often limiting to primary production in lakes (Schindler 1977). Most work has focused on direct utilization of phosphorus by algae but recently bacteria have been perceived as important in regulating phosphorus availability to phytoplankton. Through an understanding of phosphorus partitioning by bacteria and algae, we can understand how this nutrient affects planktonic and littoral zone community metabolism.

Community phosphorus fluxes have been examined in a mesotrophic lake as well as in the laboratory using phytoplankton and bacterioplankton isolated from the lake (Currie and Kalff 1984a, 1984b). When the bacteria were grown under P-limiting conditions in culture, they accounted for 95% of the initial phosphate uptake in competition with algae and the algae showed no affinity for taking up labelled phosphorus that had been incorporated into bacterial-size fractions. A similar study (Currie and Kalff 1984b) performed on lake water also demonstrated bacteria with 95% of the initial phosphate uptake, but they concluded that the algae could have an advantage in taking up excreted organic phosphorus although the evidence to support this conclusion was weak. Currie et al. (1986) examined 13 lakes of differing trophicity to determine the generality of their previous findings. In lakes that were phosphorus-limited, bacteria accounted for 95% of phosphate uptake. Alkaline phosphatase activity (APA) was found to be associated with algal particles, and the authors suggested that this would give algae an advantage in uptake of DOP through hydrolysis. However, most of the total APA was in solution, and this activity would give an advantage to bacteria if they are better able to take up orthophosphate.

Tarapchak and Moll (1988), in studies of similar processes in oligotrophic Lake Michigan, measured  $^{33}\text{P}\text{-H}_3\text{PO}_4$  uptake by algal and bacterial fractions. The filtrate, which they concluded contained the radiolabel primarily as  $^{33}\text{P}\text{-DOP}$ , was used to measure uptake of phosphorus from DOP by algae and bacteria. Their results showed that bacteria, relative to algae, had a low phosphate uptake but a high uptake of phosphorus from DOP. Phytoplankton primarily took up phosphate. These results are contradictory to those of Currie and Kalff (1984a, 1984b) and Currie et al. (1986).

To justify studying uptake of dissolved organic phosphorus (DOP) by algae and bacteria, it must be demonstrated that there are differences in the relative abilities of algae and bacteria to

obtain organic phosphorus. It is also important to quantify important variables affecting the competitive abilities of algae and bacteria to obtain DOP. Several studies have examined differences in uptake of phosphate by algae and bacteria (Rhee 1972; Currie and Kalff 1984a, 1984b), but no one has examined seasonal fluctuations of DOP uptake by algae and bacteria. Tarapchak and Moll (1988) showed that there are potentially differences in uptake of DOP by algae and bacteria in oligotrophic and eutrophic lakes. Therefore, we attempted to answer the question, "Do algae or bacteria obtain the greatest proportion of the ambient DOP pool seasonally in oligotrophic and eutrophic lakes?"

Differences in enzymatic hydrolysis of dissolved organic phosphorus and subsequent phosphorus uptake were compared by using dual-labeled ( $\gamma$ - $^{32}\text{P}$  and  $2$ - $^3\text{H}$ ) ATP in oligotrophic Lake Michigan and a moderately eutrophic lake in southeastern Michigan. More than 50% of the phosphate that was hydrolyzed was immediately taken up into bacterium-sized particles in the eutrophic lake and at a near-shore site in Lake Michigan. Less than 50% of the hydrolyzed phosphate was taken up into bacterium-sized particles at an offshore site in Lake Michigan. It is hypothesized that differences in size-fractionated uptake were the result of greater phosphorus utilization capacity in bacteria in habitats where loading of organic carbon is greater. Substantial isotope dilution of labeled phosphate uptake by unlabeled phosphate occurred, which implied that the phosphate was hydrolyzed extracellularly in both systems. Comparable nucleotidase activities were measured in the eutrophic lake and Lake Michigan, but the significance of the phosphate regenerated relative to particulate phosphorus pools was an order of magnitude greater in Lake Michigan. Seventy percent of the nucleotidase activity was inhibited by  $100 \mu\text{M}$  phosphate in the eutrophic lake, which suggests that most hydrolysis was by phosphatase. Therefore, nucleotidase

activity may be more important to phosphorus regeneration in oligotrophic habitats than phosphatase activity.

Potential sources of dissolved P to phytoplankton and bacterioplankton were examined in a small meso-eutrophic lake. Kinetic analyses of whole lake water on three dates demonstrated that the maximal rate ( $V_{max}$ ) for phosphate uptake was highest ( $5.2 \text{ nM min}^{-1}$ ) in spring. On all dates, size fractionation of plankton and kinetic analyses of uptake indicated that most ( $> 50\%$ ) uptake of phosphate was by phytoplankton at  $V_{max}$  and by bacteria at ambient concentrations. Isotope dilution assays, with either unlabelled phosphate or various dissolved organic P (DOP) compounds, demonstrated that phosphate was the preferred substrate for uptake into both algae and bacteria. Phytoplankton had greater capacity for uptake of P from both phosphate and DOP than bacterioplankton. We concluded that phytoplankton use both phosphate and DOP, particularly at high substrate concentrations, and that bacterial utilization of P may be limited by the availability of organic C or other nutrients.

APase of three strains of bacteria isolated from pelagic, epiphytic, and epipelagic habitats in a small moderately eutrophic lake had varied kinetic constants,  $K_m$ ,  $V_{max}$ , and  $K_i$  with the artificial phosphatase substrate methylumbelliferyl-phosphate. The epipelagic strain had the lowest  $K_m$  and the highest  $V_{max}$  which indicated that it had the greatest capacity to hydrolyze substrate at elevated concentrations. The APase of the pelagic strain had a relatively high  $K_m$  and  $V_{max}$  and therefore had the least potential of the strains studied to hydrolyze phosphatase substrate. All three phosphatases were competitively inhibited by phosphate at low concentrations. The epipelagic strain demonstrated partial competitive inhibition at 4 to 8  $\mu\text{M}$  concentrations of phosphate. In addition, in situ measurements of APase activity in bacteria from littoral and pelagic environments suggested that enzyme activity is greater in the littoral environment. It was suggested that

differential phosphorus loading in the habitats in which these organisms grow affects their ability to utilize DOP. The loading ratio of organic carbon to phosphorus in the habitat was hypothesized to be important to the ability of a bacterium to compete for the phosphorus moiety of DOP.

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