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Barr Report

Barr Report

with Tom Barr

Oxygen in the Planted Aquarium

Special points of interest:

- Feature Article "Oxygen in the Planted Aquarium"
- While many may not consider oxygen a nutrient, its role is often overlooked in planted aquariums.
- Photosynthesis and Respiration

Inside this issue:

While many may not consider oxygen a nutrient, its role is often overlooked in planted aquariums. While terrestrial systems are seldom ever limited by oxygen, water greatly reduces the diffusion of O2 into the aquatic environment. Fick's first law of diffusion illustrates this change and predicts the rate of flux of O2:

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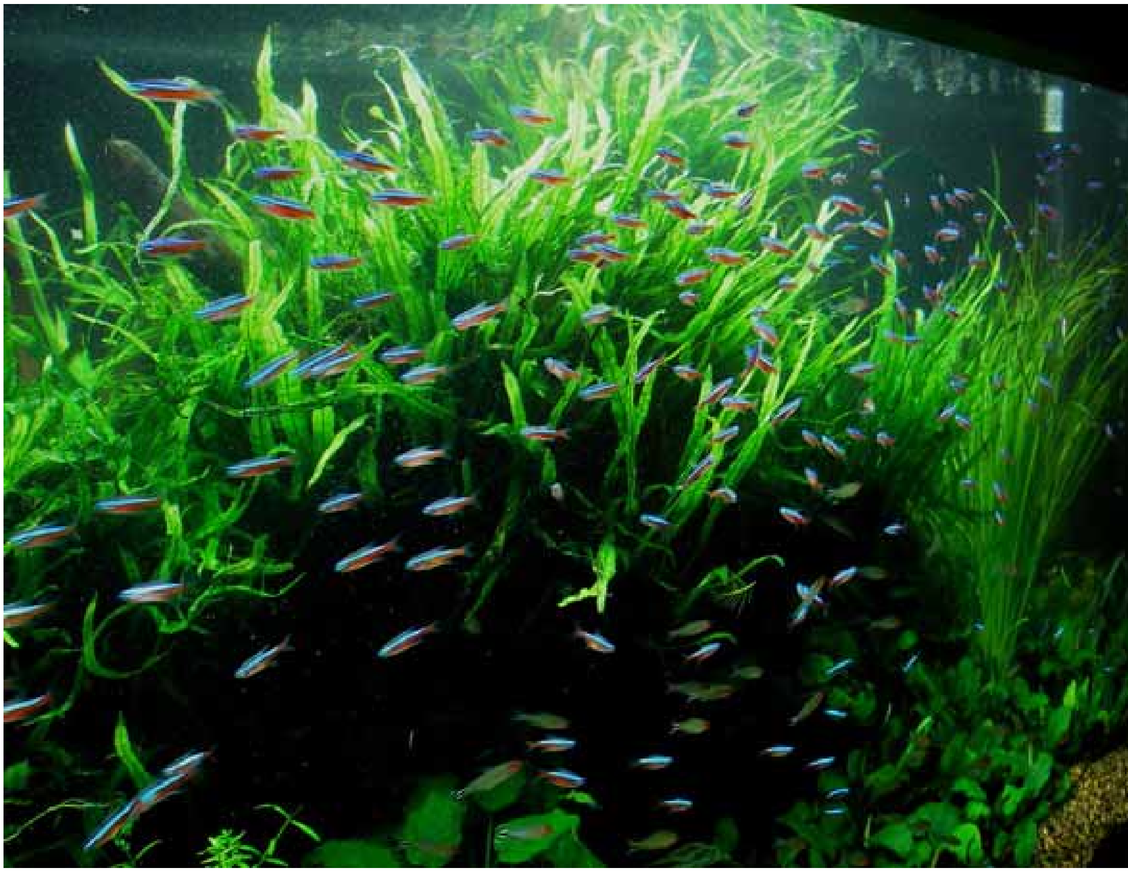
... So how much oxygen do aquatic plants require before they become oxygen limited? ....

Definition of Fick's 1st law: A relationship wherein the flux of a diffusing species is proportional to the concentration gradient:
$$J_x = -D\frac{dC}{dx}$$
where J_x = the flux of the diffusing species(how fast the gas flows in/out of the water) dC/dx = the incremental change in concentration with distance (Distance the gas has to travel-the change in distance between to points) D = diffusivity or diffusion coefficient = the proportionality constant (this changes when going from a gas phase to a liquid phase! This is the 10,000 times slower part) This relationship applies to CO2 as well as O2 and other gases traveling in/out of the water and air. This also includes bubbles in the water and water splashed up into the air such as waves and waterfalls.

While math might intimidate some, this is a rather simple equation and one that defines aquatic plants and the anaerobic nature of hydrosoil sediments.

"While many may not consider Oxygen a nutrient, its role is often overlooked in planted aquariums."



Several studies suggest that the saturation levels of oxygen required by plants for respiration was around 1-2 ppm.

Oxygen in water, (unlike the oxygen in the air) varies, sometimes greatly.

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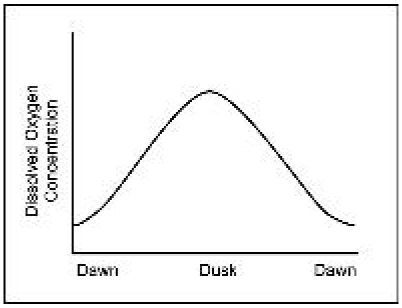
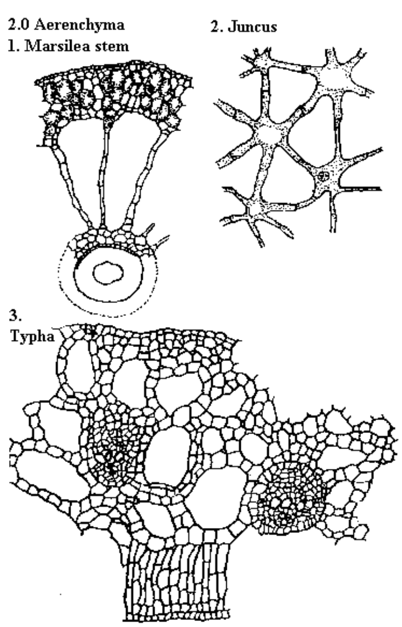


Figure 1

This is a typical shallow pond with submersed macrophytes. Note: the pH also varies with the same pattern (see the relationship between CO_2, pH and O_2?) So how much oxygen do aquatic plants require before they become oxygen limited? In several studies addressing the saturation levels required for plant respiration (O_2 demand) was around 5% or about 1-2ppm (Morris and Dacey, 1984; Sand-Jensen and Prahl 1982). Air possesses about 21% O_2. A glass of water sitting for 24 hours (at equilibrium with the air) at temperature of 26 C will have about 8.1ppm [O_2]. The equivalent concentration for oxygen limitation is about 1-2ppm, but fish will die at these levels. Several hobbyists have suggested that adding aeration or O_2 at night will help their plants grow better. Clearly, the fish and animal life would die before this becomes an issue for plants. The reduction in gas exchange is roughly 10,000 times less and unlike CO_2, O_2 is far less soluble in water. Few aquarist use pure O_2 enrichment, but it is common in aquaculture and wastewater treatment where a large volume of organic matter is loaded into treatment plants. Most fish kills in lakes are due to low oxygen levels from decay of algae, aquatic plants, sediment being suspended into the water column, rapid gas releases from anaerobic sediments such as CH_4 (methane). Aquatic plants adapt to the low levels of oxygen in the sediment a number of ways.

"Several hobbyist have suggested that adding aeration or O2 at night will help their plants grow better. Clearly, the fish and animal life would die before this becomes an issue for plants … "

Translocation to the root zone via the formation of aerenchy



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"… Most fish kills in lakes are due to low oxygen levels from decay of algae, aquatic plants, sediment being suspended into the water column, and rapid gas releases from anaerobic sediments such as CH4 (methane) ..."

Figure 2

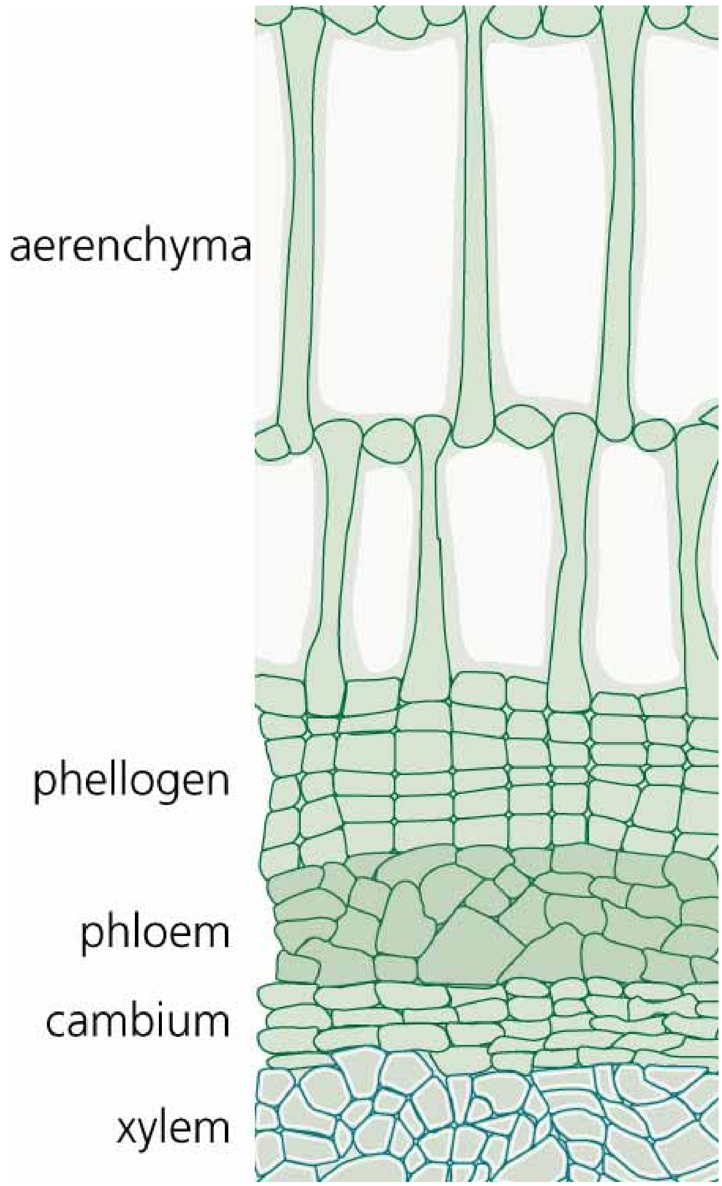

Figure 3

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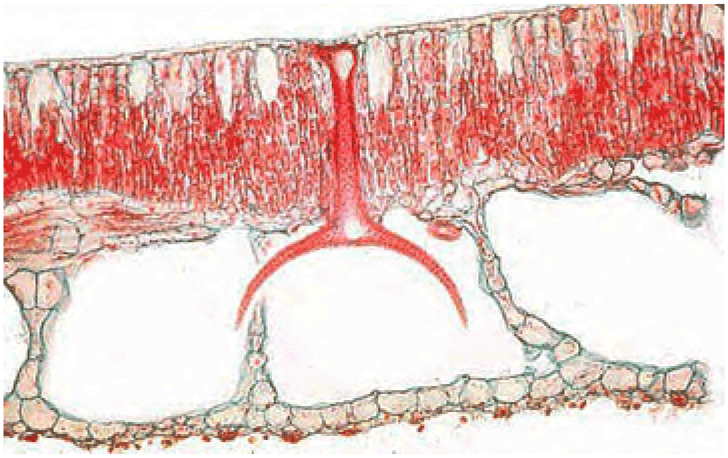

Figure 4

"… The lacunae allow rapid movement of oxygen gas to the root zone ...."

Note the large spaces in the cross sections of the stems in Figure 2 and 3 and the leaf in Figure 4. These are called lacunae (Latin for "space") and allow rapid movement of oxygen gas to the root zone. The tissue is referred to as "aerenchyma". The cup shaped structure in the second image is a sclerid. Many aquatic plants such Hyacinth possess raphids, druses(e.g. *Myriophyllum*) and many anatomical crystals and sclerified structures. See the Barr Report Calcium article for more on storage and functions of such structures.

Emergent leaves

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The stomata on these largest of leaves (*Victoria* sp.) are on the top adaxial side of the leaf, unlike most other plants which have the stomata on the lower abaxial side of the leaf. Can you see CO2 and O2 gas exchange advantages for this adaptation in aquatic plants? Some plants such as *Egeria* have no stomata, the leaves are 2 cells thick. How might they be adapted to get their CO2 and O2 requirements?

Fermentation

The basics: without O2, plants can no longer use the TCA cycle and thus they use another pathway that evolved much longer ago evolutionarily:

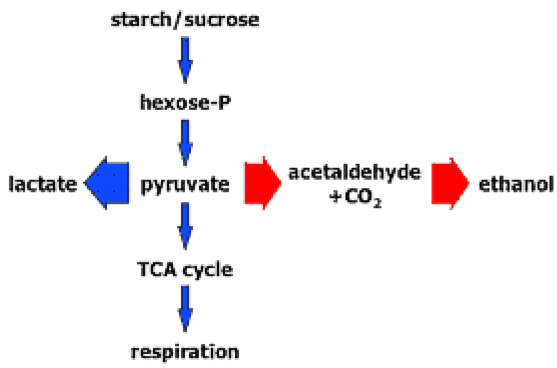

Figure 5

The flow chart shows how the plant uses the sugar (produced from photosynthesis) with O2 by using the TCA cycle and carries out oxidative respiration. A similar situation occurs when plants are subjected to flooding/submersion initially. They will quickly form the aerenchyma by dissolving internal cells to increase the O2 content to other parts of the plant.

Stay small and have a high surface to volume ratio (fine needled, highly dissected leaves).

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Obviously, one solution is to avoid the problem altogether. There are two basic mechanisms to avoid the problems of O_2 uptake in aquatic systems mentioned above, and many aquatic organisms use both to some extent. They are to stay small and to have a low metabolic rate (most all algae fit into this well adapted group). Small size avoids the problems with diffusion distances and surface/ volume ratios mentioned above; low metabolic rates decrease the need for O_2. Many aquatic organisms are small enough that simple diffusion will suffice to supply O_2. Low metabolism is also possible, and, particularly at low temperatures, almost unavoidable for small organisms. Unfortunately, warm conditions will raise metabolism rates, often above levels, which can be matched by O_2 uptake; this, coupled with the decreased solubility of O_2 at higher temperatures, may define upper temperature limits for many aquatic organisms. In order to maintain a high metabolism, or to increase body size, other strategies must come into play; increasing surface area without increasing volume, and there are two basic ways to do this. The first is to alter the shape of the organism. We looked at what happens with spheres, where surface/volume ratios decrease as size (diameter) increases. The same relationship holds for other shapes, but it is somewhat diminished for long, thin shapes. Organisms that avoid blocky, compact shapes such as spheres and cubes, and tend towards shapes with at least one dimension greatly elongated maximize surface/volume ratios while maintaining a constant volume. This in turn allows for efficient gas exchange even without some of the additional systems we will mention below, and, as a bonus, has the effect of minimizing diffusion distances. Many seaweeds and aquatic vascular plants such as *Vallisneria* have flattened blades or leaves; in fact, the leaves of terrestrial vascular plants are also examples of this phenomenon, although here the surface area is maximized to provide for light capture, not O_2 uptake. This approach is also common in nature and found in the gas exchange structures of both terrestrial and aquatic organisms, and also in filtration systems, lungs and digestive tracts.

While oxygen plays a huge role in aquatic plant anatomy and physiology, it plays important roles in the break down or organic matter such as dead leaves, bacteria, dead fish, fish waste (thus also fish food), zooplankton, and any organic matter added to the system(95%). Often when an aquarist does a large amount of substrate disturbance, the levels of O2 decline a great deal, this can be alleviated by doing a large water change immediately after any work is done on an aquarium or pond. It also plays a role as a reactant in certain biochemical reactors (5%) (Ford, 1993). These reactants are formed through processes done during photosynthesis and through respiration and must be controlled and detoxified or the cell will die. Various enzymes such as catalases, peroxidases and superoxide dimutases (SOD) detoxify many of the reactive species of oxygen and allows aerobic respiration to occur in plants, bacteria, fungi and animals without damage to the cells. The advantage is 18 times more energy than anaerobic respiration! Energy obtained from the oxidation of food is used by the cells of plants (and animals) to do work. The synthesis of new cells and tissues for growth, the transport of minerals across membranes and the opening of stomata for gas exchange are all obvious examples of cellular work requiring energy. Essentially all processes in living cells require energy.

Even though plants can make their own food through photosynthesis, they must break down some of that food (starches, sucrose etc) to supply their own energy needs. When all forms of food are metabolized in the plant, oxygen is consumed and energy is released, along with water and carbon dioxide, as seen in the equation below:

$$\textbf{Food} + O_2 \longrightarrow CO_2 + \textbf{energy}$$

If the starting material for respiratory breakdown of food is a sugar such as glucose a great deal of energy can be produced. The complete oxidation of a mole of glucose releases 673 kilocalories of energy:

$$C_6H_{12}O_6\ \textbf{(glucose)} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 673\ \textbf{kcal}$$

Notice that the amount of O_2 consumed is equal to the amount of CO_2 produced. Thus the rate of respiration of a plant oxidizing carbohydrate could be determined by measuring either the rate of O_2 consumption or the rate of CO_2 production. This is why O2 levels in water equates to aquatic macrophyte production/growth. Most aquatic animals need to obtain O_2 from the surrounding water in order to carry on cellular respiration. As we have seen, the amount of O_2 in water is limited, and both O_2 solubility and demand are correlated with temperature and organic matter loading into the ecosystem.

Net assimilation - the balance between daily photosynthetic gain and respiratory losses. The net assimilation rate (NAR), usually expressed on a per day basis, is defined as:

NAR = Total Net Photosynthesis - Total Respiration

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Plants gain dry weight only if the NAR is positive. What conditions could cause NAR to be zero or negative?
- Low light
- Low CO2
- Low nutrients
- Anything that interferes with photosynthesis
- Stress-moving the plant, salt, toxic levels of chemicals

This link will detail the biochemical explanation for more on the glycol sis, TCA and fermentative pathways. This is advanced material for those more interested in the processes.

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/glycolysis.htm

The basic reactions below show that the primary reactants are O_2 and carbohydrate and the primary end products are CO_2, H_2O, and reduced compounds such as NADH, FADH, and ATP(the chemical energy used to do the "work"). Much of the NADH and FADH are converted to ATP through what is known as the electron transport system; these reactions occur in the mitochondria. Cyanide is often used in respiration studies as it is a known blocking agent for respiration pathways, although in plants, some possess alternate pathways (but these are detectable and produce reduce efficiency). The ATP and NADH are the high energy compounds used to do chemical work in plants. Energy from respiration drives metabolic processes such as: absorption of plant nutrients (salts) into roots, transport of potassium (K) into and out of guard cells. Guard cells surround the stomata, the pore in the leaf epidermis where gas exchange occurs. Opening and closing of stomata requires the energy of ATP. Other functions: chemical synthesis=> synthesis of proteins, lipids and structural components of plants such as cellulose and other fibers al, require O2. Synthesis of storage compounds in stems, roots and seeds also require O2. Such compounds include sugars (sugarcane stems etc.), starch (in storage organs, which include stems (corms, tubers) and taro roots), proteins and lipids (many seeds, e.g. lotus, accumulate proteins and lipids). Wide variations in O2 levels can make it difficult for steady sustained growth to occur.

These are the basic biochemical pathways involved in energy production in aquatic macrophytes (as well as many organisms)

Glycolysis

$C_6H_{12}O_6$ + 2NAD + 2ADP ---> $2C_3H_4O_3$ (pyruvic acid) + 2 NADH + 2ATP

Fermentation

Pyruvate + NADH ---> CO_2 + NAD + ethanol

The TCA (Kreb's) Cycle

pyruvate + ADP + 4NAD + FAD ---> $3CO_2$ + ATP + 4NADH + FADH

The Krebs cycle recovers much of the energy contained in the carbohydrate molecule; much more than glycolysis

Electron Transport System

O_2 + NADH + 3 ADP + 3 iP ---> NAD + 3ATP + H_2O

O_2 + FADH + 2ADP + 2iP ---> FAD + 2ATP + H_2O

You will note the oxygen plays a significant role in the break down of energy used. You will also see phosphorus also playing a role in the energy pathway. Anaerobic respiration involved electron acceptors other than O2, such as Fe, Mn, NO3, SO4, and CO2. These yield very little energy compared to oxygen and are done by bacteria. All above processes are mediated by enzymes and, therefore are all temperature sensitive. Thus, rates of respiration and synthesis decline as temperature decreases and also decline at temperatures above the optimum. The optimum temperature for physiological processes and even for some biochemical reactions is

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species dependent. A water-saturated root zone inhibits nutrient uptake by excluding oxygen from the root environment, which in turn slows or stops respiration.

The primary method of O_2 transport is simple diffusion. Since all molecules are always in motion (except at 0 K), they will tend to move randomly. If they are highly concentrated in one spot, they will be least likely to move towards that spot, as opposed to moving to any of the other spots in the environment. Because the speed with which O_2 molecules move in water at normal temperatures is fixed, we can make some estimates over the distances at which simple diffusion can take place in both water and body fluids; that distance appears to be about 1 mm. If a cell is no more than 1 mm from water with sufficient O_2, then no special adaptations are needed for obtaining O_2. If the O_2 concentration of the water is low, or if the cell is greater than 2mm in diameter, or if the organism is multicellular, with some cells buried inside the body, then special measures are necessary. This is why a small alga will not have the same issues with gas transport of CO_2 as a much larger aquatic macrophyte has, they both exist and much different scales in the habitat.

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Cyclosis, the internal streaming of the cytoplasm of a cell, can be viewed with a leaf of *Egeria densa* under high light for a few minutes prior to viewing at 100-400 x magnification. The chloroplast begin to rotate and “take turns” under high light. Cyclosis can help distribute O_2 within a cell, but even it has limits, as we shall see. Consider what happens to a cell as it grows. If we are talking about a spherical cell, its volume grows according to the formula $v = 0.5236d^3$ (d = diameter), while its surface area grows according to the equation $a = 3.1416d^2$. The volume is an index of how many O_2 requiring enzymes are present; the surface area represents the "gateway" through which the O_2 must pass. If the volume increases faster than the surface area - and it does, with the volume increasing by a factor of d^3 and the surface area by only a factor of d^2 - than the cell will soon reach the point where O_2 will not be able to enter the cell fast enough. Thus the problem of respiration in aquatic systems is a combination of the amount of dissolved O_2 present, the distance over which diffusion can take place, and the surface area/volume ratio of the organism to be served. Taken together, these factors suggest that life in water should be restricted to very small organisms where diffusion distances are short and surface/volume ratios are high. The fact that large organisms are common in water suggests that there is a way around these restrictions, and, in fact, a number of methods are employed by large aquatic organisms to obtain enough O_2.

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Aeration of Roots

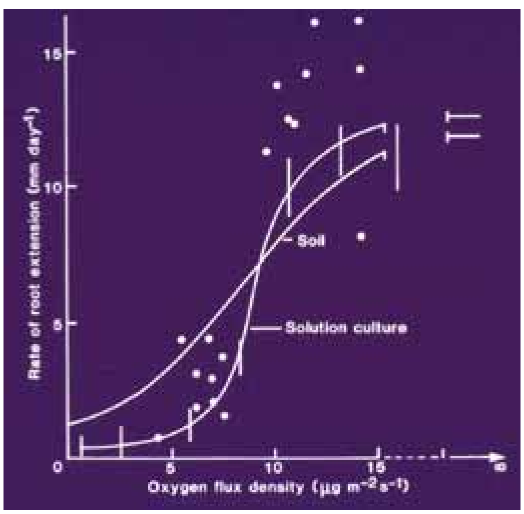

Figure 6.

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Root elongation slows dramatically as the flux of oxygen decreases. This figure shows how a plant's roots are influenced by anaerobic conditions. Aquatic plants overcome this by the formation of the aerenchyma. This takes time for these roots form and transition to flooded soil/sediments for some species such as *Cryptocoryne* species.

Oxygen is transmitted from the leaves to the roots and rhizomes by lacunae (air spaces forming channels in leaves, stems, and roots). Lacunae also have a structural role. Lacunae can take up about 60% of the plants volume. Cut a leaf off an *Echinodorus* species and view the stem, it will possess mostly spongy air fill space. A rise in ethylene concentration is the triggering factor for the formation of aerenchyma in maize. This same pathway has been shown for many submersed macrophytes as well) (Drew & Lynch, 1980; Drew, 1992; Drew at al, 1994; Drew et al, 1985). There is at least one chemical mechanism attempted to explain the rise in ethylene concentration. It begins with the conversion of the amino acid methionine to S-adenosylmethionine which is the precursor to aminoclcloprapane carboxylic acid (ACC). As ACC levels rise, so does ethylene concentrations which is the trigger for programmed cell death and aerenchyma formation. (Drew, et al, 1994). A submersed plant has 20% oxygen in its leaves, 15% in its stem, 10% in the root parts, and only 2- 5% in the root hairs. The oxygen is taken in from the air by photosynthesis and travels through the plant and out the root hairs. When low oxygen levels are present, plants use other mechanisms to adjust for respiration. Aquatic plants can use the fermentative pathway (note, this is not respiration). This has been shown experimentally by bubbling N2 or O2 into the water with rhizomes, and then measuring the ethanol production (Laing, 1940). At <3% O2, ethanol is produced by *Typha, Scirpus, Nuphar,* and others. Some aquatic plants have developed air roots along their stems for respiration in water. Aquatic trees have developed pnuematophores, which are extensions of the root system reaching above the water level. Pnuematophores take in oxygen through small holes at their tips. out) nutrient uptake, and vegetative reproduction.

What does a typical hydrosoil vertical profile look like?

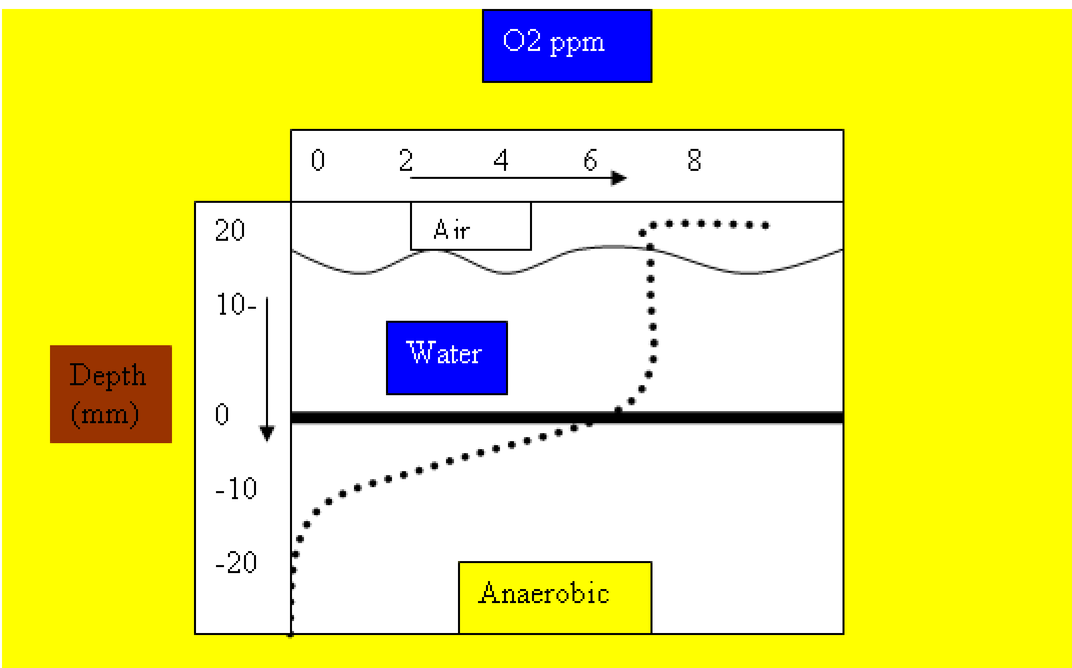

Figure 7

This shows a typical hydro soil O2 vertical profile in a wetland. The O2 drops off markedly at the hydrosoil interface. A similar profile exist for saturate soil aggregates such as porous grains as the depth increases to the center. With heavy loading from fish, low plant biomass, low plant production/growth, these profiles are not as distinct and the slope of the O2 profile is shifted to the left side with less O2 and the curve becomes more straighten. Interestingly, light also plays a role in O2 in the hydrosoil:

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O2 concentration at root surfaces with respect to increases in light

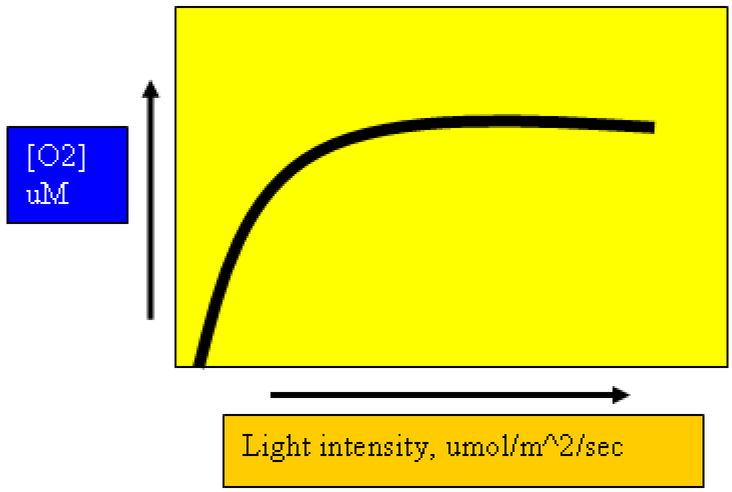

Figure 8 (adapted from Morard and Silvestre 1996)

Why would the root O2 levels increase with light and then level off? The macrophytes increase the rate of growth, which O2 is the measure, and then level off at they reach the light saturation point. Thus higher light can allow more O2 into the root zone. So how much of the O2 produced is released into the root rhizosphere? Armstrong and Armstrong (1991) showed the O2 flux in was roughly 2.08g/m^2/day and the net root respiration per day was 2.06 g/m^2/day and a net release of 0.02 g/m^2/day into the hydro soil. While many suggest that plants prefer reduce compounds found in the hydrosoil, the trade off is that the reduced compounds such as Fe2+ are now oxidized before the roots can get to these nutrients forming an iron plaque on the surface of roots, but the plant is also able to cultivate microbes and fungi that aid in acquisition of nutrients as well as detoxify Mn2+ and H2S. While this seems serious, the trade off is a small price for aforementioned benefits.

Organic matter's (OM => soil, high loading of fish waste) influence on O2 levels:

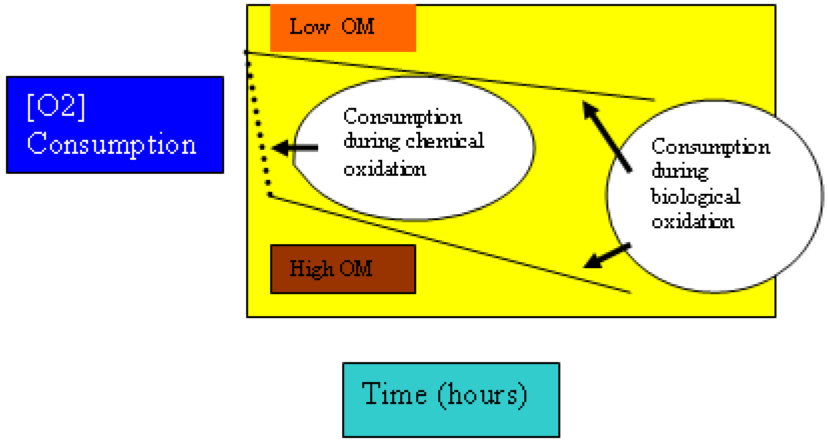

Figure 9.

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This figure shows how O2 will be affected by high loading rates and low loading rates. Most substrates start off as low OM substrates and then over time tend towards the higher OM types. This is one reason why the substrate should be periodically vacuumed and remove the excess OM; filters also become saturated with high levels of OM and require cleaning in a similar manner. Excess OM can become a drain on the plant's ability to transport O2 to the root zone as well as the O2 levels for the fish.

Given the central role in biology, oxygen is typically viewed as something for fish namely, but plants have active roles and the levels of O2 can and should be maximized for the best growth rates. Aeration for plants at night is not required nor will help the plants. O2 in the substrate is controlled by the amount of organic matter, light, anything that affects growth rates, by the flux rate of O2 and chemical species (Fe2+ etc). Substrates decline in their O2 levels rapidly where high OM is present, but very little where there is no organic matter for the bacteria to consume and thereby the bacteria will also not use any O2. Roots dramatically alter the substrate chemically and transport O2 to the substrate. This in turn increases cycling rates for decomposition. O2 levels are the best measurement for measuring growth rates of aquatic plants.

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