Increase in the oxygen concentration in Amazon waters resulting from the root exudation of two notorious water plants, *Eichhornia crassipes* (Pontederiaceae) and *Pistia stratiotes* (Araceae)

by

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Abstract

Qualitative and quantitative analyses were carried out to determine the amount of oxygen that enters the water through the root systems of two floating Neotropical plants, *Eichhornia crassipes* and *Pistia stratiotes*, under nearly anaerobic conditions. The physiological analyses were supplemented by anatomical investigations. A measurable oxygen input from both plants was detected: that from *E. crassipes* was 116 mg O$_2$·hr$^{-1}$·m$^{-2}$, and from *P. stratiotes*, 58 mg O$_2$·hr$^{-1}$·m$^{-2}$. Water surface area representing 4 kg and 2.9 kg fresh weight, respectively. The O$_2$ input from *E. crassipes* seemed to be independent of the amount of photosynthesis, suggesting that a pressure ventilation was responsible for the input. In the case of *P. stratiotes*, a relationship was found between the photosynthetic activity and the O$_2$ input. The significance of this input for the Neotropical ecosystem and the fish fauna is discussed.

Keywords: root exudation of oxygen, weeds, hypoxia, fish, Amazon.

This study is dedicated to Dr. Hans Klinge to commemorate his 60th birthday.
Introduction

Two floating plants, Eichhornia crassipes (MART.) SOLMS in the family Pontederiaceae and Pistia stratiotes (L.) in the Araceae, are found in all tropical and subtropical aquatic ecosystems without natural water-level fluctuations because of their ability to fully cover the surface of the water within a very short period of time (BOTANOUNY & EL FIKY 1975; GOPAL 1987). The thick layer of plants not only hinders ship traffic, it also becomes a danger for the entire ecosystem. The drifting beds of floating plants can dominate the submerged vegetation, rip away the floating leaves of other plants, and completely cover the littoral species, thereby greatly increasing the amount of decomposable detritus in the water (e.g. TABITA & WOODS 1962). Furthermore, the presence of the floating plants suppresses the development of phytoplankton and submerged macrophytes. Because decomposition processes frequently produce anoxic conditions beneath unbroken layers of floating plants, the death of fishes is often observed (GOPAL 1987).

Various investigations have already been undertaken in order to exploit this enormous biomass production for animal nutrition (SHIRLEY et al. 1976; OSMAN et al. 1975; SAINT-PAUL et al. 1981), paper production (NOLAN & KIRMSE 1974), an energy source (KЛАSS & GHOSHI 1980), and a means of water purification (WOLVERTON et al. 1975), but without convincing success. Other studies were undertaken to seek ways of controlling the spread of these plants, but these have not been successful.

The studies of SAINT-PAUL & SOARES (1987) in a peripheral lake along the Amazon River demonstrated that the effects of floating plants need not always have a negative effect on the ichthyofauna. The plants are native to the region investigated. From the research of BAYLEY (1982) and JUNK (1984), it is known that in the flood plain of the Amazon, the zone of macrophytes is of great importance as a "nursery" for fishes. Due to the natural diurnal fluctuations in the oxygen concentration, the fishes are periodically forced to leave the regions covered by plants and seek out open water areas for their emergency respiration. Experimental investigations, however, demonstrated that if the hypoxic conditions persist, a serrasalmid, Colosoma macropomum, and an anostomid, Schizodon fasciatum, return to the zones of plant cover and remain among the roots of the floating plants. Since continuous opercular movements are observed, suggesting a normal respiration, the oxygen availability among the roots seems to be better than in the deeper water. Although this at first seems an unusual form of behavior during a period of oxygen shortage, it may very well be explained by the presence of oxygen near the roots of some floating plants and the ability of several fish species to utilize this for respiration.

If it is true that, in addition to the negative characteristics mentioned above, several Neotropical floating plants actually have the ability of increasing the partial pressure of oxygen in the waters near their roots, this will have a very great significance for the interpretation of many ecological observations.

In order to elucidate this situation, an investigation was undertaken to determine the qualitative and quantitative oxygen input from E. crassipes and P. stratiotes in the laboratory and to provide a basis to discuss possible consequences for the entire ecosystem of the flood plain.

Materials and Methods

The Eichhornia crassipes and Pistia stratiotes strains used in the experiments were obtained from the Central Amazon region and cultured at a constant temperature in the Kiel Botanical Institute (SCHULTER 1985). The plants were illuminated for 14 hours and kept in the dark for 10. The maximum density of quanta was about 350 nmol · m⁻² · s⁻¹ (photically = scalar) corresponding to 1/4 of the early morning and 1/6 of the noon radiation in the natural habitat of the plants in the central Amazon region (FURCH & OTTO 1987). The air temperature was 27°C, and that in the experimental aquaria, 26°C. The relative humidity was 90%.

The qualitative test for an O₂ input from the macrophyte roots in the experimental aquaria employed the indicator dye, methylene blue (FURCH 1981). Substances such as sodium thionite reduce methylene blue to the colorless leucomethylene. This reaction is reversible in the presence of oxygen.

For the determinations, a 1 g l⁻¹ concentration of reduced methylene blue in an agar-agar medium was used. The plants were placed with their roots in buckets containing the medium. Because of the high viscosity of the medium, the release of oxygen from the root systems could be precisely localized by the blue color and photographically documented.

The experimental apparatus for the quantitative determination of the O₂ release is shown in Figure 1. It is essentially a closed system in which water freed from dissolved gases by boiling is used. At the beginning of the experiments, the oxygen concentration of the water was between 0.6 and 1 mg O₂ l⁻¹ at 20°C.

The O₂ concentration of the water was determined polarographically using an Orion 97-08 and a Radiometer E5046. The Orion electrode was placed in the experimental chamber for direct O₂ determination, and the Radiometer probe was installed in a flowthrough system outside of the chamber. The data were continuously recorded with a recorder. The O₂ input from the plants in the experimental chambers was calculated using the following formula:

\[ O₂ \text{ input} = (O₂ \text{ conc}_1 - O₂ \text{ conc}_2)/I(15 - 10) \times 10^{-3} \times 1000 \times 10^{-6} \equiv \text{mg O}_2 \cdot \text{m}^3 \cdot \text{h}^{-1} \]

The quantity of the O₂ input was determined according to both the wet and dry (105°C) weight of the roots. In addition, the surface area of the leaves was measured planimetrically, and the chlorophyll content was determined spectrophotometrically. The surface area of the roots was determined from planimetric measurements made using photocopies of the root system. Assuming that the roots are circular in cross-section, the total surface area could be determined from the lengths and diameters of the individual roots. The volume of the roots was calculated by a simple displacement procedure according to the principle of Archimedes (YOUNG & WERNER 1984).

The anatomical structures of the root system were investigated using a scanning electron microscope.

Results

The results of the qualitative determinations of O₂ input through the root systems were positive for both E. crassipes and P. stratiotes. The color of methylene blue in the immediate zone of contact between the roots and the medium demonstrated unequivocally that O₂ was entering the medium from the roots.

The anatomical examinations revealed that the leaf and root regions of both plant species contained extensive lacuna systems that would seem to facilitate the passage of gases (Fig. 2 and 3). A system of regularly formed chambers in the lacunae are delimited by cross-walls, and through these, gas transport all the way to the tips of the roots is possible. The septa assure an unhindered gas transport because they are not subdivided by diaphragms.
Fig. 1:
Experimental apparatus for the quantitative determination of O₂ input into the water through the root systems of floating macrophytes. 1: Irradiance, 2: O₂ meter, 3: paraffin, 4: pump, 5: stirring pill, 6: stirrer, 7: pO₂ meter, 8: registrater, 9: pH meter.

The tissue in the middle forms the central cylinder. The secondary side roots do not arise from the central cylinder but rather contact the lacunae as widely diffuse extensions. These secondary roots are 0.1 mm in diameter and consist in cross section of about four cells widely separated by intercellular spaces. The qualitative presence of O₂ as demonstrated by methylene blue is detected very quickly in the vicinity of these secondary roots as well as the in region within about 1 cm of the root tip. The large intercellular spaces and weakness of the cell walls within these secondary roots assures that there is little resistance to diffusion that might hinder the release of oxygen. The primary branch roots are surrounded by a four to five layer hypodermis, which can offer a considerable resistance to the passage of oxygen. Apparently, the large surface area of the secondary branch roots makes them capable of releasing much oxygen.

Fig. 2:
Cross-section through in the primary branch roots of *Eichhornia crassipes* (SEM, 108 x). Several layers of hypodermis, the aerenchyme sectioned by septa, and the central cylinder are arranged concentrically from the outside to the middle.

Fig. 3:
Cross-section through in the primary branch roots of *Pistia stratiotes* (SEM, 108 x). Several layers of hypodermis, the aerenchyme sectioned by septa, and the central cylinder are arranged concentrically from the outside to the middle.
In quantitative experiments with *P. stratiotes*, amounts from 0.09 to 0.7 mg O₂ · h⁻¹ were released by each plant. The amount is directly proportional to the chlorophyll content and leaf surface area of each plant. The linear relationships between the leaf and root parameters are summarized in Table 1 and illustrated in Figures 4 and 9.

Table 1: Correlation analysis of the O₂ input (mg · h⁻¹) through the root system of *Pistia stratiotes* and various leaf and root parameters. O₂ input = a + b · x; r = correlation coefficient (n = 20).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>a</th>
<th>b</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root wet weight (g)</td>
<td>-0.048</td>
<td>0.083</td>
<td>0.92</td>
</tr>
<tr>
<td>Root volume (cm³)</td>
<td>-0.029</td>
<td>0.069</td>
<td>0.93</td>
</tr>
<tr>
<td>Root surface area (cm²)</td>
<td>0.048</td>
<td>3.7 · 10⁻²</td>
<td>0.90</td>
</tr>
<tr>
<td>Surface area of the tap root (cm²)</td>
<td>-0.099</td>
<td>3.6 · 10⁻²</td>
<td>0.87</td>
</tr>
<tr>
<td>Surface area of the leaves (cm²)</td>
<td>-0.170</td>
<td>1.4 · 10⁻²</td>
<td>0.81</td>
</tr>
<tr>
<td>Chlorophyll content (mg)</td>
<td>-0.090</td>
<td>0.137</td>
<td>0.89</td>
</tr>
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</table>

As shown by the figures and table, there is a linear relationship between the amount of O₂ released and the selected parameters, and the correlation coefficients are statistically significant. The positive correlation between the O₂ input performance of the plants and both the chlorophyll content and the leaf surface area suggests that the oxygen released was produced by photosynthesis.

In the experiments with *E. crassipes*, 0.2 to 1.0 mg O₂ · h⁻¹ was released per plant. There was, however, no correlation between the amount of oxygen released and the root surface area or the chlorophyll content (Figs. 10 and 11).

**Discussion**

It was unequivocally shown for both *P. stratiotes* and *E. crassipes* that under the experimental conditions, O₂ is released from the roots into the water. The gas transport is facilitated by the extensive intercellular system. Anatomical structures that permit O₂ release are well known among swamp and water plants. Aerenchyme is often developed in those plant structures that are continually or frequently located in media where hypoxic conditions prevail. There are distinctions among the aerenchyme in leaves (e.g. *Pistia stratiotes*), petioles (e.g. *Nuphar luteum*, *Nymphaea alba* and *Nelumbo nucifera*), and in the sprouts (e.g. *Oryza sativa*). GROSSE & SCHRÖDER (1986) regard the formation of aerenchyme to be an adaptation to the habitat conditions. The facilitation of internal gas movements permits the transport of O₂ from the parts of the plants exposed to the air into those organs cut off from the atmosphere. The O₂ transported through this system may originate from photosynthesis, in which case it moves from the leaves through the large intercellular spaces into the lacunae. It may also originate from the atmosphere, in which case the transport is a mass flow. A lowered pressure in the respiring tissues, due to the relatively high solubility of CO₂ in water, produces a mass movement of air through the internal parts of the plant after the O₂ has entered by a process of pure diffusion.
Fig. 6:
Relationship between the amount of O$_2$ entering the water and the root surface area of *Pistia stratiotes*.

Fig. 7:
Relationship between the amount of O$_2$ entering the water and the tap root surface area of *Pistia stratiotes*.

Fig. 8:
Relationship between the amount of O$_2$ entering the water and the leaf surface area of *Pistia stratiotes*.

Fig. 9:
Relationship between the amount of O$_2$ entering the water and the chlorophyll content in the leaves of *Pistia stratiotes*.
An examination of the morphological and anatomical structure of *P. stratiotes*, as described by GROSSE & SCHRODER (1986) makes it clear that a thermoosmotic mass transport is not possible in this species. Because of the rosette arrangement of the leaves and their satiny-like coating of fine hairs, the necessary structural modifications for an intake of atmospheric oxygen are lacking. The most recently developed leaves in plants modified for such an O₂ intake would be located in an exposed position so that the air movement would not be interfered with. The newly formed leaves of *P. stratiotes*, however, are located in the center of the rosette and are wrapped in the older leaves. Furthermore, the fine hair coat produces a microclimate that would interfere with rather than facilitate the entry of atmospheric oxygen. However, these epidermal hairs may serve to prevent the release of the O₂ produced by photosynthesis into the atmosphere. In this way, the O₂ produced by the plant can be conserved for internal requirements. The positive correlation between the amount of oxygen released by the plant roots and the chlorophyll content suggests that the O₂ could originate from photosynthesis. Because the quantum density used in the experiment was only 1/4 to 1/6 the amount encountered in natural habitats of the plant, it seems likely that the O₂ release under ambient conditions would be considerably greater.

While the O₂ release from *P. stratiotes* shows a linear correlation with the root and leaf parameters investigated, the temporal course of the O₂ release from *E. crassipes* into the water of the experimental aquaria is better described by an exponential equation. The amount released is dependent upon the concentration of O₂ in the water surrounding the roots. In order to obtain comparable data on the O₂ release by the plants, a uniform basis on which to determine changes in the O₂ concentration of the water surrounding the roots per unit time must be established. For this purpose, a baseline concentration of 4 mg · l⁻¹ was chosen for the start of the experiment. This concentration was chosen because it had been reached in all of the experiments conducted and also because it is encountered in the natural habitats of the plants. As illustrated in Figure 12, this concentration can be reached in a várzea lake at about 9:00 a.m. (SAINT-PAUL & SOARES 1987). Thereafter, the O₂ concentration increases continuously and reaches its maximum of over 12 mg · l⁻¹ at about 3:00 p.m. Minimal concentrations below 4 mg · l⁻¹ may be reached between midnight and 6:00 a.m. However, we must state with emphasis, that such favourable conditions represent the very exception, for normally decomposing processes lead to strong impoverishment in oxygen concentration.

The potential of releasing O₂ seems to be of ecological importance for the plants. Most of the nutrients they require cannot be utilized in reduced form. Therefore, plants that continually or temporarily live under anaerobic conditions must adopt some form strategy to adapt to their habitats. GROSSE & SCHRODER (1986) were able to show that numerous species from a wide variety of taxa possess adequate adaptation mechanisms. They contain an extensive aerenchyme tissue through which O₂, either produced by photosynthesis or absorbed from the atmosphere, can be transported to the tissues in which it must be consumed. Superfluous oxygen is released into the medium, where it forms a thin film on the outer surface of the roots. In this aerobic microzone, reduced nutrients can be oxidized (BRIX 1986) to forms easily utilized by the plants.

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**Fig. 10:**
Relationship between the amount of O₂ entering the water and the root surface area of *Eichornia crassipes*.

**Fig. 11:**
Relationship between the amount of O₂ entering the water and the chlorophyll content in the leaves of *Eichornia crassipes*.

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The diurnal curve of the O$_2$ concentration in the water beneath the dense bed of floating macrophytes (Fig. 12) clearly shows fluctuation (SAINT-PAUL & SOARES 1987). Because there is certainly a horizontal exchange of water between the open water zones and the macrophyte zone, simple field studies cannot identify the source of the O$_2$. The determinations made for *E. crassipes* also fail to show that the rate of O$_2$ release is in any way related to the photosynthesis rate. However, it cannot be ruled out that some of the O$_2$ released was produced by photosynthesis. An additional explanation for this release is the mass flow of O$_2$, as described by DACEY (1979) and SCHRODER et al. (1986). This is a mass movement of atmospheric O$_2$ into the plants due to a difference in the partial pressures inside the plants and in the atmosphere.

In order to compare the oxygen concentration resulting from the performance of both plant species with that in open water zones, the average O$_2$ input per unit water surface area was calculated assuming an initial concentration of 4 mg l$^{-1}$, and the results are depicted in Figure 13. The calculations show that beneath a carpet of floating macrophytes, a measurable O$_2$ input is possible. The input beneath a layer of *E. crassipes* is nearly double that beneath an open water surface. The input from *P. stratiotes*, however, is only slightly greater than that determined in open water.

![Fig. 12: Diurnal curve of the O$_2$ concentration beneath floating macrophytes in a váraez lake.](image)

![Fig. 13: Average O$_2$ inputs by diffusion and by floating macrophytes, *Eichhornia crassipes* and *Pistia stratiotes* representing 3.6 and 2.9 kg fresh weight, and 1.2 and 0.7 kg root mass, respectively.](image)

The determinations already completed suggest the conclusion that the oxygen supply among the roots of floating plants can, under certain conditions, be greater than that in open water. This finding permits a fully new interpretation of the data on the diurnal changes in the fish densities within zones of a váraez lake (SAINT-PAUL & SOARES 1987). The increase in the fish density of the open water regions as hypoxia increases can only be explained by emigration from the zones covered by macrophytes. Through supplemental investigations, however, it was shown that apparently not all fishes are forced to take part in O$_2$ induced horizontal migrations. Several species have the ability to survive periods of hypoxia within the root masses of the floating plants, where they seem to find enough O$_2$ to meet the needs of their oxidative metabolism (Fig. 14). The two species already found to do this are the serraalmid, Colossoma macrophorum, and the anostomid, Schizodon fasciatum. These biological findings are now logically explained by the O$_2$ input from the two floating Neotropical plants.
For both of these hypoxia-tolerant species there are a series of advantages provided by the capability of remaining among the plant roots. First of all, the predator pressure is reduced, as shown by the investigations of JUNK et al. (1983). The predatory species are apparently less tolerant to hypoxia. The capability of C. macropropum to survive under hypoxic conditions beneath a bed of macrophytes gives this species the chance to utilize the rich food supply of this biotope at times that other species must avoid it. Nutritional investigations also seem to suggest that at least C. macropropum prefers to seek food at the time around sunrise, when the danger of O₂ shortages are particularly great (GOULDING, 1980; GOULDING & CARVALHO 1982; SAINT-PAUL 1985, 1988). In this way, the number of food competitors is reduced. In addition, C. macropropum beneath the macrophytes is less exposed to predation by terrestrial species than those fishes displaying emergency respiratory behavior at the surface of open water zones.

The investigations conducted showed that the floating plant species feared in many tropical ecosystems, E. crassipes and P. stratiotes, can play important roles in their natural ecosystems, and the endemic ichthyofauna seems to be well adapted to their activities. Such ecophysiological investigations in the future contribute in a significant way to the better understanding of the functional interrelationships within tropical ecosystems.

Note added in proof

After print of the present article a paper of MOORHEAD & REDDY (1988), treating a similar subject was published. They evaluated the extent of O₂ transport from aerial plant tissue into root zone for several floating (e.g. E. crassipes) and emergent aquatic macrophytes that have characteristics favorable for wastewater treatment. By use of a similar experimental set-up the authors have tried to evaluate the O₂ transport rates with regard to different morphological and anatomical structures. However, as equal to the present investigation no significant correlation between the O₂ input and the selected parameters was detected, the O₂ transport was related to the dry root mass. The overall rate of 0.91 g O₂ - kg⁻¹ dry root mass - h⁻¹ found by MOORHEAD & REDDY (1988) is similar to the amount of 0.60 g O₂ - kg⁻¹ dry root mass - h⁻¹ calculated in the present paper. Based on an estimation of the number of 200 plants - m⁻² in situ O₂ input into the water was calculated to be 116 mg O₂ - m⁻² - h⁻¹ water surface area in the present paper. Based on a specific dry root mass - m² MOORHEAD & REDDY (1988) calculated the O₂ input to be in the range of 100 - 400 mg - m⁻² - h⁻¹, thus confirming the results presented in this paper.

Resumo

Melhoramento das condições de oxigênio em lagos de várzea da Amazônia Central através de exsudação de raízes de duas macrofitas nôcias (Eichhornia crassipes (Pontederiáceas) e Pistia stratiotes (Araceáceas)).

Eichhornia crassipes e Pistia stratiotes são plantas flutuantes nativas da Amazônia tropical. A ocorrência em massa delas é geralmente nociva para os ecossistemas e também impede a utilização econômica de corpos d'água naturais e artificiais.

No presente artigo, relatam-se observações e medições indicando que ambas as plantas são capazes, sob certas condições, de enriquecer um corpo d'água com O₂, facilitando desta maneira a sobrevivência de alevinos de dois espécies importantes de peixes da Amazônia, o tamburil (Colossoma macropomum, Serrasalmidae) e o aracá (Sclhodon fasciatum, Anostomidae). Especialmente, sob condições hipóxicas que se formam de manhã, estes peixes procuram o espaço de raízes relativamente rico em oxigênio onde eles, além disso, são melhor protegidos contra predadores, in situ.

Medições do aumento de oxigênio no corpo d'água como também observações ecológicas em ambas as espécies ficam foram feitas no laboratório. Por meio de parâmetros importantes como área foliácea, conteúdo em cloreúra, peso seco, volume e comprimento e superfície das raízes conseguiu-se mostrar, que:

1. o aumento de oxigênio pelas raízes de Pistia stratiotes depende da fotossíntese, e que o fornecimento essencialmente mais forte de oxigênio por Eichhornia crassipes está ligado a uma série de mecanismos natureza desconhecida.

2. Sob condições hipóxicas, ambas as macrofitas são capazes de enriquecer o teor de O₂ de tal forma que este pode aumenatar de uma concentração inicial de 4 mg O₂ - l⁻¹ até 2,5 vezes em comparação com a concentração na superfície aquática livre e, assim, criando microhabitats nas raízes, que podem facilitar a sobrevivência de algumas espécies de peixes.

Nesta escala maior, os processos de decomposição sobrepõem-se ao incremento de oxigênio, resultando num saldo negativo no biotopo.
Zusammenfassung


Messungen zum Sauerstoffgehalt in den Wasserbiotope sowie verhaltensbiologische Beobachtungen an beiden Fischarten wurden unter Laborbedingungen durchgeführt. Mit Hilfe wichtiger Parameter wie Blattfläche, Chlorophyllgehalt, Trocken- und Nassebenen, dagegen und -oberfläche konnte für die *Pistia stratiotes* nachgewiesen werden, daß der Sauerstoffgehalt von Wurzeln photosynthetisch abhängig ist, und daß

2. Der wesentlich stärkere Sauerstoffgehalt durch *Eichhornia crassipes* an Mechanismen gebunden ist, die für diese Spezies noch nicht aufgedeckt wurden.

Beide schwimmende Macrophyten können jedoch bei hypoxischen Bedingungen den Sauerstoffgehalt im Wasser dergestalt anheben, daß dieser bei einer Ausgangskonzentration von 4 mg O₂ l⁻¹ auf das bis zu 2,5-fache im Vergleich zu einer freien Wasseroberfläche ansteigen kann und somit dem Tamabauqi und dem Araçá das Überleben im Versteck erleichtern könnten. Großvolumig wird dieser Effekt jedoch von Zeitzuschlägen überlagert, so daß im Biotop gewöhnlich negative Bilanzen anstehen werden.

References


