Underwater photosynthesis of submerged plants – recent advances and methods

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We describe the general background and the recent advances in research on underwater photosynthesis of leaf segments, whole communities, and plant dominated aquatic ecosystems and present contemporary methods tailor made to quantify photosynthesis and carbon fixation under water. The majority of studies of aquatic photosynthesis have been carried out with detached leaves or thalli and this selectiveness influences the perception of the regulation of aquatic photosynthesis. We thus recommend assessing the influence of inorganic carbon and temperature on natural aquatic communities of variable density in addition to studying detached leaves in the scenarios of rising CO2 and temperature. Moreover, a growing number of researchers are interested in tolerance of terrestrial plants during flooding as torrential rains sometimes result in overland floods that inundate terrestrial plants. We propose to undertake studies to elucidate the importance of leaf acclimation of terrestrial plants to facilitate gas exchange and light utilization under water as these acclimations influence underwater photosynthesis as well as internal aeration of plant tissues during submergence.

Keywords: flooding tolerance, light extinction, carbon dioxide, wetland plants, photorespiration

Knowledge of plant and environmental factors determining photosynthesis by submerged plants is essential for understanding aquatic plant ecophysiology and ecosystem productivity, as well as submergence tolerance of terrestrial plants. Following the pioneering studies by Arens (1933) and Steemann Nielsen (1946) on the use of dissolved inorganic carbon (DIC) for photosynthesis of aquatic plants, numerous studies on the regulatory role of light and DIC for underwater photosynthesis of aquatic plants have been conducted. Particularly, the use of DIC by aquatic plants has fascinated researchers and been reviewed several times (e.g., Madsen and Sand-Jensen, 1991; Maberly and Madsen, 2002; Raven and Hurd, 2012) because this process is important for growth and survival and the uptake mechanisms are very different from those of terrestrial, amphibious, and floating leaved plants exposed to atmospheric air (definitions of these life forms and examples of species are in Sculthorpe (1967)). Since the physical conditions differ markedly between water and air, we have often been approached by researchers asking for practical advice, unavailable in the literature, before engaging in work with underwater photosynthesis. Thus, this review serves to offer the background and a practical guide for measurements of carbon fixation by plants when under water.

Moreover, a growing number of researchers are interested in tolerance of terrestrial plants during flooding (Figure 1A). Torrential rains sometimes result in overland floods that inundate terrestrial plants (Vervuren et al., 2003) and with the current projection on climate change, the frequency of such flooding events are expected to increase (Parry et al., 2007). We therefore predict that research on underwater photosynthesis will extend greatly beyond its current focus on aquatic plants as natural wetlands and many crops will become submerged in future flooding events. Researchers engaging in underwater photosynthesis should be aware that physical restrictions on light availability and gas exchange are much more profound under water than on land (Sand-Jensen and Krause-Jensen, 1997). Moreover, the aquatic sources and mechanisms of inorganic carbon use are complex, difficult to study, and often challenging to fully understand (Madsen and Sand-Jensen, 1991; Raven and Hurd, 2012).

Photosynthesis provides sugars and O2. The importance of underwater photosynthesis to internal O2 status (Figure 1B), including via internal long-distance transport into roots growing in anoxic sediments, has been demonstrated for aquatic species (e.g., Borum et al., 2005; Sand-Jensen et al., 2005; Holmer et al., 2009; Pedersen et al., 2011) and terrestrial wetland plants when completely submerged (Pedersen et al., 2006; Winkel et al., 2011, 2013). By contrast, during the night submerged plants rely on O2 uptake from the surrounding water to sustain their respiration and belowground organs can suffer from O2 deficiency.

The majority of studies on photosynthesis by submerged aquatic plants have been carried out on detached leaves and algal thalli. These may experience very different environmental conditions than entire communities of submerged plants or plant dominated ecosystems, which have rarely been examined (e.g., Sand-Jensen et al., 2007; Christensen et al., 2013). We thus recommend undertaking studies on communities and ecosystems because they may reveal very different principles of regulation of greater relevance for the ecology and natural performance of...
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FIGURE 1 | Completely submerged terrestrial vegetation (A), white flakes of CaCO$_3$ on leaves of a pondweed (Potamogeton lucens) (B) and an incubator with a vertically rotating wheel holding vials with leaf segments for measurements of underwater net photosynthesis (C) or dark respiration when in complete darkness. The bubbles on the submerged mosses (A) are obvious signs that underwater photosynthesis takes place with O$_2$ evolution causing the bubble formation. Moreover, the submerged grasses possess superhydrophobic self cleansing leaf surfaces that retain a thin gas film when under water, evident as a silvery reflecting surface. In (B), high pH on the adaxial leaf surfaces following extensive underwater photosynthesis has resulted in precipitation of CaCO$_3$ (See “Challenges Under Water – Reduced Gas Diffusion and Light Penetration”). Photos: a shallow puddle on Öland, Sweden (A), the bicarbonate rich (1.8 mmol DIC L$^{-1}$) Lake Slåen, Denmark (B) and the custom built wheel incubator by Ray Scott at the University of Western Australia (C), photos by Ole Pedersen.

submerged aquatic plants, as well as survival of terrestrial species during overland floods.

With the present review, we describe the general background and the recent advances in underwater photosynthesis of phytoelements (shoots, excised thalli, or leaf portions), communities, and plant dominated aquatic ecosystems and present contemporary methods tailor made to quantify photosynthesis and carbon fixation under water.

UNDERWATER PHOTOSYNTHESIS

CHALLENGES UNDER WATER – REDUCED GAS DIFFUSION AND LIGHT PENETRATION

The $10^4$-fold lower diffusion coefficient of gases in water, compared with in air, presents a major challenge to submerged plants (Armstrong, 1979; Maberly and Madsen, 2002). Diffusive boundary layers (DBL) develop on all surfaces and their thickness adjacent to leaves in water is of the same order of magnitude as those for leaves in air (Vogel, 1994; Raven and Hurd, 2012). Although the transport distance for gases across the DBL is similar, the much lower diffusion coefficient in water results in a $10^4$-fold lower flux for the same concentration gradient and thus the DBL constitutes a much larger proportion of the total resistance to gas exchange for leaves under water than in air (Maberly and Madsen, 2002). The “bottleneck effect” of the DBL on underwater gas exchange was demonstrated in a study of four submerged aquatic species, where the DBL accounted for 90% of the total resistance to carbon fixation (Black et al., 1981). Hence, inorganic carbon limitation of photosynthesis is a much more common and prominent feature for aquatic than terrestrial leaves (Stirling et al., 1997). On average, the underwater photosynthesis increased threefold in a study of 14 submerged freshwater species at saturating relative to ambient supply of DIC supply (Nielsen and Sand-Jensen, 1989). The immediate acclimation of photosynthesis of five fast growing annual terrestrial species by doubling of atmospheric CO$_2$ was 1.6- to 2.1-fold while the average increase of relative growth rate over 56 day was 1.25-fold and only significant for one of the five species (Stirling et al., 1998).

The restricted gas exchange under water has resulted in evolution of a suite of adaptive features in aquatic leaves and macroalgal thalli to reduce the influence of DBL on the exchange of O$_2$ and
CO\(_2\), including the supplementary use of HCO\(_3^-\) (bicarbonate) (Sculthorpe, 1967; Maberly, 1990; Colmer et al., 2011). In seawater and in many freshwaters, the pool of HCO\(_3^-\) is several fold higher than that of CO\(_2\) and thus presents an attractive alternative to CO\(_2\). Use of HCO\(_3^-\) has evolved many times among unicellular algae, macroalgae, and angiosperms in freshwater and marine environments (Raven and Hurd, 2012) and can involve direct uptake into the cells or external conversion to CO\(_2\) in the DBL catalyzed by surface bound carbonic anhydrase and/or extrusion of protons in acids bands (charophytes; Lucas and Smith, 1973) or lower leaf surfaces in e.g., species of Potamogeton and Elodea (Prins et al., 1980) often with precipitation of CaCO\(_3\) on the alkaline upper leaf surfaces (Figure 2B). While the DBL reduces the direct HCO\(_3^-\) flux to the leaf surface, the “stagnant” layer is required to forming high CO\(_2\) concentrations by acid titration of HCO\(_3^-\) (Helder, 1985). Use of HCO\(_3^-\) is prominent for marine macroalgae and seagrasses, freshwater charophytes, and in 50% of 80 tested species of freshwater angiosperms (Sand-Jensen and Gordon, 1984; Maberly and Madsen, 2002), but the ability is absent among mosses and pteridophytes. Also 12 amphibious species alternating between emergent and submerged forms in Danish lowland streams relied solely on CO\(_2\) use although high HCO\(_3^-\) concentrations may still benefit photosynthesis by stabilizing pH and permitting rapid uncatalyzed replenishment of the CO\(_2\) consumed (Maberly, 1990). The proportion of HCO\(_3^-\) users among angiosperm species in lakes increases significantly with alkalinity and, thus, availability of HCO\(_3^-\) (Maberly and Madsen, 2002) in accordance with the increasing advantage of HCO\(_3^-\) use for photosynthesis and growth. Assuming for simplicity a 10-fold higher apparent preference for CO\(_2\) than HCO\(_3^-\) by leaves in alkaline water containing 0.015 mmol L\(^{-1}\) CO\(_2\) and 1.5 mmol L\(^{-1}\) HCO\(_3^-\), the supply rate of HCO\(_3^-\) would be 10-fold higher than that of CO\(_2\). In softwater containing only 0.15 mmol L\(^{-1}\) HCO\(_3^-\) the supply rate of the two carbon species would be the same. Terrestrial plant species lack these adaptive features for aquatic life, and when their leaves undergo dramatic reduced net photosynthesis (Sand-Jensen et al., 1992; Nielsen, 1993) and dark respiration (Colmer and Pedersen, 2008; Pedersen et al., 2009). Thus, 13 terrestrial species submerged in CO\(_2\) rich stream water were unable to use HCO\(_3^-\) and median rates of underwater net photosynthesis were sevenfold lower than of 10 permanently submerged stream plants and the terrestrial species were unable to support substantial growth (Sand-Jensen et al., 1992; Figure 2).

The extraction capacity of the DIC pool is only some 1–4% for obligate CO\(_2\) users while it is typically 40–70% among HCO\(_3^-\) users; 16 of 19 species (Madsen and Sand-Jensen, 1991). This is because of the ability of HCO\(_3^-\) users to continue photosynthesizing despite very high external pH and low DIC in the water. In vegetation rich water bodies of high pH, HCO\(_3^-\) users can eventually out compete all obligate CO\(_2\) users (Sand-Jensen et al., 2010). Submerged aquatic plants unable to use HCO\(_3^-\) typically have final pH’s in the external medium of the order of 8.6–9.8 in alkaline solutions and final CO\(_2\) concentrations equivalent to CO\(_2\) compensation points of 2–10 µmol L\(^{-1}\), while active HCO\(_3^-\) users typically have final pH’s of 9.8–11.0 and final CO\(_2\) concentrations mostly below 0.3 µmol L\(^{-1}\) (Sand-Jensen et al., 1992).

For a large collection of stream plants, median final CO\(_2\) values among the supposedly obligate CO\(_2\) users were 6.0 µmol L\(^{-1}\) for homophyllous and 4.8 µmol L\(^{-1}\) for heterophyllous amphibious plants, within the typical range of CO\(_2\) compensation points, while the median final CO\(_2\) concentration for the putative HCO\(_3^-\) users was only 0.04 µmol L\(^{-1}\) reflecting the supplementary use of HCO\(_3^-\) (Figure 2). Heterophyllous amphibious species form morphologically and anatomically distinct leaf types under water...
as compared to in air (Sculthorpe, 1967). The underwater leaf forms are an acclimation that enhances underwater gas exchange (Sand-Jensen et al., 1992; Colmer et al., 2011).

Photosynthesis of submerged aquatic plants and flooded terrestrial plants can also be severely limited by light (Kirk, 1994). In water, light is exponentially attenuated with depth following the equation: \( I_z = I_0 (1 - e^{-z/\alpha}) \), where \( I_z \) is the available irradiance at a given depth \( z \), \( I_0 \) is the irradiance at the surface, and \( \alpha \) is the attenuation coefficient. The proportional reflection and backscattering at the water surface \( (f) \) is variable but typically about 0.1 such that the proportion of downwelling irradiance is 0.9 (Kirk, 1994). The attenuation coefficient of pure water averaged across the photosynthetic spectrum is about 0.03 m\(^{-1}\), so in ultra clear water such as oligotrophic oceanic water, rooted plants could grow as deep as 70 m with 10% of surface irradiance still being available, which happens to be the approximate lower depth limit of seagrasses (Duarte, 1991). In most cases, however, colored dissolved organic matter (CDOM), pigments in planktonic algae and suspended particles, together reduce light penetration much more profoundly (Staehr et al., 2012b). Because freshwaters compared with marine waters are typically richer in nutrients, phytoplankton, CDOM exported from land and particles suspended from shallow sediments, attenuation coefficients typically range from 0.3 to 10 m\(^{-1}\) and thus have lower depth limits of rooted plants from 7 m to only 0.2 m (Middelboe and Markager, 1997). Flooding after heavy rain is commonly associated with erosion, high particle loads and high release of CDOM from inundated terrestrial soils. Flooded terrestrial plants can, therefore, experience extreme shading corresponding to attenuation coefficients between 1 and 8 m\(^{-1}\) (Parolin, 2009) making light limitation also of terrestrial plants a prominent feature during flooding events (Colmer et al., 2011).

**UNDERWATER PHOTOSYNTHESIS IN SUBMERGED AQUATIC PLANTS AND RECENT ADVANCES**

The net process of photosynthesis (Eq. 1) is often described simply as the fixation of CO\(_2\) (or HCO\(_3^-\)) in water; Eq. 2 catalyzed by several enzymes, including Rubisco, driven by light and resulting in production of organic matter, O\(_2\) and OH\(^-\):

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{CH}_2\text{O} + \text{O}_2 \\
\text{HCO}_3^- + \text{H}_2\text{O} & \rightarrow \text{CH}_2\text{O} + \text{O}_2 + \text{OH}^- 
\end{align*}
\]

The rate of the process can be determined by the production of O\(_2\) and new organic matter (e.g., by isotopic tracing with \(^{13}\)C and \(^{14}\)C) and the consumption of CO\(_2\), HCO\(_3^-\), or more generally the pool of DIC: CO\(_2\), HCO\(_3^-\) and CO\(_3^{2-}\). Photosynthesis is an alkalinization process as reflected by the release of OH\(^-\) in Eq. 2 and the equivalent consumption of CO\(_2\) and protons in Eq. 1 (i.e., CO\(_2\) + H\(_2\)O \rightarrow H\(^+\) + HCO\(_3^-\)). Photosynthesis can be determined by the pH increase when converted to DIC consumption accounting for the buffer capacity [mainly due to carbonate alkalinity (CA), see, The CO\(_2\) Equilibrium in Water].

In charophytic macroalgae, use of HCO\(_3^-\) in photosynthesis can be closely coupled stoichiometrically to carbonate precipitation (McConnaughey, 1991):

\[
\text{Ca}^{2+} + 2\text{HCO}_3^- + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2 + \text{CaCO}_3
\]

This process is pH neutral because conversion of HCO\(_3^-\) to CO\(_2\) generates the necessary proton for conversion of HCO\(_3^-\) to CO\(_2\) for assimilation. Thus, HCO\(_3^-\) is equally divided between production of organic matter and CaCO\(_3\) and the photosynthetic quotient (PQ: mol O\(_2\) evolved mol\(^{-1}\) DIC consumed) is only about 0.5 compared with the typical value of 1.0 in Eqs 1 and 2. The active processes are apparently active antiprot of H\(^+\) and Ca\(^{2+}\) in the acid bands and Ca\(^{2+}\) extrusion in the alkaline bands resulting in gradual accumulation of CaCO\(_3\) from inside the carbonate layer (McConnaughey, 1991). Carbonate precipitation closely coupled to photosynthesis is also found in coralline red algae, several green algae, and numerous freshwater angiosperms developing polar leaves with high pH and carbonate precipitation being confined to the upper leaf surfaces (Raven and Hurd, 2012). However, it remains to be tested whether active Ca\(^{2+}\) extrusion is involved in angiosperm use of HCO\(_3^-\) as in charophytes (Prins et al., 1980). Even though photosynthesis and carbonate precipitation may not be closely coupled, the alkalinization process in Eqs 1 and 2 may still result in carbonate precipitation on leaf surfaces (Figure 1B) or in the surrounding water because of increase of pH and CO\(_3^{2-}\), though with a variable ratio to the fixation of CO\(_2\) in organic matter. Consequently, O\(_2\) evolution is a more reliable measure of underwater photosynthesis, while DIC use and production of organic matter and carbonate are essential parameters in the analysis of plant growth and carbon dynamics in ecosystems and on regional and global scales (McConnaughey et al., 1994; Cole et al., 2007). While coupled calcification photosynthesis leads to extensive drawdown of DIC and sediment accumulation of organic carbon and carbonates, carbonate formation per se generates H\(^+\) tending to reduce pH and increase CO\(_2\). During geological periods of intense formation of coral reefs, CO\(_2\) concentrations are suggested to have increased in the ocean and the atmosphere (Opdyke and Walker, 1992). The coupled photosynthesis calcification process has three major ecological or physiological implications: (i) in coral and coralline algae carbonates are directly used to build up the structural skeleton, (ii) in all phototrophs, surface precipitates will protect them against grazing animals, and (iii) calcification will prevent alkalinization during intensive photosynthesis which could otherwise have led to such high pH levels that tissues are damaged and photosynthesis is severely inhibited.

The photosynthetic capacity under optimum light, temperature, and DIC conditions varies among species and within species depending on their investment in active transport processes and catalytic machinery. On dry mass basis, maximum rates of photosynthesis of detached leaves of submerged aquatic plants from lakes typically vary 25-fold and dark respiration only fourfold between slow-growing, oligotrophic isoeitid species and fast growing, eutrophic elodeid species (Nielsen and Sand-Jensen, 1989). Photosynthetic rates per unit dry mass increase significantly with reduced leaf thickness, higher relative surface area, and higher concentrations of pigments and nitrogen in structural and catalytic proteins, including Rubisco (Madsen et al., 1993). Because metabolism on a dry mass basis increases with declining leaf thickness, photosynthesis expressed per surface area only varies eightfold among species (Nielsen and Sand-Jensen, 1989). A comparison
with terrestrial leaves characterizes the aquatic leaves by their lower chlorophyll and Rubisco concentrations and lower photosynthetic rates per surface area mainly due to the thin leaves of most aquatic species. This finding has been interpreted by Maberly and Madsen (2002) as a result of selection of submerged plants to match the low rates of carbon influx predominantly because of high transport resistance. Thin submerged leaves with chloroplasts in epidermal layers will also increase the cost effectiveness of light use and can also be regarded as a particular advantage in a low light aquatic environment with no risk of desiccation damage to the epidermal layers.

Realized rates of underwater photosynthesis for a given plant tissue varies from zero at compensation levels for light and DIC to maximum rates at saturating light and DIC. Light and DIC levels required to saturate photosynthesis increase with temperature and are highly dependent on the extent of self shading and, therefore, the scale of analysis of either detached leaves, individuals or populations (See Underwater Photosynthesis – Approaches and Methods). On a daily level, light limitation takes place early in the morning (low light but plenty of CO$_2$, Figure 3) and coinhibition of both light and CO$_2$ takes place late in the afternoon where also CO$_2$ is low (Figure 3). On a seasonal level, light limitation is present from late autumn to early spring outside the tropical regions. Populations in deep or turbid waters and dense populations with high self shading face permanent light limitation. Photosynthetic rates at saturating light and DIC increase with temperature due to stimulation of enzyme activity up to an optimum level depending on the adaptation and acclimation of the species but are usually located between 25 and 32°C for temperate submerged aquatic plants (Santamaria and van Vierssen, 1997). The temperature exponent gradually declines and reaches zero as temperature approaches the optimum temperature and it turns negative above the optimum due to increasing influence of photorespiration (Long, 1991) and risk of damage of macromolecules, membranes, and structural organization of membrane proteins (Johnson et al., 1974; Lambers et al., 2008). Respiration continues to increase up to higher temperatures than photosynthesis resulting in proportionally greater losses of organic matter and optima for growth being located at lower temperature than optima for photosynthesis (Olesen and Madsen, 2000; Pilon and Santamaria, 2001).

Ninety nine percent of all studies of aquatic photosynthesis have been carried out with detached leaves or thalli and this selectiveness influences the perception of the regulation of aquatic photosynthesis (Sand-Jensen and Krause-Jensen, 1997). The influence of light, DIC, and temperature on underwater photosynthesis show mutual interdependencies and are, moreover, strongly dependent on the spatial scale. From detached phytoelements to closed communities, light compensation points typically increase three- to eightfold and light saturation levels increase from 200 to 400 µmol m$^{-2}$ s$^{-1}$ to more than the maximum irradiances at noon of about 1500 µmol m$^{-2}$ s$^{-1}$ (Table 1; Sand-Jensen et al., 2007). The stimulation of photosynthesis in alkaline water by rising CO$_2$ concentrations from 20 (close to air equilibrium) to 250 µmol L$^{-1}$ (more than 10-fold above air equilibrium) is about ninefold for detached leaves and only 1.9- to 2.5-fold for dense communities of freshwater CO$_2$ users while for efficient HCO$_3^−$ users the CO$_2$ stimulation is only about twofold for individual leaves and insignificant for dense communities (Table 2; Sand-Jensen et al., 2007). Open communities of less self shading take an immediate position between detached individual leaves and dense communities. Profound self shading and light limitation of photosynthesis in dense aquatic communities imply that the influence of temperature and inorganic carbon supply is smaller than observed for well illuminated phytoelements. The full scale influence of temperature and CO$_2$ on community photosynthesis is confined to tissues in the upper part of the canopy receiving irradiances above light saturation.

Up scaling of metabolic analyses from communities of submerged aquatic plants to entire ecosystems dominated by rooted plants have only been done in a few instances. Kelly et al. (1983) studied a shallow, densely vegetated stream (Grande Stream, Denmark) by open water O$_2$ measurements and confirmed that incoming irradiance was the main determinant of daily and seasonal variations of underwater photosynthesis which was only light saturated for a few hours at noon on clear summer days. The high CO$_2$ concentrations (typically 10-fold air equilibrium) in lowland streams is a prerequisite for the high photosaturated rates and strong light dependency of submerged plants in general and CO$_2$ users in particular (Sand-Jensen and Frost-Christensen, 1998). With natural CO$_2$ concentrations close to air equilibrium, as observed in most lakes and ponds and in streams in the
Table 1 | Photosynthetic parameters for thallus segments and communities of Fucus serratus of varying leaf area index (LAI).

|                | Thallus (mean) | Community LAI (m² m⁻²) |
|----------------|----------------|------------------------|
|                | 3.0            | 6.3                    | 8.9          | 13.8         |
| GPₚmax (µmol O₂ m⁻² s⁻¹) | 795            | 11.5                   | 174          | 22.0         | 23.7         |
| A (µmol O₂ mol⁻¹ photon) | 0.084          | 0.031                  | 0.049        | 0.089        | 0.072        |
| Eₜ (µmol photon m⁻² s⁻¹) | 22             | 67                     | 99           | 94           | 175          |
| Eₚ (µmol photon m⁻² s⁻¹) | Approx. 300    | Not sat.               | Not sat.     | Not sat.     | Not sat.     |

GPₚmax: light saturated photosynthesis, A: the initial linear slope of photosynthesis versus irradiance, Eₜ: the light compensation points when net photosynthesis is zero, Eₚ: the irradiance for onset of light saturated photosynthesis. From Binzer and Sand-Jensen (2002b).

Table 2 | Increase (x-fold) of maximum gross production of O₂ at high (250 µmol L⁻¹) relative to low (20 µmol L⁻¹) CO₂ concentration in alkaline water (5000 µmol L⁻¹ DIC) of leaves and freshwater plant communities at two densities (LAI; 2 or 10 m² m⁻²).

|                | Temperature (˚C) | Leaves | Community (LAI 2 m² m⁻²) | Community (LAI 10 m² m⁻²) |
|----------------|-----------------|--------|--------------------------|---------------------------|
| HCO₃⁻ USERS    |                 |        |                          |                           |
| Potamogeton crispus | 15              | 2.1    | 1.45                      | 1.16                      |
| Potamogeton pectinatus | 15              | 2.0    | 1.33                      | 0.96                      |
| CO₂ USERS      |                 |        |                          |                           |
| Callitriche cophocarpa | 15              | 9.0    | 3.32                      | 2.46                      |
|                 | 25              |        | 2.14                      | 1.91                      |
| Sparganium simplex | 15              | 9.3    | 4.24                      | 1.91                      |
|                 | 25              |        | 4.16                      | 1.54                      |

The ratio GPₚmax (high CO₂)/GPₚmax (low CO₂) is shown. Leaf values are from Sand-Jensen (1983) and community values from Sand-Jensen et al. (2007).

afternoon after several hours of planktonic photosynthesis (Sand-Jensen and Frost-Christensen, 1998; Christensen et al., 2013), CO₂ plays a stronger regulatory role for photosynthesis particularly in open plant stands of low self shading (Sand-Jensen and Frost-Christensen, 1998). Moreover, the species rich group of terrestrial plants in lowland streams would be unable to survive if the water had not been greatly supersaturated as their CO₂ compensation points resemble or exceed the CO₂ concentrations at air equilibrium (Sand-Jensen and Frost-Christensen, 1999). Recent use of open water measurements of O₂ and pH in shallow, alkaline ponds dominated entirely by charophytes documents that high biomass densities in late summer are attained by sustained slow growth over the preceding 3 months at very low nutrients concentrations in the water, and that daily photosynthesis is mostly limited by light (Figure 4) and only briefly by DIC at high pH (>9.5), and with virtually no CO₂ available in the afternoon (Christensen et al., 2013). Only submerged aquatic plants capable of using HCO₃⁻ and concentrating CO₂ internally at the site of Rubisco can thrive in this environment (Sand-Jensen et al., 2010). Plant species forming dense communities in shallow ponds must also be able to tolerate substantial diurnal variations in temperature (e.g., 18–32°C) and O₂ (hypoxic to twice air equilibrium) (Christensen et al., 2013). Daily photosynthesis and respiration were high in the pond and closely interrelated showing that newly produced organic matter was mostly rapidly respired by plants and bacteria. Overall, the analyses of individual phytoelements, communities, and ecosystems confirm that the relative roles of light and DIC for determining photosynthesis are closely interrelated and highly dependent on plant density and species affinities for CO₂ and HCO₃⁻. Maximum photosynthetic rates under light and inorganic carbon saturation are quite variable both between and within species depending on selected strategies and the investment in catalytic machinery coupled to supply of resources (e.g., nutrients). While photosaturated photosynthetic rates are strongly dependent on species, acclimation, and temperature, light limited rates are rather temperature independent and relatively similar among species (Frost-Christensen and Sand-Jensen, 1992). As the importance of light limitation for community photosynthesis...
in dense plant stands, the influence of species, temperature, and DIC supply decline and enable us to predict community photosynthesis primarily from the overall distribution and absorpance of light in the canopy (Binzen and Sand-Jensen, 2002a; b; Binzer et al., 2006).

RECENT ADVANCES IN UNDERWATER PHOTOSYNTHESIS IN TERRESTRIAL WETLAND PLANTS

Terrestrial wetland plants grow in waterlogged soils and/or sediments with shallow standing water, so that a large proportion of the shoot is in contact with air. So, aerial photosynthesis predominates but these plants can experience episodes of complete submergence during floods. Although much more tolerant of submergence than non-wetland terrestrial species, submergence is regarded as a serious abiotic stress for terrestrial wetland plants, but species (and genotypes within a species) differ markedly in submergence tolerance (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). The impeded gas exchange under water restricts respiratory and photosynthesis (See Challenges Under Water – Reduced Gas Diffusion and Light Penetration; photosynthesis can also be limited by low light when submerged (See Challenges Under Water – Reduced Gas Diffusion and Light Penetration and Underwater Photosynthesis in Submerged Aquatic plants and Recent Advances). Thus, submergence disrupts energy metabolism of terrestrial plant species as a result of a reduced O$_2$ supply (at least during the night, in some tissues) and/or diminished carbohydrate status because of the restricted photosynthesis under water. Terrestrial wetland species lack most of the adaptive leaf features for inorganic carbon acquisition for photosynthesis as described in Section “Underwater Photosynthesis in Submerged Aquatic Plants and Recent Advances” for aquatic and acclimated amphibious plants. Thus, when compared with leaves of aquatic plants, those of terrestrial plants generally have larger overall apparent resistance to diffusion of CO$_2$ from the bulk medium to chloroplasts, so that slow CO$_2$ uptake restricts underwater photosynthesis. Underwater photosynthesis by leaves of terrestrial wetland species is lower than that achieved by aquatic species, when compared per unit of leaf dry mass (Sand-Jensen et al., 1992; Colmer et al., 2011).

The few studies available show, however, that the low photosynthesis when under water enhances survival of submerged terrestrial plants (Vervuren et al., 1999; Mommer et al., 2006b; Vashish et al., 2011). Both the sugars and O$_2$ produced would likely contribute to enhanced survival when submerged (Mommer and Visser, 2005), and in the case of sugars especially when submergence lasts more than a few days and internal carbohydrates become depleted. Depletion of carbohydrates during submergence is considered a major factor influencing survival of submerged rice (Setter and Laureles, 1996) and determining recovery following desubmergence and ultimately grain yield in flood-prone areas (Bailey-Serres et al., 2010; Mackll et al., 2012). The O$_2$ produced in photosynthesis can travel from leaves to roots via aerenchyma, and so this endogenously produced O$_2$ improves the internal aeration of submerged plants (e.g., rice; Pedersen et al., 2009; Winkel et al., 2013).

A recent review (Colmer et al., 2011) highlighted there are few studies of underwater photosynthesis by terrestrial wetland plants, and few of these compared rates underwater with those achieved by leaves in air. Similarly, a quantitative understanding of the potential role of underwater photosynthesis to whole plant carbon budgets during submergence seems to be lacking for terrestrial wetland species, whereas carbon budgets for several aquatic species (e.g., van der Bijl et al., 1989) and systems (e.g., Christensen et al., 2013) have been evaluated. For some terrestrial wetland species, only a few crude leaf level estimates of carbon budgets have been considered (e.g., in Colmer and Pedersen, 2008), but the potential contribution of underwater photosynthesis to carbon gain was demonstrated in growth studies of completely submerged rice, albeit under controlled conditions (e.g., Pedersen et al., 2009). Studies of whole plant carbon budgets in field conditions are generally lacking, even understanding of this aspect for the important wetland crop rice submerged in various field scenarios appears to be incomplete.

Detailed studies of underwater photosynthesis of terrestrial wetland species have focused on production and performance of submergence acclimated leaves. New leaves produced when under water by some terrestrial wetland species are better acclimated for underwater photosynthesis than the aerial leaves (Mommer et al., 2007). The acclimated leaves have a thin cuticle and overall are also thinner and of less breath (Mommer and Visser, 2005). These morphological and anatomical differences as compared with the usual leaves produced in air, reduce the resistance to CO$_2$ (and O$_2$) diffusion between the bulk medium and chloroplasts in submerged leaves, owing to narrower DBLs (suggested by Colmer et al., 2011), lower cuticle resistance (Mommer et al., 2006b), and shorter internal diffusion path lengths (Mommer et al., 2006a). However, although a reduced cuticle that enhanced underwater gas exchange occurs in several terrestrial wetland species (few species have been evaluated to date), the magnitude of the reduction in apparent resistance to gas exchange with the medium was not correlated with submergence tolerance for the species tested (Mommer et al., 2007), highlighting the need for further experimental investigations.

Some recent work on underwater photosynthesis by submerged terrestrial wetland plants has evaluated the contribution of gas films on superhydrophobic leaf surfaces to gas exchange with floodwaters. Leaf surface hydrophobicity (i.e. water repellence) is a feature that sheds off water in wet aerial environments (Smith and McClean, 1989; Brewer and Smith, 1997) and promotes "self cleansing," enhancing leaf performance and reputedly lowering susceptibility to pathogens (Neinhuis and Barthlott, 1997). Some terrestrial wetland species have super hydrophobic leaves that when submerged retain a gas film, e.g., rice (Raskin and Kende, 1983) and Phragmites australis and others (Colmer and Pedersen, 2008). Gas films enhance CO$_2$ uptake for underwater photosynthesis (Raskin and Kende, 1983; Colmer and Pedersen, 2008; Pedersen et al., 2009) and O$_2$ entry for respiration in darkness (Colmer and Pedersen, 2008; Pedersen and Colmer, 2012). The enhancement by leaf gas films of CO$_2$ uptake (in light) and of O$_2$ (in darkness) was demonstrated by the marked declines in underwater photosynthesis and respiration when the films were experimentally removed (Colmer and Pedersen, 2008; Pedersen et al., 2009; Pedersen and Colmer, 2012). In addition, leaves produced in air by terrestrial wetland species that did not form gas films

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when submerged (i.e. leaves of these species were not sufficiently hydrophobic), had lower rates of underwater photosynthesis than those that did form gas films (Colmer and Pedersen, 2008; Colmer et al., 2011). As one example, leaf segments of rice with dissolved CO\textsubscript{2} set as in a field pond and with underwater photosynthesis measured as described in Section “The Rotating Wheel Incubator,” showed rates four- to fivefold higher for leaf segments with intact gas films compared to those with the films experimentally removed (Winkel et al., 2013). Moreover, a field study using in situ monitoring of O\textsubscript{2} in rhizomes of Spartina anglica demonstrated the benefit of having leaf gas films to internal aeration during complete submergence, both during day and night tides (Winkel et al., 2011). Summing up, leaf gas films enhance underwater photosynthesis and internal aeration of some terrestrial wetland plants when submerged, with benefits also demonstrated to growth when submerged in controlled experiments (e.g., rice; Pedersen et al., 2009).

**UNDERWATER PHOTOSYNTHESIS – APPROACHES AND METHODS**

Conventional infrared gas analyzer (IRGA) systems following CO\textsubscript{2} exchange in air do not work under water, so dedicated measuring systems are required to quantify underwater net photosynthesis and dark respiration. DIC can be measured by injection of small aliquots of water into concentrated acid in a bubble chamber purged with gaseous N\textsubscript{2} carrying the released CO\textsubscript{2} into an IRGA (Vermaat and Sand-Jensen, 1987). However, photosynthesis measurements based on DIC determinations are thus based on discrete measurements at selected times and can be complicated because of the large and variable combined pool of DIC in water (See Underwater Photosynthesis in Submerged Aquatic plants and Recent Advances and The CO\textsubscript{2} Equilibria in Water). Indirect methods to track DIC changes can be based on continuous measurements of pH in solution (Maberly, 1996). The DIC technique to measure photosynthesis has potential errors if: (i) DIC is removed by external carbonate precipitation, (ii) internal DIC accumulates in tissues or colony gels, (iii) DIC dissolution of solid carbonates occurs, or (iv) DIC is released from internal pools (McConnaughey et al., 1994; Sand-Jensen et al., 2009). External measurements of pH to estimate DIC changes have the same potential errors as above and, moreover, also due to direct exchange of protons from tissues not always being closely coupled to DIC exchange. Therefore, most methods for studies of underwater net photosynthesis are based upon O\textsubscript{2} detection.

In contrast to gas exchange measurements of photosynthesis by leaves in air using open systems and CO\textsubscript{2} detection, underwater measurements commonly use closed systems and detection of O\textsubscript{2}. In addition to the rationale for O\textsubscript{2} detection described in the preceding paragraph, O\textsubscript{2} detection also enables measurements in waters of substantially different DIC concentrations (e.g., soft-water lakes up to 100 \(\mu\)mol L\(^{-1}\), ocean approximately 2000 \(\mu\)mol L\(^{-1}\) and hardwater lakes up to 10000 \(\mu\)mol L\(^{-1}\)). The drawback of closed systems is that these are non-steady-state (i.e. DIC declining and O\textsubscript{2} increasing with time). Use of open systems with O\textsubscript{2} detection is constrained by reliable continuous detection of differences in O\textsubscript{2} concentrations between incoming and outgoing solutions from an appropriate chamber.

Changes in O\textsubscript{2} concentration over time are straightforward to measure with Clark type amperometric electrodes or more recently by use of O\textsubscript{2} sensitive optodes. Oxygen partial pressure (pO\textsubscript{2}) or dissolved O\textsubscript{2} can be continuously monitored in water with an accuracy of 0.01 kPa or 0.2 \(\mu\)mol L\(^{-1}\) (Strickland and Parsons, 1972). Photosynthesis determined from changes in O\textsubscript{2} and DIC pools dissolved in the surrounding water requires that those are much greater than changes in such pools within the plant tissue (Sand-Jensen and Prahl, 1982). This is best achieved by having large incubation volumes relative to plant volumes. Alternatively, changes in tissue pools can be measured (Sand-Jensen et al., 2005) or be deduced by establishment of true steady state where tissue concentrations remain constant or quasi steady state where tissue concentrations changes proportionally to external concentrations (Sand-Jensen and Prahl, 1982). Measurements of underwater photosynthesis based upon O\textsubscript{2} evolution can include great error when plants with highly porous tissues (perhaps variable in volume and having much higher “solubility” of O\textsubscript{2} than water; See Medium and Tissue) are incubated in small chambers (Hartman and Brown, 1967; Richardson et al., 1984). On the other hand, measurements of underwater photosynthesis based upon changes in DIC can include extreme error when plant tissues (or colony matrices in the case of algae and cyanobacteria) hold very large pools of DIC that do not change in concert with those in the surrounding water. For example, DIC in the colony gel of Nostoc zetsersteldis continues to support photosynthesis after water pools have been exhausted, and in darkness respiratory CO\textsubscript{2} replenishes this internal pool before being released to the water (Sand-Jensen et al., 2009).

Measurements of radioactive labeling of the DIC pool with \(^{14}\text{C}\) and the use of pulse amplitude modulated (PAM) fluorometry are also methods to measure photosynthetic performance under water; these technique are beyond the focus of the present paper so readers are referred to e.g., Adams et al. (1978) or Kemp et al. (1986) for methods on \(^{14}\text{C}\) and to Silva et al. (2009) or Suggett et al. (2011) and chapters therein for PAM approaches.

**THE CO\textsubscript{2} EQUILIBRIA IN WATER**

Understanding the chemistry of dissolved DIC and the proportional changes in its three constituents (CO\textsubscript{2}, HCO\textsubscript{3}\textsuperscript{−} and CO\textsubscript{3}\textsuperscript{2−}) depending on ionic strength, temperature, and primarily pH (Mackereth et al., 1978) is essential because it determines the availability of the preferred CO\textsubscript{2} source and the supplementary HCO\textsubscript{3}\textsuperscript{−} source for underwater net photosynthesis. When CO\textsubscript{2} dissolves in water, the following equilibriums is established:

\[
\text{CO}_2(aq) + H_2O \leftrightarrow (H_2CO_3) \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+
\]

(4)

CO\textsubscript{2}’s reaction with water (H\textsubscript{2}O) forming carbonic acid (H\textsubscript{2}CO\textsubscript{3}) is a time dependent process which in some organisms is catalyzed by the enzyme carbonic anhydrase. H\textsubscript{2}CO\textsubscript{3} can dissociate immediately into a proton (H\textsuperscript{+}) and bicarbonate (HCO\textsubscript{3}\textsuperscript{−}) so the dissolution of CO\textsubscript{2} into water causes pH to drop. At high pH, HCO\textsubscript{3}\textsuperscript{−} can further dissociate into a second H\textsuperscript{+} and carbonate (CO\textsubscript{3}\textsuperscript{2−}). The relative distribution of the three main inorganic carbon species with pH is shown (Figure 5). The pK\textsubscript{a1} is 6.532 and
OH \text{above 9, OH}^− \text{phosphate, and with some contribution by borate in seawater. At pH systems with insignificant contribution from silicate and phosphate, carbon species that can be used as an additional carbon source, synthesis than HCO}_3^− \text{under well defined light and temperature conditions. O}_2 \text{ produced during incubation is measured by an electrode/optode and under water net photosynthesis can be calculated based on e.g., leaf area, fresh mass, dry mass, and/or chlorophyll. Alternatively, consumption of DIC can be used as a photosynthetic measure. Incubation in darkness provides data on dark respiration.}

**Medium and tissue**

The choice of medium is basically between an artificial medium with a well defined ion and gas composition or ambient water with the ion and gas composition of natural habitats (essential chemical parameters such as pH, DIC, and alkalinity should be characterized). An example of an artificial medium is the Smart and Barko (1985) general purpose culture medium. This medium contains (mmol L\(^{-1}\)) 0.62 Ca\(^{2+}\), 0.28 Mg\(^{2+}\), 0.28 SO\(_4^{2−}\) and 1.24 Cl\(^{−}\) and KHCO\(_3\) (sometimes mixed with NaHCO\(_3\)) is used to generate the required DIC. HCl, NaOH (or KOH), atmospheric air or gas mixtures of known pCO\(_2\) can be used to adjust pH to a required value based on the desired amount of free CO\(_2\). Since all incubations are short term, there are no micro nutrients or vitamins in this medium. Some studies have also used submergence solutions or “ambient” water from streams or lakes in order to establish a rate of photosynthesis under specific conditions (Sand-Jensen et al., 1992; Nielsen, 1993; Sand-Jensen and Frost-Christensen, 1998) and these can also be adjusted to predefined pH, CO\(_2\) and/or O\(_2\) levels. Any production of O\(_2\) by microalgae or consumption by microbial organisms in ambient water is accounted for in the blanks; micro-filtration of water is commonly used to remove background microflora.

Photosynthesis, as previously demonstrated for rice (Setter et al., 1989) and the aquatic pteridophyte, *Isoetes australis*, (Pedersen et al., 2011), during incubation is a potential issue as the
| pH   | DIC (mmol L⁻¹) | CO₂ (mmol L⁻¹) | HCO⁻³ (mmol L⁻¹) | CO₂⁻³ (mmol L⁻¹) | OH⁻ (mmol L⁻¹) |
|------|---------------|----------------|------------------|------------------|---------------|
| 6.00 | 6.5073        | 4.5074         | 1.9998           | 0.0001           | 0.0000        |
| 6.05 | 6.0170        | 4.0172         | 1.9997           | 0.0001           | 0.0000        |
| 6.10 | 5.5801        | 3.5803         | 1.9997           | 0.0001           | 0.0000        |
| 6.15 | 5.1907        | 3.1909         | 1.9997           | 0.0002           | 0.0000        |
| 6.20 | 4.8436        | 2.8438         | 1.9996           | 0.0002           | 0.0000        |
| 6.25 | 4.5343        | 2.5345         | 1.9996           | 0.0002           | 0.0000        |
| 6.30 | 4.2586        | 2.2588         | 1.9995           | 0.0002           | 0.0000        |
| 6.35 | 4.0128        | 2.0131         | 1.9995           | 0.0002           | 0.0000        |
| 6.40 | 3.7938        | 1.7941         | 1.9994           | 0.0003           | 0.0000        |
| 6.45 | 3.5986        | 1.5990         | 1.9994           | 0.0003           | 0.0000        |
| 6.50 | 3.4246        | 1.4250         | 1.9993           | 0.0004           | 0.0000        |
| 6.55 | 3.2696        | 1.2700         | 1.9992           | 0.0004           | 0.0000        |
| 6.60 | 3.1313        | 1.1318         | 1.9991           | 0.0004           | 0.0000        |
| 6.65 | 3.0082        | 1.0087         | 1.9990           | 0.0005           | 0.0000        |
| 6.70 | 2.8983        | 0.8989         | 1.9989           | 0.0006           | 0.0000        |
| 6.75 | 2.8004        | 0.8011         | 1.9987           | 0.0006           | 0.0000        |
| 6.80 | 2.7132        | 0.7139         | 1.9985           | 0.0007           | 0.0000        |
| 6.85 | 2.6354        | 0.6362         | 1.9984           | 0.0008           | 0.0001        |
| 6.90 | 2.5661        | 0.5670         | 1.9982           | 0.0009           | 0.0001        |
| 6.95 | 2.5042        | 0.5053         | 1.9979           | 0.0010           | 0.0001        |
| 7.00 | 2.4491        | 0.4503         | 1.9977           | 0.0011           | 0.0001        |
| 7.05 | 2.3999        | 0.4013         | 1.9974           | 0.0012           | 0.0001        |
| 7.10 | 2.3561        | 0.3576         | 1.9971           | 0.0014           | 0.0001        |
| 7.15 | 2.3169        | 0.3186         | 1.9968           | 0.0016           | 0.0001        |
| 7.20 | 2.2820        | 0.2839         | 1.9964           | 0.0018           | 0.0001        |
| 7.25 | 2.2509        | 0.2530         | 1.9959           | 0.0020           | 0.0001        |
| 7.30 | 2.2230        | 0.2254         | 1.9954           | 0.0022           | 0.0001        |
| 7.35 | 2.1982        | 0.2006         | 1.9949           | 0.0025           | 0.0002        |
| 7.40 | 2.1780        | 0.1789         | 1.9942           | 0.0028           | 0.0002        |
| 7.45 | 2.1561        | 0.1584         | 1.9935           | 0.0031           | 0.0002        |
| 7.50 | 2.1383        | 0.1420         | 1.9927           | 0.0035           | 0.0002        |
| 7.55 | 2.1223        | 0.1265         | 1.9919           | 0.0039           | 0.0003        |
| 7.60 | 2.1080        | 0.1127         | 1.9909           | 0.0044           | 0.0003        |
| 7.65 | 2.0951        | 0.1004         | 1.9898           | 0.0050           | 0.0003        |
| 7.70 | 2.0835        | 0.0894         | 1.9885           | 0.0056           | 0.0004        |
| 7.75 | 2.0730        | 0.0796         | 1.9871           | 0.0062           | 0.0004        |
| 7.80 | 2.0635        | 0.0709         | 1.9856           | 0.0070           | 0.0005        |
| 7.85 | 2.0548        | 0.0632         | 1.9838           | 0.0078           | 0.0005        |
| 7.90 | 2.0469        | 0.0562         | 1.9819           | 0.0088           | 0.0006        |
| 7.95 | 2.0396        | 0.0501         | 1.9797           | 0.0098           | 0.0007        |
| 8.00 | 2.0328        | 0.0446         | 1.9772           | 0.0100           | 0.0007        |
| 8.05 | 2.0265        | 0.0397         | 1.9745           | 0.0123           | 0.0008        |
| 8.10 | 2.0205        | 0.0353         | 1.9714           | 0.0138           | 0.0009        |
| 8.15 | 2.0149        | 0.0314         | 1.9680           | 0.0155           | 0.0010        |
| 8.20 | 2.0094        | 0.0279         | 1.9641           | 0.0173           | 0.0012        |
| 8.25 | 2.0041        | 0.0248         | 1.9596           | 0.0194           | 0.0013        |
| 8.30 | 1.9989        | 0.0221         | 1.9550           | 0.0217           | 0.0015        |
| 8.35 | 1.9937        | 0.0196         | 1.9497           | 0.0243           | 0.0017        |
| 8.40 | 1.9884        | 0.0174         | 1.9437           | 0.0272           | 0.0019        |
| 8.45 | 1.9830        | 0.0155         | 1.9371           | 0.0304           | 0.0021        |
| 8.50 | 1.9774        | 0.0138         | 1.9297           | 0.0340           | 0.0023        |
Table 3 | Continued

| pH  | DIC (mmol L$^{-1}$) | CO$_2$ (mmol L$^{-1}$) | HCO$_3^-$ (mmol L$^{-1}$) | CO$_3^{2-}$ (mmol L$^{-1}$) | OH$^-$ (mmol L$^{-1}$) |
|-----|--------------------|-------------------------|---------------------------|---------------------------|-------------------------|
| 8.55| 1.9716             | 0.0122                  | 1.9214                    | 0.0380                    | 0.0026                  |
| 8.60| 1.9655             | 0.0108                  | 1.9122                    | 0.0424                    | 0.0029                  |
| 8.65| 1.9590             | 0.0096                  | 1.9020                    | 0.0474                    | 0.0033                  |
| 8.70| 1.9520             | 0.0085                  | 1.8907                    | 0.0528                    | 0.0037                  |
| 8.75| 1.9445             | 0.0075                  | 1.8781                    | 0.0589                    | 0.0041                  |
| 8.80| 1.9364             | 0.0067                  | 1.8642                    | 0.0656                    | 0.0047                  |
| 8.85| 1.9277             | 0.0059                  | 1.8489                    | 0.0729                    | 0.0052                  |
| 8.90| 1.9182             | 0.0052                  | 1.8319                    | 0.0811                    | 0.0059                  |
| 8.95| 1.9079             | 0.0046                  | 1.8133                    | 0.0901                    | 0.0066                  |
| 9.00| 1.8967             | 0.0040                  | 1.7928                    | 0.0999                    | 0.0074                  |
| 9.05| 1.8846             | 0.0036                  | 1.7703                    | 0.1107                    | 0.0083                  |
| 9.10| 1.8714             | 0.0031                  | 1.7457                    | 0.1225                    | 0.0093                  |
| 9.15| 1.8570             | 0.0027                  | 1.7189                    | 0.1353                    | 0.0104                  |
| 9.20| 1.8415             | 0.0024                  | 1.6898                    | 0.1493                    | 0.0117                  |
| 9.25| 1.8246             | 0.0021                  | 1.6582                    | 0.1643                    | 0.0131                  |
| 9.30| 1.8065             | 0.0018                  | 1.6241                    | 0.1806                    | 0.0147                  |
| 9.35| 1.7870             | 0.0016                  | 1.5874                    | 0.1981                    | 0.0165                  |
| 9.40| 1.7661             | 0.0014                  | 1.5480                    | 0.2167                    | 0.0185                  |
| 9.45| 1.7438             | 0.0012                  | 1.5061                    | 0.2366                    | 0.0208                  |
| 9.50| 1.7201             | 0.0010                  | 1.4615                    | 0.2576                    | 0.0233                  |
| 9.55| 1.6950             | 0.0009                  | 1.4144                    | 0.2797                    | 0.0262                  |
| 9.60| 1.6686             | 0.0008                  | 1.3649                    | 0.3028                    | 0.0294                  |
| 9.65| 1.6408             | 0.0007                  | 1.3132                    | 0.3269                    | 0.0329                  |
| 9.70| 1.6118             | 0.0006                  | 1.2594                    | 0.3518                    | 0.0370                  |
| 9.75| 1.5817             | 0.0005                  | 1.2039                    | 0.3773                    | 0.0415                  |
| 9.80| 1.5506             | 0.0004                  | 1.1469                    | 0.4033                    | 0.0465                  |
| 9.85| 1.5186             | 0.0003                  | 1.0887                    | 0.4295                    | 0.0522                  |
| 9.90| 1.4858             | 0.0003                  | 1.0297                    | 0.4559                    | 0.0586                  |
| 9.95| 1.4525             | 0.0002                  | 0.9703                    | 0.4820                    | 0.0657                  |
| 10.00| 1.4188             | 0.0002                  | 0.9109                    | 0.5077                    | 0.0738                  |
| 10.05| 1.3847             | 0.0002                  | 0.8519                    | 0.5327                    | 0.0828                  |
| 10.10| 1.3506             | 0.0001                  | 0.7936                    | 0.5668                    | 0.0929                  |
| 10.15| 1.3162             | 0.0001                  | 0.7364                    | 0.5797                    | 0.1042                  |
| 10.20| 1.2820             | 0.0001                  | 0.6807                    | 0.6012                    | 0.1169                  |
| 10.25| 1.2478             | 0.0001                  | 0.6267                    | 0.6211                    | 0.1312                  |
| 10.30| 1.2138             | 0.0001                  | 0.5747                    | 0.6391                    | 0.1472                  |
| 10.35| 1.1800             | 0.0001                  | 0.5249                    | 0.6550                    | 0.1651                  |
| 10.40| 1.1462             | 0.0000                  | 0.4776                    | 0.6686                    | 0.1853                  |
| 10.45| 1.1124             | 0.0000                  | 0.4327                    | 0.6797                    | 0.2079                  |
| 10.50| 1.0786             | 0.0000                  | 0.3905                    | 0.6881                    | 0.2333                  |
| 10.55| 1.0446             | 0.0000                  | 0.3508                    | 0.6937                    | 0.2617                  |
| 10.60| 1.0101             | 0.0000                  | 0.3138                    | 0.6963                    | 0.2937                  |
| 10.65| 0.9750             | 0.0000                  | 0.2794                    | 0.6956                    | 0.3295                  |
| 10.70| 0.9389             | 0.0000                  | 0.2475                    | 0.6914                    | 0.3697                  |
| 10.75| 0.9017             | 0.0000                  | 0.2181                    | 0.6835                    | 0.4148                  |
| 10.80| 0.8628             | 0.0000                  | 0.1910                    | 0.6718                    | 0.4654                  |
| 10.85| 0.8220             | 0.0000                  | 0.1662                    | 0.6558                    | 0.5222                  |
| 10.90| 0.7828             | 0.0000                  | 0.1435                    | 0.6353                    | 0.5859                  |
| 10.95| 0.7327             | 0.0000                  | 0.1228                    | 0.6099                    | 0.6574                  |
| 11.00| 0.6831             | 0.0000                  | 0.1039                    | 0.5792                    | 0.7377                  |

Calculated from Mackereth et al. (1978) and Gutz (2012). See Figure 5 for the relative distribution between CO$_2$, HCO$_3^-$ and CO$_3^{2-}$ versus pH. The yellow highlight refers to Example 1, Section “Medium and Tissue.”
evolved O₂ is trapped in solution of the closed glass vial. The risk of photorespiration is increased during experiments at high temperature (Long, 1991) and with very low DIC and CO₂ concentrations leading to low ratios of CO₂ to O₂ at the site of Rubisco (Maberly and Spence, 1989; Sand-Jensen and Frost-Christensen, 1999). Therefore, the starting partial pressure of O₂ (pO₂) should be brought down to approximately 50% of air equilibrium, i.e., 10 kPa. This is sufficient to address the issue of photorespiration (provided that incubation do not last long periods so that O₂ produced increases above air equilibrium) and at the same time there is still enough O₂ in the medium to prevent tissue anoxia before photosynthesis starts producing O₂ (Colmer and Pedersen, 2008). In practice, equal volumes of medium (including all ions) are bubbled with either air or N₂. After mixing the two solutions, the pO₂ will be approximately 10 kPa and HCO₃⁻ can be added to the medium and pH adjusted accordingly to achieve the desired amount of free CO₂ (see example below).

In some situations, an organic buffer may be used to maintain a constant pH in the medium during incubation. In practice, however, HCO₃⁻ is a natural and often sufficient buffer in itself and we do not recommend using buffers if the CA is above 1 mmol L⁻¹ as HCO₃⁻ would be sufficient to buffer against large pH fluctuations during incubation (Sand-Jensen et al., 1992; Colmer and Pedersen, 2008). Moreover, organic buffers can also modify membrane porters and pH at plant surfaces modifying HCO₃⁻ use and influx/efflux of CO₂ (Price and Badger, 1985; Larsson and Axelson, 1999; Moulin et al., 2011). pH of the medium should be measured in a sample taken of the initial solution and then also in vials after incubations. With the ongoing advancement of optodes, pH may even be measured without opening the vials if applying pH sensitive microdots (See “O₂ Measurements” for description of O₂ sensitive microdots). If additional buffering is required, i.e. pH measurements after incubation show unacceptable drift in pH, then MES or TES buffers may be used, e.g., at a concentration of 5 mmol L⁻¹ (Pedersen et al., 2009, 2010), though the possible influence of these buffers on HCO₃⁻ use must be kept in mind.

The vials (10–100 mL glass vials with ground glass stoppers) are filled with medium using a siphon. By siphoning the medium into the bottom of each vial, exchange of O₂ and particularly CO₂ with the atmosphere is minimized; prepare sufficient medium to flush the vials at least twice the volume, and fill the vials completely. An air bubble can hold 36-fold more O₂ as the same volume of deionized (DI) water at 25°C, so bubbles in the vials introduce significant error to the net photosynthesis measurements. A set of small tissue samples are preferred to prevent self shading and to promote good mixing in the vials so that tissues are well exposed to light and chemicals during incubation.

Example 1: Preparation of artificial floodwater with CA of 2.0 mmol L⁻¹ and 200 μmol free CO₂ L⁻¹. Prepare a solution of DI water containing Ca²⁺, Mg²⁺, SO₄²⁻, and Cl⁻ at the concentrations described above. Divide the solution into two containers and bubble one half of the solution with air and the other half with N₂ for 20 min and then mix the two solutions. Add the required amount of DIC (Table 3, highlighted in yellow for this example) which is 2.2 mmol L⁻¹. Add the DIC in the form of KHCO₃, NaHCO₃ or a mixture, and acidify the solution to pH 7.35 using HCl. This results in a solution with a CA of 2 mmol L⁻¹ (in mmol L⁻¹: 1.995 HCO₃⁻ + 0.002 CO₂⁻) and 200 μmol L⁻¹ CO₂ (Table 3).

**Incubator with light and temperature control**

The incubator provides constant temperature and mixing throughout the incubation. It consists of a vertically rotating wheel where glass bottles or vials can be clipped on facing the light source. The wheel rotates at about 10 rpm in a tank with temperature controlled water and a transparent glass or Perspex wall for illumination at various irradiances (Figure 1C).

The rotating wheel incubator was originally invented for photosynthesis measurements in phytoplankton (Steemann Nielsen, 1952) and the typical light source in commercially available models consists of a rack of fluorescent tubes. However, it is hard to achieve PAR levels much above 500 μmol photons m⁻² s⁻¹ with fluorescent light so high pressure metal halide lamps (mercury or sodium) or light emitting plasma lamps are required to provide the levels of light needed to light saturate net photosynthesis by leaves of many terrestrial species and some macroalgae with thick thalli.

**O₂ measurements**

The O₂ produced or consumed during incubation can be measured directly in the glass vials using O₂ electrodes or optodes. In the absence of good electrodes or optodes, the Winkler titration can also be applied; see Strickland and Parsons (1972) for details. Contemporary methods for O₂ measurements in water involve either Clark type electrodes or optodes. A Clark type O₂ electrode measures pO₂ as molecular O₂ transverses a membrane before the electrochemical reaction on the cathode results in a current which is linearly proportional to the pO₂ of the medium. Since the electrode consumes O₂, a conventional large O₂ electrode is quite
After measuring O$_2$ base while a fitted pH electrode allows calculation of the exact amount of free CO$_2$ chamber with internal mixing and possessing injection ports and

**Principle:** a leaf or algal thalli sample is incubated in a closed chamber with light and temperature control (Klimant et al., 1997). Alternatively, the fluorophore can be coated onto tiny plastic patches which (microdots) can be mounted directly in the medium where O$_2$ is to be measured; the microdot with the fluorophore is then illuminated from the outside through the transparent wall of the container. Molecular O$_2$ quenches the florescence so that the transmitted signal can be calibrated toward O$_2$ in the medium; the relationships between quenching and pO$_2$ is non-linear. Optodes do not consume O$_2$ and are thus completely insensitive to stirring. However, O$_2$ optodes can have higher temperature coefficients than Clark type microelectrodes and require even better temperature control during measurements (Kragh et al., 2008). On the other hand, optodes can be built into the individual glass vials (microdots glued onto the glass wall inside the vial) and the O$_2$ concentration can be measured in a non-destructive manner (Kragh et al., 2008). The great advance of this approach is that vials can remain sealed and be returned to the rotating wheel if a preliminary reading shows that longer incubation is required in order to obtain the necessary accuracy, or O$_2$ evolution can be followed over time to ensure *quasi* steady state measurements or to elucidate possible temporal patterns.

**Supporting measurements and calculations**

After measuring O$_2$ of each vial, the tissue must be processed according to standard procedures to establish the area, the fresh mass or dry mass, the chlorophyll concentration, or all of the above. The underwater net photosynthesis is calculated as the net O$_2$ evolution rate per unit tissue per unit time. In practice, the change in O$_2$ content in each vial (change in O$_2$ concentration multiplied by the volume of the vial; individual volumes of vials (i.e. minus the volume of the glass beads, etc.) must be established) divided by the incubation time and divided by the amount of tissue (i.e., mass, area or any of the other above mentioned parameters used to scale photosynthesis per sample unit). An example of a CO$_2$ response curve established with the technique described here in Section "The Rotating Wheel Incubator" is shown in Figure 6.

**THE CLOSED CHAMBER WITH INJECTION PORTS**

Principle: a leaf or algal thalli sample is incubated in a closed chamber with internal mixing and possessing injection ports and fitted with an electrode/optode that follows O$_2$ concentration. The amount of free CO$_2$ can be manipulated by injection of acid or base while a fitted pH electrode allows calculation of the exact CO$_2$ level. The approach enables production of a complete light or CO$_2$ response curve based on the same sample, and underwater net photosynthesis can be calculated based on e.g., leaf area, fresh mass, dry mass, and/or chlorophyll concentration. Incubation in darkness can provide data on dark respiration.

**Chamber with light and temperature control**

The chamber for measurements of underwater net photosynthesis enables measurements with light, temperature, and CO$_2$ manipulations in water, with monitoring of O$_2$ with time. Chambers are commercially available for underwater photosynthesis measurements on macro algae, phytoplankton, or isolated chloroplasts and these are made from glass, acrylic glass, or acetal. These chambers can also be custom built to match specific electrodes, light sources, and fitted with extra ports for temperature and PAR sensors and injection of acid/bases or inhibitors. The chamber must be made from a material the can be sterilized and also have at least one transparent side to enable illumination of the sample. The light source can be diode based (650 nm red diode) or “full spectrum” halogen light to simulate white sunlight. Pay attention to the fact that some lighting devices are unable to produce sufficient light to saturate the photosynthesis of some terrestrial leaves or thick macroalgae thalli. Illumination (even by means of fiber optics) produces heat, so cooling of the chamber by a water jacket is crucial.

A light sensor small enough to measure inside the chamber is also essential. The spherical PAR sensor US-SQS/L (Walz, Effeltrich, Germany) is of a size (Ø = 3.7 mm) that enables permanent installation in most chambers.

Finally, the issue of mixing must be addressed. The simplest solution is to use a glass coated stir bar (avoid Teflon coated stir bars as these can hold O$_2$ which is isolated from the sample with a coarse mesh to prevent contact with the tissue. It may be necessary
to fix the tissue in the swirling current; if the tissue rotates with the water current in the chamber, the DBL will be larger than if the tissue is fixed. The thicker DBL increases the apparent resistance to CO\textsubscript{2} uptake or O\textsubscript{2} escape.

**O\textsubscript{2} and pH measurements**

O\textsubscript{2} measurements in the closed chamber are similar to O\textsubscript{2} measurements in the vials described in Section "O\textsubscript{2} Measurements." An O\textsubscript{2} sensor (Clark type electrode or optode) is fixed in the chamber in one of the ports, or if an optode is used, a patch with fluorophore can be glued onto the interior wall. A pH electrode is fitted in a second port and the signals from both sensors are logged onto a computer with data acquisition software. Calibration of both O\textsubscript{2} and pH sensors should be performed in the chamber to avoid stirring-related artifacts to the calibrations. Remember to pay extra attention to temperature if using O\textsubscript{2} optodes. It may take a while for the temperature of the solution inside the chamber to equilibrate with that of the cooling jacket, and working in a constant temperature room or keeping solutions in a thermostated water bath will significantly reduce the time it takes before a temperature steady state is obtained; always measure temperature directly in the chambers. Temperature influences electrode or optode performance, solubility of gases, and metabolic rate of the tissues (see Section "O\textsubscript{2} Measurements"). After insertion of tissue and filling of the chamber with medium (see below), pH can be manipulated by injection of small amounts of acid or base through one of the injection ports. Fit a 27G needle in one of the injection ports and let it function as "over pressure valve" to prevent pressurization during injection of acid or base (or inhibitors); the needle may be left in the stopper during the experiment as diffusion of gases in water is too slow to result in experimental artifacts.

As described in Section "The Rotating Wheel Incubator" for incubations of tissues in closed vials on the wheel, substantial photorespiration can occur if O\textsubscript{2} is allowed to build up in the medium. Therefore, the susceptibility to photorespiration should initially be established for each tissue type. The linearity of O\textsubscript{2} production with increasing external pO\textsubscript{2} is easily tested the following way: a medium with total DIC of 5.0 mmol L\textsuperscript{-1} is prepared from KHCO\textsubscript{3} in a 5.0 mmol L\textsuperscript{-1} TES buffer adjusted to pH 8.00 and with a pO\textsubscript{2} of 10 kPa. The tissue is then allowed to photosynthesize up to a pO\textsubscript{2} of 30 kPa. Here, approximately 500 µmol O\textsubscript{2} has been produced from 500 µmol CO\textsubscript{2} and because of the TES buffer the pH has remained at 8.0. Although the DIC pool has declined to 4.5 mmol L\textsuperscript{-1}, free CO\textsubscript{2} has changed by only 10% from 110 to 100 µmol L\textsuperscript{-1}. If the O\textsubscript{2} evolution occurs linearly in this range, it means that the approximately threefold lower CO\textsubscript{2}:O\textsubscript{2} in the medium, with likely even greater changes in internal CO\textsubscript{2}:O\textsubscript{2}, has not increased photorespiration. If the curve exhibits a saturation tendency (i.e. declining rate of net O\textsubscript{2} production with increasing pO\textsubscript{2}), photorespiration has probably increased with increasing pO\textsubscript{2} in the chamber.

Medium and tissue may be prepared as described in Section "Medium and Tissue." However, as a CO\textsubscript{2} response curve in the closed photosynthesis chamber often involves conversion of HCO\textsubscript{3}\textsuperscript{-} into free CO\textsubscript{2} (dissolved), e.g., by manipulation of pH, enough HCO\textsubscript{3}\textsuperscript{-} must initially be present in the medium to produce the required levels of free CO\textsubscript{2}. Following injection of small amounts of acid or base to manipulate free CO\textsubscript{2}, the rate of net photosynthesis changes accordingly so that a new rate of net O\textsubscript{2} production (slope of dissolved O\textsubscript{2} with time) is established at each dissolved CO\textsubscript{2}. However, pH may also change slightly in the time interval because CO\textsubscript{2} is extracted from the system as it is fixed via photosynthesis (Eqs 1 and 4). Hence, for every rate of underwater net photosynthesis determined in a time interval, the mean CO\textsubscript{2} concentration must be calculated in order to present the CO\textsubscript{2} response curve of the tissue.

Example 2: average free CO\textsubscript{2} concentration in the pH range from 7.25 to 7.30 in a medium with total DIC of 2.0 mmol L\textsuperscript{-1}. According to Gutz (2012), CA of such a solution at pH 7.25 would be 1.77 mmol L\textsuperscript{-1} having 223 µmol CO\textsubscript{2} L\textsuperscript{-1}; at pH 7.30 CA would be 1.80 mmol L\textsuperscript{-1} and have 203 µmol CO\textsubscript{2} L\textsuperscript{-1}. Consequently, the average free CO\textsubscript{2} concentration in the pH range was 213 µmol CO\textsubscript{2} L\textsuperscript{-1}.

After each experiment, the incubated tissue must be characterized to enable calculation of underwater net photosynthesis rates; the supporting measurements are as those described in section "Supporting Measurements and Calculations."

**PH DRIFT APPROACH TO ESTABLISH CO\textsubscript{2} COMPENSATION POINTS**

Principle: Leaf or algal thalli samples are incubated in glass vials for 16–18 h where after pH and CA or DIC are measured. CO\textsubscript{2} compensation points and carbon extraction capacity of tissues can be calculated. The method is also used as a diagnostic test for bicarbonate (HCO\textsubscript{3}\textsuperscript{-}) use in underwater photosynthesis.

These long term incubations are used to test how far net photosynthesis of a given plant sample at saturating light can extract DIC, i.e. to deplete CO\textsubscript{2} and HCO\textsubscript{3}\textsuperscript{-} and drive up pH. Because the objective is to determine the ultimate DIC extraction capacity and maximum upper pH in a standardized way, all incubation vials are prepared to have an equal standard DIC concentration (usually 1–2 mmol L\textsuperscript{-1} for alkaline waters and 0.1–0.3 mmol L\textsuperscript{-1} for softwaters) and a pH, CO\textsubscript{2}, and O\textsubscript{2} concentration corresponding to air equilibrium (Sand-Jensen et al., 1992, 2009). Artificial media and natural waters can be applied. However, to minimize O\textsubscript{2} build up and the risk of photorespiration during extended incubation the initial O\textsubscript{2} can be reduced to 20–50% of air equilibrium. To ensure the maximum possible DIC depletion, the amount of plant material is typically three times larger than in the incubations described in Sections "The rotating Wheel Incubator" and "The Closed Chamber with Injection Ports" though it must still be able to move freely in the vials to ensure adequate mixing.

The initial and final DIC and pH must be determined in order to calculate the DIC extraction capacity during incubation and the CO\textsubscript{2} compensation point after incubation. Provided no internal pools of DIC and protons interfere with conditions in the water/medium and no precipitation or dissolution of carbonates takes place, DIC can be determined in the medium from CA, pH, temperature, and ionic strength; CA in turn can be determined by acidimetric titration (Stumm and Morgan, 1996). The risk of carbonate precipitation is small in artificial media of KHCO\textsubscript{3} and much larger in natural waters and artificial media where Ca(HCO\textsubscript{3})\textsubscript{2} dominates, the reason being that K\textsubscript{2}CO\textsubscript{3} is highly soluble and CaCO\textsubscript{3} is poorly soluble. Calcium carbonate precipitation is likely to take place in pH drift experiments where final
pH exceeds 10. Therefore, it is always recommended to directly measure DIC. This can be done by injecting of small water samples into concentrated acid in a bubble chamber purged with \( \text{N}_2 \) gas carrying the released \( \text{CO}_2 \) into an IRGA (Vermaat and Sand-Jensen, 1987). Water samples may need to be filtered (with no atmospheric contact) if minute \( \text{CaCO}_3 \) crystals have been formed in the external water of high pH. It is generally recommendable to determine (or check) \( \text{CO}_2 \) compensation points by depletion experiments in media of low initial DIC (<50 \( \mu \text{mol L}^{-1} \)) and low pH (<6.5) where the interference by \( \text{HCO}_3^- \) is low and \( \text{CaCO}_3 \) is not formed.

The pH drift technique has also been used to determine DIC consumption at intervals during the ongoing drift of pH upwards (Maberly and Spence, 1983; Spence and Maberly, 1985). DIC, pH, the proportion of carbon species and \( \text{O}_2 \) change during the time of incubation. Because all parameters may influence photosynthesis, and exchange with internal DIC and proton pools in the incubated tissue may interfere with calculations, we cannot recommend the procedure for determining rates of net photosynthesis considering the much more accurate and straightforward methods being available today (as described in this review).

**COMMUNITY PHOTOSYNTHESIS IN LARGE CHAMBERS**

Principle: Community photosynthesis is measured in large closed chambers with linear dimensions of 0.5–0.6 m, or larger, to minimize edge effects and make certain that natural changes of plant density, tissue capacity and irradiance through the canopy are maintained. Photosynthetic rates are measured by \( \text{O}_2 \) and DIC, as for phytoelements in small chambers (See The Closed Chamber with Injection Ports), but photosynthetic parameters and their dependence on DIC and temperature are markedly different for communities than phytoelements.

Submerged aquatic plants grow in communities of variable density where the spatial structure and self shading are prominent features (Sand-Jensen, 1989). Light limitation is substantial and the efficiency of photosynthesis at low light is therefore important (Binzer and Sand-Jensen, 2002a,b). The photosynthetic chamber needs to be large enough to include tall communities (Binzer et al., 2006; Middelboe et al., 2006). It is made of glass or transparent acrylic glass and viewed from above, the shape of photosynthetic chambers can be cylindrical, rectangular, or quadratic. The cylindrical shape can be advantageous because the surface area of side walls relative to chamber volume is smaller than in rectangular or quadratic chambers, and these two latter types may also have “dead corners” with stagnant waters. The light sources are high pressure metal halide lamps (mercury or sodium) or light emitting plasma lamps because only those provide a sufficiently high irradiance (>1000 \( \mu \text{mol photon m}^{-2} \text{s}^{-1} \)). The light sources must be placed more than 0.5 m above the photosynthetic chamber and the light path both above the chamber and around the chamber walls are surrounded by totally reflecting material to reduce the influence of distance with depth in the chamber both when plants are absent or present. Irradiance is measured with depth in the water and through canopies of different densities using a small spherical PAR sensor. To ensure statistically reliable determinations of vertical attenuation a series (e.g., 10) of measurements are performed at different positions (Middelboe et al., 2006).

Temperature, \( \text{O}_2 \), DIC, and pH are set and measured as described in Section “The Closed Chamber with Injection Ports,” while mixing is provided by large submersible pumps ensuring current velocities above 2 cm s\(^{-1}\). Temperature control is attained by direct cooling and warming of the water in the incubation chamber or by placing it in a larger temperature controlled holding tank. In the latter case some temperature variations (1–3°C) is difficult to avoid between light and darkness.

Algal communities for measurements can be collected attached to stones or established over a period of one or several years on artificial tiles of desired size set out in the field and later brought to the laboratory for measurements in the photosynthetic chamber (Binzer et al., 2006; Middelboe et al., 2006). Rooted submerged plants can be harvested from natural stands with the 3D structure kept intact when roots and rhizomes are interwoven. In other cases, individual plants are placed in a homogeneous pattern on the chamber bottom in small plastic bags surrounding the root system. Alternatively the individuals are tied to a frame on the chamber bottom. Plant density is determined as fresh mass, dry mass, or plant surface area normalized to bottom area. Leaf area indices (LAI) ranging from 1 to 12 are useful for comparisons among species. Vertical distribution of plant biomass and surface can be determined by cutting the plants sequentially in well defined strata starting at the top of the canopy.

The setup is suited to evaluate the influence on community photosynthesis by variable irradiance, temperature, DIC (including variable \( \text{CO}_2 \) and \( \text{HCO}_3^- \)), canopy density, and spatial structure (Sand-Jensen et al., 2007).

Community photosynthesis can also be determined over longer periods of time by employing the large chambers in an open mode. This allows for exchange of \( \text{O}_2 \) and \( \text{CO}_2 \) with the atmosphere to prevent that the chambers undergo too extensive accumulation and depletion in the water during several days of alternating light or dark periods. For calculation of photosynthesis and respiration, exchange rates between air and water must be determined. The flux (\( F_{\text{exch}} \), mol m\(^{-2} \) s\(^{-1} \)) between water and air for \( \text{O}_2 \) is given by the equation:

\[
F_{\text{exch}} = K (C_{\text{act}} - C_{\text{equ}})
\]  

where \( K \) is the exchange coefficient (piston velocity, ms\(^{-1} \)), \( C_{\text{act}} \) is the actual and \( C_{\text{equ}} \) is the equilibrium concentration of \( \text{O}_2 \) (mol m\(^{-3} \)) in water at the actual temperature (Stach et al., 2012b). Piston velocity is controlled by surface turbulence and can, therefore, be considered a constant for a given mixing regime determined by the strength and location of the pumps and the dampening influence of the plant community. Thus, \( K \) must be directly measured for a given plant density and mixing regime. This is best done in the dark, where only dark respiration (mol m\(^{-2} \) s\(^{-1} \)) takes place, by modifying \( C_{\text{act}} \) to for example 10 or 30 kPa and measuring the total \( \text{O}_2 \) flux (\( F \)) over time as a result of respiration and exchange with the atmosphere above from time changes in \( \text{O}_2 \) concentrations in the water:

\[
F = R + K(C_{\text{act}} - C_{\text{equ}})
\]
From 30 kPa the actual pO\(_2\) will first rapidly decline as a combined result of respiration and loss to the atmosphere and gradually decline less rapidly as pO\(_2\) approaches equilibrium with the atmosphere and respiration alone drives pO\(_2\) further downwards. Calculations of pO\(_2\) changes over time in relation to differences in the pO\(_2\) gradient between water and air produces a straight line (Eq. 8) permitting calculation of R and K assuming that they remain constant for a given mixing intensity and plant density.

Community measurements operated in an open mode have the main advantage for future application that fluxes of O\(_2\), DIC, Ca\(^{2+}\), H\(^+\), and nutrient ions (NH\(_4^+\), NO\(_3^-\), and PO\(_4^{3-}\)) can be determined during repeated diel light dark cycles for several weeks while the submerged plants may also grow. Combined field measurements have been operated in open chambers and mesocosms under a strict mixing regime under natural temperature and light conditions both for phytoplankton (e.g., Markager and Sand-Jensen, 1989), submerged aquatic plants (e.g., Liboriussen et al., 2005), and flooded terrestrial plants (e.g., Setter et al., 1988).

**The Open Natural System**

Principle: Natural ecosystems dominated by submerged aquatic plants have free undisturbed gas exchange with the atmosphere and input/output of water. Determination of ecosystem metabolism by open water measurements requires accurate calculations of atmospheric exchange of O\(_2\) and CO\(_2\). The main advantages of the ecosystem approach is that environmental conditions and processes are natural and temporal patterns can be followed over months or years, while allowing plant density and acclimation to gradients in light, DIC, and other environmental variables to develop.

Photosynthesis of submerged aquatic plants derived from analysis of ecosystems can only be determined when rooted plants or macroalgae are the main phototrophs responsible of more than 90% of ecosystem photosynthesis. Only then can the patterns obtained be referred to the metabolism of macrophytes accepting that a minor error (<10%) due to photosynthesis of microalgae may be present. The dominance of submerged aquatic plants can be realized in shallow plant rich ponds, lakes, streams, and coastal lagoons. Open water measurements are used to follow changes in O\(_2\), DIC, pH, temperature, and irradiance, and enable calculation of ecosystem net production, plant gross production, and community respiration assuming fully mixed conditions (Odum, 1956; Staehr et al., 2012a). Meteorological observations of wind direction, wind velocity, atmospheric pressure, etc., in standing waters and current velocity, water depth, slope, and bed roughness in flowing waters, can be used to estimate physical exchange coefficients of gases (i.e. piston velocities) and thus calculate fluxes between water and atmosphere using empirical models (Sand-Jensen and Staehr, 2011). Flow chambers, floating chambers, inert gases, and coverage of water surfaces by impermeable floating plastic can be used for direct determination of exchange coefficients which are critical in all determinations of ecosystem metabolism (Staehr et al., 2012a,b). Rooted plants with gas filled lacunae formation and release of gas bubbles can introduce error. Oxygen storage may delay establishment of steady state exchange of O\(_2\) following dark light switches by some 10–20 min for most rooted plants (Westlake, 1978) and loss of bubbles is negligible in swift flowing waters, while bubble release may account for 10% of net O\(_2\) release in slow flowing waters (Kragh et al., unpublished data).

The strength of these measurements is that they provide natural rates under fully realistic and undisturbed environmental conditions. They can reveal the coupling between O\(_2\) and carbon metabolism, the natural precipitation and dissolution of carbonates and the direct involvement of accumulation and release of acids in the photosynthetic process. Measurements have shown fast exchange rates of protons between macrophytes and water following diurnal light dark switches partly uncoupled from exchanges of DIC during photosynthesis and respiration; a phenomenon that is not unraveled in short term laboratory measurements with detached phototrodes (Kragh et al., unpublished data). Ecosystem measurements can also reveal how early summer growth in biomass and late summer senescence influence plant metabolism and how ongoing desication of ponds may suddenly stop photosynthesis and accelerate decomposition, while refilling may restart photosynthesis and growth (Christensen et al., 2013). Modeling approaches, as successfully used for canopy level understanding of terrestrial systems systems (Ainsworth and Long, 2005), should also be applied more widely in studies of aquatic systems (e.g., Binzer and Sand-Jensen, 2002a,b). All techniques for measuring and calculating ecosystem process are basically available (Staehr et al., 2012a) and awaits broad scale application.

**Outlook**

Studies of photosynthesis by aquatic and submerged wetland plants are few compared with research on photosynthesis in air, but underwater systems are attracting more attention. Light and CO\(_2\) availability under water are often low to submerged plants. Low CO\(_2\) together with impeded escape of O\(_2\) can result in high photorespiration as a component determining net photosynthesis. Focus studies of contrasting species and systems are required to develop our understanding of “models” since the environment under water is more complex than in air and there is a diversity of photosynthetic mechanisms (i.e. C\(_3\), C\(_4\), CAM, and bicarbonate use) in aquatic species.

The physical and chemical environments of overland floods are only poorly known and few data exist on light extinction and CO\(_2\) and O\(_2\) concentrations in floodwaters. Such data are crucial to design relevant laboratory experiments on submergence tolerance of terrestrial plants and to establish, for example, carbon budgets during submergence on leaf lamina as well as for whole plants. Also, studies on leaf acclimation of terrestrial plants to facilitate gas exchange and light utilization under water are also only in their infancy; these acclimations influence underwater photosynthesis as well as internal aeration of plant tissues during submergence.

Finally, a challenge also exists to assess the influence of light, inorganic carbon, and temperature on natural aquatic communities of variable density instead of only studying detached leaves in the scenarios of rising CO\(_2\) and temperature. Use of mathematical modeling, both at the leaf and community levels, will provide valuable additional understanding of underwater photosynthesis.
Improved knowledge of plant and environmental factors determining rate of underwater net photosynthesis at various scales (leaf-to-community) is essential for understanding aquatic plant ecophysiology, submergence tolerance of terrestrial plants, and productivity of the many aquatic and flood-plain ecosystems worldwide.

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REFERENCES

Adams, M. S., Guulizzi, P., and Adams, S. (1978). Relationship of dissolved inorganic carbon to macrophyte photosynthesis in some Italian lakes. Limnol. Oceanogr. 23, 912–919.

Ainsworth, E. A., and Long, S. P. (2005). A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165, 351–372.

Arens, K. (1933). Physiologisch polarisierter Massenaustrausch und Photosynthese bei submersen Wasserpflanzen. I. Planta 20, 621–658.

Armstrong, W. (1979). Aeration in aquatic macrophytes. J. Exp. Bot. 30, 1–11.

Bailey-Serres, J., Fukao, T., Ismail, A. M., Sack, L. A., and旱, S. P. (2005). A perspective on under-owed carbon in photosynthesis. New Phytol. 169, 918–926.

Brewer, C. A., and Smith, W. K. (1997). Patterns of leaf surface wetness for montane and subalpine plants. Plant Cell Environ. 20, 1–11.

Christensen, J. P. A., Sand-Jensen, K., and Steen, S. P. (2013). Fluctuating water levels control water chemistry and metabolism of a charophyte pond. Freshw. Biol. doi:10.1111/feb.12132

Cole, J. L., Caneva, E. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegel, R. G., et al. (2007). Pluming the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10, 172–185.

Colmer, T. D., and Pedersen, O. (2008). Underwater photosynthesis and respiration in leaves of submerged wetland plants: gas films improve CO2 and O2 exchange. New Phytol. 177, 918–926.

Colmer, T. D., and Voesenek, L. A. C. J. (2009). Floating tolerance: suites of plant traits in variable environments. Funct. Plant Biol. 36, 665–681.

Dickson, A. G. (1981). An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. Deep Sea Res. A 28, 609–623.

Duarte, C. M. (1991). Seagrass depth limits. Aquat. Bot. 40, 363–377.

Frost-Christensen, H., and Sand-Jensen, K. (1992). The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. Oecologia 91, 377–384.

Gundersen, J. K., Ramsing, N. B., and Glud, R. N. (1998). Predicting the signal of O2 microsensors from physical dimensions, temperature, salinity, and O2 concentration. Limnol. Oceanogr. 43, 1932–1937.

Gutz, I. G. R. (2012). CurTiPot – pH and Acid-base Titration Curves: Analysis and Simulation Software, Version 3.6.1 [Online]. Available at: http://www2.iq.usp.br/docente/gutz/Curtipot.html [accessed 16 Dec 2012].

Hartman, R. T., and Brown, D. L. (1967). Changes in internal atmosphere of submersed vascular hydrophytes in relation to photosynthesis. Ecology 48, 252–258.

Helder, R. J. (1985). Diffusion of inorganic carbon across an unstirred layer: a simplified quantitative approach. Plant Cell Environ. 8, 399–408.

Holmer, M., Pedersen, O., Krause-Jensen, D., Olesen, B., Petersen, M. H., Schopmeyer, S., et al. (2009). Sulfide intrusion in the tropical seagrasses Thalassia testudinum and Syringodium filiforme. Estuar. Coast. Shelf Sci. 85, 319–326.

Johnson, F. H., Eyre, H., and Stover, B. J. (1974). The Theory of Rate Processes in Biology and Medicine. New York: Wiley.

Kelly, M. G., Thysen, N., and Moeslund, B. (1983). Light and the annual variation of oxygen- and carbon-based measurements of productivity in a macrophyte-dominated river. Limnol. Oceanogr. 28, 503–515.

Kemp, W. M., Marlon, R. L., and Jones, T. W. (1986). Comparison of methods for measuring production by the submersed macrophyte Potamogeton perfoliatus L. Limnol. Oceanogr. 31, 1322–1334.

Kirk, I. T. O. (1994). Light and Photosynthesis in Aquatic Ecosystems. New York: Cambridge Univ Press.

Klimant, I., Kühl, M., Glud, R. N., and Holst, G. (1997). Optical measurement of oxygen and temperature in microscale: strategies and biological applications. Sens. Actuators B Chem. 39, 28–37.

Kragh, T., Søndergaard, M., and Christensen, B. I. (2008). Exposure to sunlight and phosphorus-limitation on bacterial degradation of coloured dissolved organic matter (CDOM) in freshwater. FEMS Microbiol. Ecol. 64, 230–239.

Lammers, H., Chapin, F. S. III, and Pons, T. L. (2008). Plant Physiological Ecology. Heidelberg: Springer-Verlag.

Larsson, C., and Axelsson, L. (1999). Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. J. Phycol. 26, 439–449.

Maberly, S. C. (1996). Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. Freshw. Biol. 35, 579–598.

Maberly, S. C., and Madsen, T. V. (2002). Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. Funct. Plant Biol. 29, 393–405.

Maberly, S. C., and Spence, D. H. N. (1983). Photosynthetic inorganic carbon use by freshwater plants. J. Ecol. 71, 705–724.

Maberly, S. C., and Spence, D. H. N. (1989). Photosynthesis and photosorption in freshwater organ- isms: amphibious plants. Aquat. Bot. 34, 267–286.

Mackereth, F. J. H., Heron, J., and Talling, J. F. (1978). Water Analysis: Some Revised Methods for Limnolo- gists. Cumbria: Freshwater Biological Association.

Mackill, D. J., Ismail, A. M., Singh, U. S., Lubious, A. V., and Paris, T. R. (2012). Development and Rapid Adoption of Submergence-Tolerant (Subel) Rice Varieties. San Diego: Academic Press. 299–352.

Madsen, T. V., and Sand-Jensen, K. (1993). Photosynthetic carbon assimilation in aquatic macrophytes. Aquat. Bot. 41, 5–40.

Madsen, T. V., Sand-Jensen, K., and Beer, S. (1993). Comparison of photosyn- thetic performance and carboxyla- tion capacity in a range of aquatic macrophytes of different growth forms. Aquat. Bot. 44, 373–384.

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Markager, S., and Sand-Jensen, K. (1989). Patterns of night-time respiration in a dense phytoplankton community under a natural light regime. J. Ecol. 77, 49–61.

McConnaughey, T. A. (1991). Calcification in Chara corallina: CO2 hydration reaction generates protons for bicarbonate assimilation. Limnol. Oceanogr. 36, 619–628.

McConnaughey, T. A., La Baugh, J. W., Rosenberry, D. O., Striegel, R. G., Reddy, M. M., Schuster, P. F., et al. (1994). Carbon budget for a groundwater-fed lake: calcification supports summer photosynthesis. Limnol. Oceanogr. 39, 1319–1332.

Middelboe, A. L., and Markager, S. (1997). Depth limits and minimum light requirements of freshwater macrophytes. Freshw. Biol. 37, 553–568.

Middelboe, A. L., Sand-Jensen, K., and Binzer, T. (2006). Highly predictable photosynthetic production in natural macroagal communities from incoming and absorbed light. Oecologia 150, 464–476.

Mommer, L., Lenssen, J. P. M., Huber, H., Visser, E. J. W., and De Kroon, H. (2006a). Ecophysiological determinants of plant performance under flooding; a comparative study of seven plant families. J. Ecol. 94, 1117–1129.

Mommer, L., Pons, T. L., and Visser, E. J. W. (2006b). Photosynthetic consequences of phenotypic plasticity in response to submergence: Rumex palustris as a case study. J. Exp. Bot. 57, 283–290.

Mommer, L., and Visser, E. J. W. (2005). Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. Ann. Bot. 96, 581–589.

Mommer, L., Wolters-Arts, M., Andersen, C., Visser, E. J. W., and Pedersen, O. (2007). Submergence-induced leaf acclimation in terrestrial species varying in flooding tolerance. New Phytol. 176, 337–345.

Moulin, P., Andria, R. J., Axelsson, L., and Mercado, J. M. (2011). Differentiation mechanisms of inorganic carbon acquisition in red macroalgae (Rhodophyta) revealed by the use of TRIS buffer. Aquat. Bot. 95, 31–38.

Nehnhaus, C., and Bartholw, W. (1997). Co2 utilization and distribution of water-repellent, self-cleaning plant surfaces. Ann. Bot. 79, 667–677.

Nielsen, S. L. (1993). A comparison of aerial and submersed photosynthesis in some Danish amphibious plants. Aquat. Bot. 45, 27–40.

Nielsen, S. L., and Sand-Jensen, K. (1989). Regulation of photosynthetic rates of submerged rooted macrophytes. Oecologia 81, 364–368.

Odum, H. T. (1956). Primary production in flowing waters. Limnol. Oceanogr. 1, 102–117.

Olesen, B., and Madsen, T. V. (2000). Growth and physiological acclimation to temperature and inorganic carbon availability by two submersed aquatic macrophyte species, Callitriche cophocarpa and Elodea canadensis. Punct. Ecol. 14, 253–260.

Odyke, B. N., and Walker, J. C. G. (1992). Return of the coral reef hypothesis: basin to shelf partitioning of CaCO3 and its effect on atmospheric CO2. Geology 20, 733–736.

Parolin, P. (2009). Submerged in darkness: adaptations to prolonged submergence by woody species of the Amazonian floodplains. Ann. Bot. 103, 339–376.

Parry, M. L., Cazzaniga, O. F., Palutikof, J. P., van der Linden, P. L., and Hansom, C. E. (eds) (2007). Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the Intergovernmental Panel on Climate Change (Cambridge: Cambridge University Press), 982.

Pedersen, O., and Colmer, T. D. (2012). Physical gills prevent drowning of many wetland insects, spiders and plants. J. Exp. Biol. 215, 705–709.

Pedersen, O., Malik, A. I., and Colmer, T. D. (2010). Submergence tolerance in Hordeum marinum: dissolved CO2 determines underwater photosynthesis and growth. Punct. Plant. Biol. 37, 524–531.

Pedersen, O., Pulido, C., Rich, S. M., and Colmer, T. D. (2011). In situ O2 dynamics in submerged Isoetes aus-tralis: varied leaf gas permeability influences underwater photosynthesis and internal O2, J. Exp. Bot. 62, 4691–4700.

Pedersen, O., Rich, S. M., and Colmer, T. D. (2009). Surviving floods: leaf gas films improve O2 and CO2 exchange, root aeration, and growth of completely submersed rice. Plant J. 58, 147–156.

Pedersen, O., Vos, H., and Colmer, T. D. (2006). Oxygen dynamics during submergence in the halophytic stem succulent Halosarcia pergranulata. Plant Cell Environ. 29, 1388–1399.

Pilon, J., and Santamaría, L. (2001). Seasonal acclimation in the photosynthetic and respiratory temperature responses of three submerged freshwater macrophyte species. New Phytol. 151, 659–670.

Price, G. D., and Badger, M. R. (1985). Inhibition by proton buffers of photosynthetic utilization of bicarbon- ate in Chara corallina. Aust. J. Plant Physiol. 12, 257–267.

Prins, H. B. A., Snell, J. F. H., Helder, R. I., and Zanstra, P. E. (1980). Photosynthetic HCO3− utilization and OH− excretion in aquatic angiosperms: light-induced pH changes at the leaf surface. Plant Physiol. 66, 818–822.

Raskin, I., and Kende, H. (1985). How does deep water rice solve its air-ation problem? Plant Physiol. 72, 447–454.

Raven, J. A., and Hurd, C. L. (2012). Ecophysiology of photosynthesis in macroalgae. Photosyn. Res. 113, 105–125.

Revsbech, N. P. (1987). An oxygen microelectrode with a guard cathode. Limnol. Oceanogr. 34, 474–478.

Revsbech, N. P., and Jorgensen, B. B. (1986). "Microeletrodes: their use in microbial ecology," in Advances in Microbial Ecology, ed. K. C. Marshall (New York: Plenum Press), 293–352.

Rich, S. M., Pedersen, O., Ludwig, M., and Colmer, T. D. (2013). Shoot atmospheric contact is of little importance to aeration of deeper portions of the wetland plant Mesioneetes browningii; submersed organs mainly acquire O2 from the water column or produce it endogenously in underwater photosynthesis. Plant Cell Environ. 36, 213–223.

Richardson, K., Griffiths, H., Reed, M. L., Raven, J. A., and Griffiths, N. M. (1984). Inorganic carbon assimilation in the Isoetid, Isoetes lacustris L. and Leloria dornmanniana L. Oecologia 61, 115–121.

Sand-Jensen, K. (1983). Photosynthetic carbon-sources of stream macrophytes, J. Exp. Bot. 34, 198–210.

Sand-Jensen, K. (1989). Environmental variables and their effect on photosynthesis of aquatic plant communities. Aquat. Bot. 34, 5–25.

Sand-Jensen, K., Bastrup-Spohr, L., Winckel, A., Moller, C. L., Borum, J., Brodersen, K. P., et al. (2010). Plant distribution patterns and adaptations in a limestone quarry on Oland. Svensk Botanisk Tidskrift 104, 23–31.

Sand-Jensen, K., Binzer, T., and Middelboe, A. L. (2007). Scaling of phenotypic plasticity sequences of phenotypic plasticity in many wetland insects, spiders and plants. Ann. Bot. 109, 302–312.

Sculthorpe, C. D. (1967). The Biology of Aquatic Vascular Plants. London: Edward Arnold Ltd.

Setter, T. L., Kupkanchakul, T., Waters, I., and Greenway, H. (1988). Evaluation of factors contributing to diurnal changes in O2 concentrations in freshwater of deepwater rice fields. New Phytol. 110, 151–162.

Setter, T. L., and Laurenț, E. V. (1996). The beneficial effect of reduced
elongation growth on submergence tolerance of rice. J. Exp. Bot. 47, 1551–1559.
Setter, T. L., Waters, L., Wallace, L., Bekhassan, P., and Greenway, H. (1989). Submergence of rice. I. Growth and photosynthetic response to CO2 enrichment of floodwater. Aust. J. Plant Physiol. 16, 251–263.
Siva, J., Sharon, Y., Santos, R., and Beer, S. (2009). Measuring seagrass photosynthesis: methods and applications. Aquatic Biol. 7, 127–141.
Smart, R., and Barko, J. (1985). Laboratory culture of submersed freshwater macrophytes on natural sediments. Aquat. Bot. 21, 251–263.
Smith, W. K., and McClean, T. (2011). Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications. Dordrecht: Springer.
Sparks, T. H. (1998). Effects of temperature and CO2 concentration on growth and allometry of replicate Populus tremuloides trees. New Phytol. 140, 2363–2367.
Winkel, A., Colmer, T. D., and Pedersen, O. (2011). Leaf gas films of Spartina anglica enhance rhizome and root oxygen during tidal submergence. Plant Cell Environ. 34, 2083–2092.

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