

Diffusive boundary layers do not limit the photosynthesis of the aquatic macrophyte *Vallisneria americana* at moderate flows and saturating light levels

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Abstract

Hydrodynamic models of mass transport assume that diffusive processes next to the surface limit transport and that there are no biological and chemical processes that control the supply and demand of the scalar. The validity of these assumptions was examined by measuring the momentum boundary layer (via particle image velocimetry) and the concentration boundary layer (via O₂ microsensors) over the leaves of *Vallisneria americana*. The O₂ flux (J_{obs}) was highest at $x = 2$ cm downstream from the leading edge of the leaf and was 1.8 to 1.4 times higher than J_{obs} measured at the trailing edge of the leaf at 0.5 cm s⁻¹ and 6.6 cm s⁻¹ mean velocity (U), respectively. The maximum J_{obs} was 0.44 ± 0.07 (mean \pm SE) vs. 0.50 ± 0.09 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 0.5 vs. 6.6 cm s⁻¹. Interestingly, the surface O₂ potential ($\Delta[\text{O}_2] = [\text{O}_2]_{\text{surface}} - [\text{O}_2]_{\text{bulk}}$) was also unimodal at the low velocity ($\Delta[\text{O}_2]_{\text{max}} = 36 \pm 5$ mmol m⁻³ at $x = 3$ cm) but was uniform at the higher velocity ($\Delta[\text{O}_2] = 9 \pm 0.7$ mmol m⁻³). An analysis of the time scale of nutrient diffusion (τ_D) vs. nutrient uptake (τ_{up}) through the measured diffusion boundary layer revealed that uptake was always the slower process (i.e., $\tau_D < \tau_{up}$; τ_D and τ_{up} increased with x and decreased with U). Under moderate water velocities and saturating irradiance, uptake rates rather than diffusive transport processes appear to control mass transfer rates regardless of the location on the leaf and the water velocity.

Many aquatic organisms rely on the fluid environment for the transport of scalar quantities (e.g., nutrients and metabolites). In the case of aquatic macrophytes, photosynthetic surfaces can serve as sinks (CO₂) or sources (O₂) of scalars that are physiological important. Scalar transport is governed by (1) fluid motion, which influences the rates of advection, and (2) the concentration gradient, which affects the rates of diffusion. The rates of mass transport to aquatic organisms can also be influenced by interactions between and among morphological features, fluid flow, and physiological processes (Nielsen et al. 2006; Nishihara and Ackerman 2006, 2007b).

Regardless of these observations, the basic model underlying the coupling of mass transport and physiological processes was suggested more than 30 yr ago when film theory (a solution to the advection-diffusion equation) was used to describe the transport of scalars to biological surfaces (Lieb and Stein 1972; Winne 1973). Film theory assumes that diffusive transport is the only significant process and that a homogeneous stagnant “film” exists adjacent to the surface. There are, however, violations to these assumptions, namely, that advection also contributes to mass transport (Levich 1962; Campbell and Hanratty 1983) and that scalar boundary layer thickness (i.e., “film thickness” or concentration boundary layer [CBL]) may not be uniform in the streamwise direction at biologically relevant spatial scales (Nishihara and Ackerman 2006, 2007b, 2008). Evidence also indicates that processes such as the conversion of HCO₃⁻ to CO₂ in the CBL of phytoplankton can enhance mass transport (Wolf-Gladrow and Riebesell 1997; Tortell et al. 2006). In other words,

processes unrelated to hydrodynamics may also contribute to the flux of scalar quantities to the surfaces of aquatic organisms.

Whereas a variety of alternative solutions to the advection-diffusion equation have been developed (Acrivos and Chambré 1957; Levich 1962; Libby and Liu 1966; e.g., surface renewal theory, Campbell and Hanratty 1983), film theory has remained the principal model used in studies of aquatic organisms (e.g., aquatic macrophytes: Wheeler 1980; Koch 1993; Larkum et al. 2003; aquatic animals: Jørgensen and Des Marais 1990; Gardella and Edmunds 2001).

Film theory seeks to estimate mass transfer rates through the application of theoretical and/or analytical models of the advection-diffusion equation (Hurd et al. 1996; Sanford and Crawford 2000). The two boundary conditions, which are incorporated to simplify the solution, neglect linear and nonlinear processes that can occur close to the surface (e.g., nutrient uptake kinetics) and that can vary in space and time. The first of these is the Dirichlet boundary condition, in which the concentration of the scalar (C) is constant at the water-surface interface (C_{surface}); the second is the Neumann boundary condition, in which there is a constant mass flux of the scalar to or from the surface (Bird et al. 2002). For the former, it is commonly assumed that $C_{\text{surface}} = 0$ (i.e., the “perfect sink” condition; Vogel 1994; Hurd et al. 1996; Larkum et al. 2003), which is valid when uptake rates (J) are mass transfer limited (i.e., unsaturated J). In the case of the latter, the saturation of uptake rates is usually assumed to be sufficient even though surface reactions (i.e., heterogeneous reactions) may indicate spatial heterogeneity of C_{surface} and mass flux (i.e., mixed boundary condition; Schlichting and Gersten 2000), and chemical reactions within the boundary layer (i.e., homogeneous reactions) are not considered (Wolf-Gladrow and Riebesell 1997). It is evident that mass transfer processes

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modeled by film theory require further empirical investigation.

A conceptual model of mass transport and mixed boundary conditions

Although analytical solutions coupling mass transport and nonlinear mixed boundary conditions do not exist (Gervais and Jensen 2006), the problem can be conceptualized as follows. Consider a leaf oriented parallel to a unidirectional flow field with a uniform velocity (U) distribution and nutrient concentration (C_{bulk}). For saturating kinetics (i.e., photosynthesis rates and nutrient uptake rates), the uptake rate (J) can be described as an asymptotic function

$$J = \frac{J_{max} C_{surface}}{K_m + C_{surface}}$$

of $C_{surface}$, where K_m is the concentration when the uptake is 50% of the maximum uptake rate (J_{max}) (Sanford and Crawford 2000). As water travels over the leaf, a momentum boundary layer (MBL), which contains the velocity gradient, forms because of the interaction between the fluid and the leaf surface (i.e., the no-slip condition). A CBL, which contains the concentration gradient, will develop if and only if the leaf acts as a sink for nutrients (e.g., dissolved inorganic carbon [DIC] and/or source of photosynthetic product (i.e., O_2)). Close to the leading edge of the leaf, $C_{surface} \approx C_{bulk}$, and the supply of C will be sufficient so that $J \approx J_{max}$, and hence a Neumann boundary condition occurs. However, as the leaf extracts nutrients from the overlying water, $C_{surface}$ will approach 0, and for a sufficiently large downstream distance (x), uptake can be mass transfer limited, and a Dirichlet boundary condition (i.e., “perfect sink”) can occur (i.e., $C_{surface} = 0$). Whether and how this conceptual model of a mixed boundary condition applies remains to be determined.

The purpose of this study is to investigate the fundamental assumptions of the film theory model of mass transport by examining the MBL and CBL over the flat blade-like leaves of the freshwater macrophyte *Vallisneria americana* Michx. Specifically, we will determine whether transport by diffusive processes limit the photosynthesis of aquatic macrophytes.

Methods

Determination of mass transfer—Physiological and mass transport processes can be determined from the O_2 profiles measured over photosynthesizing leaves of *V. americana* by fitting a hyperbolic tangent model to the profiles,

$$\theta = \alpha \tanh\left(\frac{\beta}{\alpha} z\right)$$

where

$$\theta = \frac{C_{surface} - C}{C_{surface} - C_{bulk}}$$

α and β are constants, and z is the height above the leaf (Nishihara and Ackerman 2007a). The O_2 flux (J_{obs}) can be determined as

$$J_{obs} = -D\Delta[O_2] \frac{d\theta}{dz}$$

where the molecular diffusivity of O_2 [D] at 24°C is $2.4 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ and $\Delta[O_2]$ is the O_2 potential (i.e., $C_{surface} - C_{bulk}$) by applying Fick’s first law at the surface of the leaf ($z = 0$). The mass transfer coefficient is

$$k_c = \frac{J_{obs}}{\Delta[O_2]}$$

and the local Sherwood number is

$$Sh_x = \frac{k_c x}{D}$$

which is the ratio of the advective to diffusive mass transfer (Nishihara and Ackerman 2007a).

Experimental setup—Leaves from the freshwater macrophyte *V. americana* were carefully selected to provide a uniform size and shape (7-cm-long lengths were cut from leaves that were 0.7 cm wide), with no undulations in the leaf or visible epiphytes. Experiments were conducted in a $10 \times 10 \times 100$ -cm recirculating flow chamber that provided well-behaved and well-characterized flow at the location of the leaf (Nishihara and Ackerman 2006). The chamber was filled with local well water and adjusted with distilled water to a pH of 7.49, $[HCO_3^-]$ of 460 mmol m^{-3} , and $[CO_2]$ of 17.1 mmol m^{-3} (Stumm and Morgan 1996). The water was maintained at 24°C and aerated to maintain O_2 and CO_2 saturation. Given that decreases in pH or $[HCO_3^-]$ were not detected during the course of an experiment (~1 h), the [DIC] was assumed to be constant (Nishihara and Ackerman 2006). Saturating rates of photosynthesis were achieved by supplying photosynthetically active radiation with a slide projector that was equipped with a quartz lamp ($156 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

Measurement of the scalar and velocity profiles and boundary layers— O_2 profiles (motorized OX25 microsensor; Unisense) were obtained over leaves that were attached to a wire stand (3 cm above flow-chamber floor) and placed 56 cm downstream from the flow straighteners in the flow chamber. A series of vertical profiles were taken at $x = 1, 2, 3, 4, 5, 6,$ and 7 cm downstream from the leading edge of each leaf at low and high free-stream velocities ($U = 0.5$ and 6.6 cm s^{-1} ; six leaves were examined at each velocity). Velocity profiles were obtained above leaves using a digital particle image velocimetry (PIV) system at the same U (Nishihara and Ackerman 2006). The nature of the MBL was assessed from the dimensionless velocity profiles (local u/U , where u is the downstream velocity) using both laminar and turbulent formulations (White 1999). Mass transfer rates (k_c) for laminar flow were determined by recognizing that $Sh_x = k_c x D^{-1}$ and $Sh_x = 0.336 Re_x^{0.5} Sc^{0.33}$ for laminar conditions (i.e., $k_c = 0.336 Re_x^{0.5} Sc^{0.33} x^{-1} D$), where Re_x is the local Reynolds number

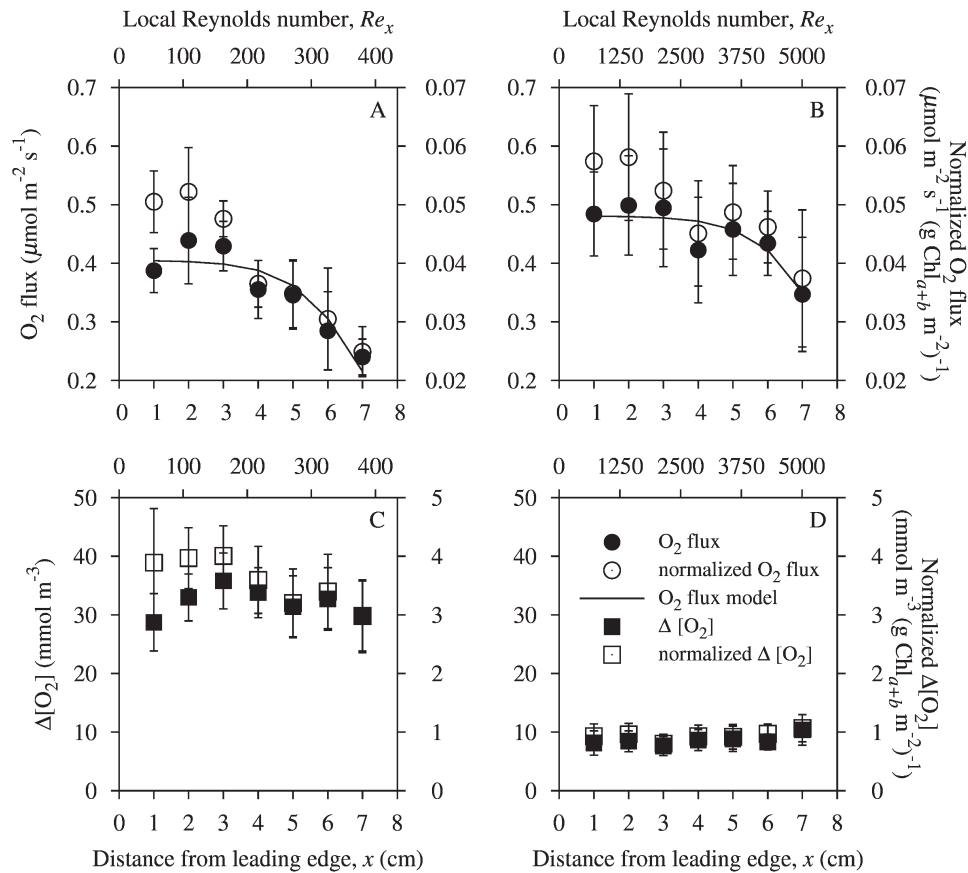


Fig. 1. The O_2 flux (J_{obs}) and chlorophyll $a + b$ (Chl_{a+b})–normalized O_2 flux determined for *Vallisneria americana* at (A) 0.5 and (B) 6.6 $cm\ s^{-1}$. The $[O_2]$ potential ($\Delta[O_2]$) and the Chl_{a+b} –normalized $\Delta[O_2]$ above leaves at (C) 0.5 and (D) 6.6 $cm\ s^{-1}$. Solid lines indicate an empirical model fit to J_{obs} (i.e., $J_{obs} = J_{max}' (1 - (1 + e^{-(x-\phi)})^{-1})$). Error bars indicate one standard error.

($Re_x = Ux/\nu$), Sc is the Schmidt number = ν/D , and ν is the kinematic viscosity of water, which at 24°C is $0.922 \times 10^{-6} m^2\ s^{-1}$ (Nishihara and Ackerman 2006). The raw velocity data were used to determine the shear velocity (u_*) through the law of the wall (e.g., Ackerman and Hoover 2001), which was needed for the turbulent formulation of k_c ($k_c = 0.1 u_* Sc^{-0.67}$). u_* was also needed to determine the thickness of the theoretical diffusive boundary layer (DBL) based on the diffusive sublayer (DSL) of the MBL ($\delta_{DSL} = 10[\nu/u_*]Sc^{-0.33}$) (Dade 1993). It is important to note that k_c so determined assumes a constant surface concentration or a constant flux boundary condition.

Chlorophyll determination—A $\sim 1\text{-cm}^2$ section of the leaf centered under each measurement location was used to determine the local chlorophyll $a + b$ concentration (Chl_{a+b}) via extraction in 90% acetone (Ackerman and Nishihara 2007a).

Data analysis—The O_2 flux (J_{obs}), local Sherwood number (Sh_x), the thickness of the CBL (δ_{CBL}), and the thickness of the DBL of the CBL (δ_{DBL}) were determined using existing mathematical and hydrodynamic models (Nishihara and Ackerman 2006, 2007a, 2008). The thickness of the MBL (δ_{MBL}) was determined as the height

where u is 99% of U (White 1999). Theoretical values of the oxygen potential ($\Delta[O_2]$), δ_{DSL} , and Sh_x were determined from flat plate theory (White 1999) using the measured values of u_* . A repeated-measures analysis of variance (rANOVA) was used to examine the effects of U and x on J_{obs} and $\Delta[O_2]$, and an analysis of covariance (ANCOVA) was used to examine the relationship between Sh_x and Re_x . J_{obs} and $\Delta[O_2]$ were normalized by the $[Chl_{a+b}]$ determined at each measurement location to minimize potential variations resulting from physiological differences (e.g., chlorophyll content due to age) along the length of the leaves.

Results

The O_2 flux (J_{obs}) was not uniform across the surface of *V. americana* leaves but was highest at $x = 2\text{ cm}$ downstream from the leading edge for both velocities (Fig. 1). At low velocity, J_{obs} increased from $0.39 \pm 0.04\ \mu mol\ m^{-2}\ s^{-1}$ (mean \pm SE) to $0.44 \pm 0.07\ \mu mol\ m^{-2}\ s^{-1}$ and then decreased monotonically to $0.25 \pm 0.03\ \mu mol\ m^{-2}\ s^{-1}$. At the high velocity, J_{obs} increased from $0.48 \pm 0.07\ \mu mol\ m^{-2}\ s^{-1}$ to $0.50 \pm 0.09\ \mu mol\ m^{-2}\ s^{-1}$ and then decreased to $0.35 \pm 0.10\ \mu mol\ m^{-2}\ s^{-1}$. rANOVA revealed that J_{obs} decreased

significantly with downstream distance (x) regardless of velocity ($F_{6,60} = 3.73$, $p < 0.01$); however, significant differences in J_{obs} were not found between velocities ($F_{1,10} = 3.08$, $p = 0.11$), nor was there a significant interaction between downstream distance and velocity ($F_{6,60} = 0.22$, $p = 0.097$). A similar analysis of the Chl_{a+b} -normalized J_{obs} confirmed these patterns (statistical results not provided; Fig. 1A,B).

The magnitude of the surface O_2 potential ($\Delta[\text{O}_2] = C_{surface} - C_{bulk}$) along leaf surfaces was greater at the low velocity, and the pattern of $\Delta[\text{O}_2]$ downstream was different between velocities (Fig. 1C,D). At the low velocity, $\Delta[\text{O}_2]$ was highest 3 cm downstream from the leading edge, increasing from 29 ± 5 to 36 ± 5 mmol m^{-3} and then decreasing to 30 ± 6 mmol m^{-3} . In contrast, $\Delta[\text{O}_2]$ was relatively constant at the high velocity (9.0 ± 0.7 mmol m^{-3}). rANOVA revealed a significant effect of velocity on $\Delta[\text{O}_2]$ ($F_{1,10} = 43.0$, $p < 0.0001$); however, the variation in $\Delta[\text{O}_2]$ was not significant with downstream distance ($F_{6,60} = 0.88$, $p = 0.515$), nor was there a significant interaction between downstream distance and velocity ($F_{6,60} = 1.71$, $p = 0.135$). An analysis of the Chl_{a+b} -normalized $\Delta[\text{O}_2]$ confirmed the significant effect of velocity on $\Delta[\text{O}_2]$ ($F_{1,10} = 23.8$, $p < 0.001$) but also revealed a significant effect of downstream distance on $\Delta[\text{O}_2]$ ($F_{6,60} = 4.49$, $p < 0.001$). Given the differences between low and high velocities, it was not surprising to find a significant interaction between downstream distance and velocity ($F_{6,60} = 3.51$, $p < 0.01$).

The effect of momentum transport on the mass transport of O_2 was examined using the relationship between the local Sherwood number (Sh_x) and the local Reynolds number (Re_x ; i.e., $Sh_x = a Re_x^b Sc^{0.33}$), which were determined from k_c based on the O_2 profile data (i.e., $k_c = J_{obs}/\Delta[\text{O}_2]$; Fig. 2). A log-log regression of $Sh_x Sc^{-0.33}$ on Re_x revealed that the intercept $\log(a)$ was -1.1 ± 0.3 at low velocity and -0.7 ± 0.4 at the high velocity and that the slope b was 0.81 ± 0.06 at low velocity and 0.70 ± 0.05 at high velocity. The slopes and intercept were not significantly different under ANCOVA ($F_{1,11} = 0.836$, $p = 0.380$); therefore, pooled values of the intercept, $\log(a) = -0.4 \pm 0.2$ and the slope $b = 0.66 \pm 0.03$ were obtained (i.e., $Sh_x = 0.39 Re_x^{0.66} Sc^{0.33}$).

The dimensionless velocity measurements, obtained through PIV at the low and high velocities, provided reasonably well behaved steeply increasing profiles (Fig. 3A). The maximum Re_x were 379 and 5011 at the low and high velocities, respectively. The laminar MBL thickness (δ_{MBL}) was found to fit the following relationship, $\delta_{MBL} = 6 \pm 1 \times Re_x^{-0.5}$ (mean \pm 95% confidence interval; Fig. 3A). The δ_{MBL} increased with x from 8.4 to 21 mm at low velocity and from 2.2 to 5.8 mm at high velocity. The DSL thickness (δ_{DSL}) estimated using u_* was relatively constant at both 0.5 cm s^{-1} (0.39 ± 0.04 mm) and 6.6 cm s^{-1} (0.12 ± 0.04 mm).

The dimensionless concentration measurements obtained through O_2 microelectrodes measurements followed a logarithmic-like profile that was steeper at the high velocity (Fig. 3B). The CBL thickness (δ_{CBL}) determined using the hyperbolic tangent model increased with x from

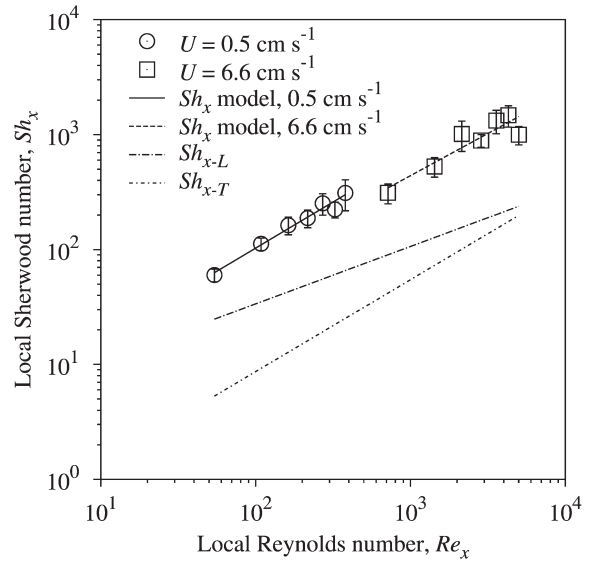


Fig. 2. The local Sherwood number (Sh_x) of O_2 vs. the local Reynolds number (Re_x) determined over the leaves of *Vallisneria americana* at 0.5 and 6.6 cm s^{-1} . Sh_x for laminar (Sh_{x-L}) and turbulent (Sh_{x-T}) boundary layers were determined from flat plate theory, where $Sh_{x-L} = 0.464 Re_x^{0.5} Sc^{0.33}$ and $Sh_{x-T} = 0.030 Re_x^{0.8} Sc^{0.33}$ under uniform O_2 flux adjacent to the leaf surface. Error bars indicate one standard error.

0.34 to 0.90 mm at the low velocity and from 0.15 to 0.32 mm at the high velocity. A model, analogous to that of the δ_{MBL} , of the form $\delta_{CBL} = A \times Re_x^B Sc^{-0.33}$, was fit to the O_2 concentration data. In this case, the constant $\log(A) = 0.07 \pm 0.03$ at low velocity and $\log(A) = 0.12 \pm 0.17$ at high velocity, and the exponent $B = -0.6 \pm 0.1$ at low velocity and $B = -0.7 \pm 0.2$ at high velocity (i.e., $\delta_{DSL} = 1.2 Re_x^{-0.6} Sc^{-0.33}$ vs. $\delta_{CBL} = 1.3 Re_x^{-0.7} Sc^{-0.33}$). The thickness of the measured DBL, $\delta_{DBL} = \theta (d\theta/dz)^{-1}$, determined from the O_2 profiles, was not uniform; rather, it increased from 0.07 to 0.18 mm at the low velocity and from 0.03 to 0.06 mm at the high velocity.

Discussion

The MBL—The flow over the leaves of *V. americana* was reasonable well behaved and appeared to be consistent with theoretical expectations for flow over a flat plate (Bird et al. 2002). For example, the MBL increased in thickness with distance from the leading edge and was thicker at lower velocities (Fig. 3). The MBL did not appear to be turbulent in nature; rather, the data fit a model (e.g., $\delta_{MBL} = 6 \pm 1 \times Re_x^{-0.5}$) that was laminar ($\delta_{MBL} \sim 5 \times Re_x^{-0.5}$) rather than turbulent ($\delta_{MBL} \sim 0.16 \times Re_x^{-0.143}$; i.e., the $1/7$ power law; Incropera and DeWitt 1996; White 1999). This would be consistent with theory given that the maximum Re_x values were considerably lower than the critical value for transition to turbulence over a flat plate (i.e., $Re_x \sim 3 - 5 \times 10^5$; White 1999). The shear velocity (u_*) did not change appreciably over the length of the leaf, which is also consistent for the u_* determined using a laminar formulation (u_* decreased from 0.11 to 0.07 cm s^{-1} at $U = 0.5 \text{ cm s}^{-1}$ [$Re_x = 54$ and 380 , respectively] and from

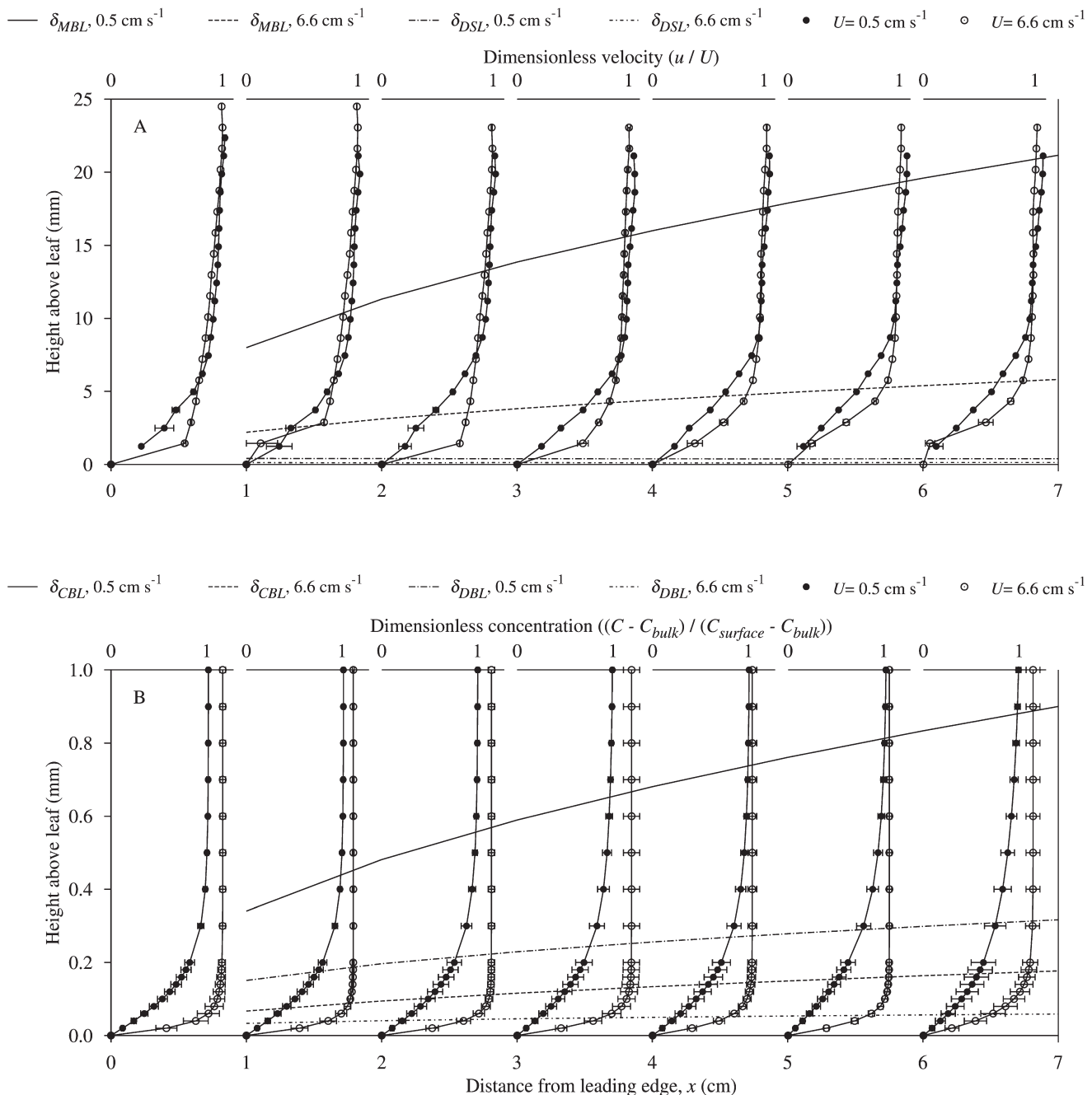


Fig. 3. The boundary layers above the leaves of *Vallisneria americana* determined at 0.5 and 6.6 cm s⁻¹. (A) The dimensionless momentum boundary layer (MBL) based on local velocities (u) measured by particle image velocimetry (PIV) normalized to the flow chamber free-stream velocity (U). (B) The dimensionless concentration boundary layer (CBL) based on the [O₂] measured at the leaf surface ($C_{surface}$) and the bulk fluid (C_{bulk}) using an O₂ microsensor (Nishihara and Ackerman 2007a). Error bars indicate one standard error.

0.74 cm s⁻¹ to 0.45 cm s⁻¹ at $U = 6.6$ cm s⁻¹ [$Re_x = 715$ and 5011, respectively; White 1999]. These results would confirm that the physical environment used in the experiments is consistent with the flat plate model of fluid dynamics (Nishihara and Ackerman 2006, 2007a).

The CBL—The CBL over the leaves of *V. americana* were remarkably similar to the MBL in pattern but $\sim 1/25$ of the magnitude in the vertical direction (note the

difference in the scale of the ordinate in Fig. 3A,B). Specifically, the thickness of the CBL (δ_{CBL}) grew in the downstream direction (x) and was thicker at the lower velocity. The exponent b from the local Sherwood–local Reynolds number relationship (i.e., $b = 0.66 \pm 0.03$) indicated that the CBL was neither laminar ($b = 0.5$) nor turbulent ($b = 0.8$) in nature (Bird et al. 2002). Moreover, empirical values of Sh_x were five times greater than theoretical values determined at low velocity (e.g., 187 vs.

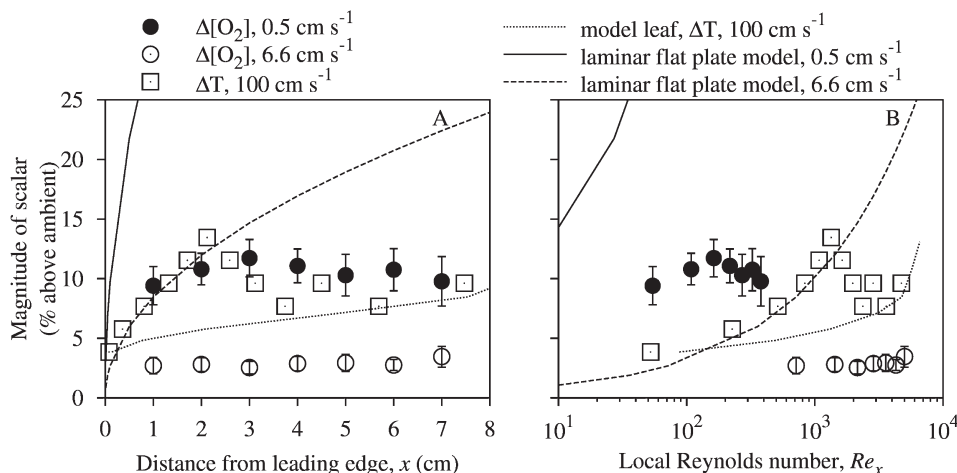


Fig. 4. The relative magnitude of the scalars above the ambient $[O_2]$ (280 mmol m^{-3}) measured over the leaves of *Vallisneria americana* and above the ambient temperature (26°C) measured over a *Phaseolus vulgaris* leaf (fig. 1 in Wigley and Clark 1974). The $[O_2]$ at 0.5 and 6.6 $cm\ s^{-1}$ for *V. americana*, $[O_2]$ determined from laminar flat plate theory and temperature at 100 $cm\ s^{-1}$ for *P. vulgaris* (in air) vs. (A) downstream distance (x) and (B) local Reynolds number (Re_x). Error bars indicate one standard error.

34) and seven times greater at the higher velocity (i.e., different intercept values, a ; Fig. 2). This discrepancy was also evident in the empirically determined $\delta_{CBL} - Re_x$ model exponent B (-0.6 ± 0.1 and -0.7 ± 0.2 for the low and high velocities, respectively), which was consistent with laminar ($B = -0.5$) rather than turbulent conditions ($B = -0.2$), whereas the values of the constant A (1.17 [1.09, 1.26] [mean - SE, mean + SE] and 1.32 [0.89, 1.95] for the low and high velocities, respectively) were intermediate between laminar ($A = 5$) and turbulent ($A = 0.37$) predictions for mass transport (Incropera and DeWitt 1996).

In contrast to the δ_{DSL} of the MBL, there was an increase in the measured thickness of the DBL (δ_{DBL}) downstream. The developing CBL (δ_{CBL}) over leaves of *V. americana* was an average of 36% (at low velocity) and 26% (at high velocity) of the theoretical predictions for a CBL over a flat plate, which is consistent with earlier reports (e.g., 37%) for *Vallisneria* species (Nishihara and Ackerman 2007b). Although the MBL was laminar over the leaf surface, mass transport processes in the CBL did not necessarily follow a laminar model, nor was the O_2 flux constant spatially. The differences in the empirical and theoretical values of Sh_x and the thickness and structure of the CBL demonstrate the inability of film theory to describe mass fluxes to photosynthesizing leaves. Moreover, estimates of δ_{CBL} and δ_{DBL} based on velocity data using laminar or turbulent formulations do not reflect the true nature of the CBL over a photosynthetically active leaf.

Mechanism of mass transport and physiological interaction—The raw and normalized O_2 flux (J_{obs}) were relatively high near the leading edge of *V. americana* leaves and decreased downstream of $x = 2$ cm at both velocities (Fig. 1). This would indicate that the rate of DIC ($CO_2 + HCO_3^-$) supply was not sufficient to maintain maximal rates of photosynthesis (J_{max}) over the entire length of the

leaf. Given that $\Delta[O_2]$ did not increase downstream of $x = 2$ cm, advection was sufficient to prevent an accumulation of O_2 at the leaf surface. It is, therefore, likely that the $[DIC]$ was sufficient to support J_{max} near the leading edge, whereas the supply of DIC decreased farther downstream, leading to a decrease in J_{obs} . The boundary conditions describing the mass transport of nutrients in a laminar CBL typically assumes that $C_{surface} = 0$, which implies that if J is equal to the rate of mass transport, it should decrease proportional to $x^{-0.5}$ or that if the flux at the surface is constant, C should increase at a rate proportional to $x^{0.5}$. Given that neither of these trends were observed and J and $C_{surface}$ varied over the surface (Fig. 1), estimates of δ_{CBL} or δ_{DBL} based on the assumption of $C_{surface} = 0$ or $J = \text{constant}$ should be interpreted with caution.

The coupling of mass transport and photosynthesis is evident when the idealized boundary conditions (i.e., constant surface flux and no reactions) are compared with the data. A steady increase in the surface $[O_2]$ is predicted for O_2 production independent of the bulk $[CO_2]$ and HCO_3^- (flat plate model in Fig. 4). However, the $\Delta[O_2]$ results for the low velocity indicate that the scalar concentrations at the surface show a peak at low velocities (Fig. 4). This type of observation has also been made with respect to heat flux data from the leaves of a terrestrial plant, *Phaseolus vulgaris* (Wigley and Clark 1974) (Fig. 4). As indicated previously, $C_{surface}$ (i.e., O_2) was not constant over the leaves, nor did it increase proportionally to $x^{0.5}$; however, this was not the case when the data were normalized to Chl_{a+b} . These results are also consistent with results from chemically reactive surfaces (e.g., finite-order reaction kinetics), which show a decline in $C_{surface}$ of the substrate (Acrivos and Chambré 1957; Libby and Liu 1966).

The relationship between J_{obs} and x posited by the conceptual model presented previously can be parameterized by an empirical sigmoidal model ($J_{obs} = J_{max}' (1 - (1 + e^{-(x - \phi)})^{-1})$), where J_{max}' is the maximum value of J_{obs}

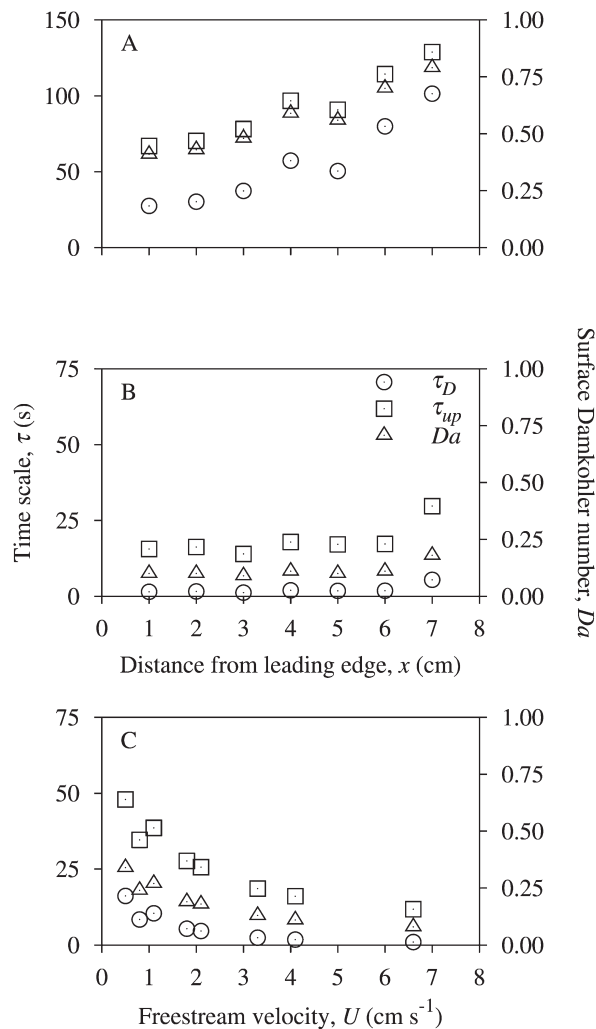


Fig. 5. A time scale analysis of dissolved inorganic carbon (DIC) transport and uptake over the surface of *Vallisneria americana* leaves. The time scale of DIC diffusion (τ_D) and DIC uptake (τ_{up}) through the measured diffusive boundary layer and the surface Damkohler number ($Da = \tau_D/\tau_{up}$) determined at (A) 0.5 cm s^{-1} , (B) 6.6 cm s^{-1} , and (C) at eight velocities (U) at a single location $x = 2.5$ downstream (data from Nishihara and Ackerman 2006).

near the leading edge and φ is the distance where $J_{obs} = 0.5 J_{max}'$. A nonlinear regression revealed that $J_{max}' = 0.41 \pm 0.02 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and $\varphi = 7.1 \pm 0.3 \text{ cm}$ ($r^2 = 0.92$; $F_{2,5} = 459$; $p < 0.001$) at low velocity, whereas $J_{max}' = 0.48 \pm 0.01 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and $\varphi = 8.0 \pm 0.3 \text{ cm}$ ($r^2 = 0.90$; $F_{2,5} = 1041$; $p < 0.0001$) at the higher velocity. Three regions can be thus be defined over the leaf surface where uptake rates are: (1) kinetically limited ($x < x_{99}$) and therefore limited by carbon uptake, (2) mass transfer limited ($x > x_{01}$) and therefore limited by carbon supply, or (3) intermediate in nature ($x_{99} < x < x_{01}$, where x_{99} and x_{01} are the downstream distances when J is 99% and 1% of J_{max}' , respectively). Kinetic limitation occurred within the first 2.5 cm of *V. americana* leaves at low velocity vs. 3.4 cm at the higher velocity (Fig. 1A,B). Beyond these distances,

limitation to photosynthesis were intermediate in nature, given that mass transfer limitation was predicted to occur beyond $x = 11.7$ and 12.6 cm at low and high velocity, respectively. Since increasing mass transfer rates increase DIC supply (i.e., CO_2 and HCO_3^-), such increases would lead to an increase in the amount of leaf surface area undergoing kinetic limitation. Given this scenario, it is doubtful that mass transfer limitation (i.e., $C_{surface} = 0$) would occur at high mass transfer rates or over photosynthetic surfaces (i.e., leaves) of small spatial scale, such as those examined for *V. americana* for $Sh_x \gg 1$.

Time scale of nutrient diffusion and uptake

The fundamental prediction of film theory that transport by diffusive processes limits the photosynthesis of aquatic macrophytes can be examined by comparing time scales of nutrient diffusion ($\tau_D = \delta_{DBL}^2 D^{-1}$) and nutrient uptake ($\tau_{up} = \delta_{DBL} C_{bulk} J_{max}^{-1}$) through the measured DBL, where $J_{adv} \ll J_{diff}$ (i.e., when diffusion dominates). τ_{up} and τ_D were determined for *V. americana* leaves examined in this study and an earlier study (Nishihara and Ackerman 2006, 2007a,b) using a pooled J_{max} mean of $1.3 \mu\text{mol m}^{-2} \text{ s}^{-1}$ given that the plants in both studies were grown under identical conditions (this represents a conservative estimate as lower values of J_{max} increase τ_{up}). The analysis revealed that $\tau_{up} > \tau_D$, both time scales increased with x regardless of velocity, and the magnitudes of the time scales were larger at the lower velocity (Fig. 5A,B). This pattern of τ_{up} and τ_D decreasing with velocity was also observed at $x = 2.5 \text{ cm}$ when velocities were increased systematically (Fig. 5C; Nishihara and Ackerman 2006). This is supported by the ratio of τ_D to τ_{up} , known as the surface Damkohler number ($Da = J_{max} \delta_{DBL} C_{bulk}^{-1} D^{-1}$) (Allen and Bhatia 2003), which ranged from 0.4 to 0.8 along the surface of the leaf at low velocity vs. 0.1 to 0.2 at the higher velocity (Fig. 5A,B) and declined from 0.4 to 0.05 at $x = 2.5 \text{ cm}$ as velocities were increased (Fig. 5C). This pattern also holds using a more conservative estimate of the distance over which nutrient transport and uptake occurs given by the δ_{DSL} . In this case, Da was 0.9 ± 0.1 at low velocity and 0.3 ± 0.1 at high velocity because the δ_{DSL} did not vary along the surface of the leaf (data not provided). The result that $Da < 1$, which indicates that nutrient uptake is the slower process and uptake rates are not mass transfer limited, is not definitive, as Da was expected to be > 1 at $x < 2 \text{ cm}$, given that the O_2 flux was kinetically limited (Fig. 1A,B), and observations of mass transfer-limited uptake rates have been demonstrated under these conditions (Nishihara and Ackerman 2006). Regardless, it would appear that DIC uptake occurs at longer time scales than the time scale for diffusion of DIC through the DBL, and, consequently, the mass transport of DIC does not appear to limit the photosynthetic rates of macrophytes.

Of the five processes involved in the diffusion, uptake, and conversion of DIC related to macrophyte photosynthesis, only nutrient uptake ($\text{O}(10^1 \text{ s})$) and the uncatalyzed conversion of HCO_3^- ($\text{O}(10^2 \text{ s})$) occur at rates slower than nutrient diffusion in the DBL (Table 1). Given that the $[\text{CO}_2]$ found in a number of aquatic environments

Table 1. The time scales of diffusion, uptake, and conversion of CO₂ and HCO₃⁻ for macrophyte photosynthesis.*

Process	τ (s)	Source
DIC uptake in <i>Vallisneria americana</i> (i.e., τ_{up})	O(10 ¹)	Present study
DIC diffusion through the DBL in <i>V. americana</i> (i.e., τ_D)	O(10 ⁰)	Present study
CO ₂ diffusion through the cuticular membrane	O(10 ⁻¹)	Frost-Christensen et al. (2003)
Uncatalyzed HCO ₃ ⁻ conversion to CO ₂	O(10 ²)	Wolf-Gladrow and Riebesell (1997)
Catalyzed HCO ₃ ⁻ conversion to CO ₂ via carbonic anhydrase	O(10 ⁻⁶)	Lindskog (1997)

* DIC, dissolved inorganic carbon; DBL, diffusive boundary layer.

can be relatively low (e.g., 17 mmol m⁻³ of CO₂ vs. 460 mmol m⁻³ of HCO₃⁻ at pH = 7.5), HCO₃⁻-to-CO₂ conversion is needed to support the photosynthesis rates observed in several macrophytes (Madsen and Maberly 2003). Regardless of the mechanism responsible for the conversion of HCO₃⁻ to CO₂, it is clear that physicochemical processes can limit the supply of carbon to HCO₃⁻-using macrophytes and that these processes will control the photosynthesis rate at low velocity when the CO₂ is supplied predominantly through diffusive rather than advective transport. There are a few mechanisms, however, that may explain the drop in O₂ flux: CO₂ diffusing out of the leaf and into the CO₂-depleted CBL downstream of the leading edge, which may occur through the increase in CO₂ concentration within the leaf as a result of carbon-concentrating mechanisms (Frost-Christensen et al. 2003; Madsen and Maberly 2003) and/or extruded H⁺ may be advected away, which would reduce the ability of the leaf to either acidify the water overlying the surface or decrease the rate of HCO₃⁻ cotransport (Prins and Elezenga 1989; Madsen and Maberly 2003). Moreover, it is relevant to note that uncertainties in the pH measurement as well as the value of the dissociation constant (pK_a) used will cause the [CO₂] to vary (15 to 30 mmol m⁻³ for pK_a ranging from 6.1 to 6.3). However, this will not change the [DIC] (i.e., change C_{bulk}) or the values of Da .

These results suggests that the assumptions used by film theory to examine mass transport may not hold for aquatic macrophytes, especially in the case of HCO₃⁻-using macrophytes with varying rates of uptake on their surfaces. The substrate concentration (e.g., CO₂ and HCO₃⁻) does not appear to be constant over leaf surfaces, nor is the leaf surface a perfect sink (i.e., the Dirichlet boundary condition). Given that the MBL over the leaves were well characterized and laminar in nature, it is doubtful that these variations were physical in origin, although variation in environmental conditions (e.g., irradiance, [DIC], temperature) can play a role. An analysis of τ_{up} and τ_D indicated that CO₂ + HCO₃⁻ diffusion through the DBL is not the rate-limiting step in mass transport, and it may vary spatially over the surface of a leaf at moderate flows and saturating light conditions. Future studies are needed to relate mass transfer to the hydrodynamics of physiologically active aquatic organisms under ecologically relevant conditions and to identify whether other waterborne nutrients have similar rate-limiting processes.

Acknowledgments

We thank Mark Loewen for comments regarding the time scale analysis and several anonymous reviewers for their comments on

the manuscript. This research was supported in part by funding from the University of Guelph and the Natural Sciences and Engineering Research Council of Canada to J.D.A.

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Associate editor: Chris Rehmann

Received: 11 March 2008

Accepted: 08 May 2009

Amended: 01 June 2009