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Kinetics of Dark Oxygen Uptake of *Pocillopora damicornis*[1]

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ABSTRACT: Colonies of *Pocillopora damicornis* were placed in a sealed aquarium in the dark. Water velocity was altered and measured in 10 different experiments. During each experiment, seawater in the aquarium was supersaturated with oxygen (O_2) and then O_2 concentration was measured through time until the concentration in the aquarium decreased to 0.3 mg O_2 l^{-1}. Resulting O_2 uptake curves were interpreted as a function of water velocity. Rate of O_2 uptake fit a hyperbolic equation ($dO_2/dt = V_m O_2/K_s + O_2$). Maximum uptake rate ($V_m$) varied between 0.12 and 0.27 mg O_2 l^{-1} min^{-1} (mean = 0.18), and the half-saturation constant (K_s) varied between 0.86 and 2.52 mg O_2 l^{-1}. Both V_m and K_s did not vary with water velocity, indicating that in these experiments, water motion had little influence on either diffusive boundary layers near the coral tissue or the metabolic rate of O_2 uptake. Even supersaturated concentrations of O_2 did not completely saturate the uptake capacity of this enzyme system.

DARK RESPIRATION RATES OF CORALS are often measured by the rate of O_2 uptake in enclosed, sealed aquaria. It is commonly observed that a plot of O_2versus time begins to curve upward at concentrations below ca. 2 mg O_2 l^{-1} or about 30% of saturation. The curvilinear feature indicates that the rate of O_2 uptake decreases as the concentration of O_2 decreases. This observation has led to the practice of measuring respiration rates at O_2 concentrations near saturation (McCloskey et al. 1978). As yet there has been no research to show whether the curvilinear feature of the plot is a result of (1) the actual metabolic requirement of coral diminishing as there is less O_2 in the water, or (2) the rate of O_2 uptake decreasing because of diffusive boundary layers of O_2 around the coral tissue. In the former case the coral would have a metabolic response to low O_2 in its environment; in the latter case the coral would not be getting sufficient O_2 to satisfy its metabolic demand.

A diffusive O_2 boundary layer is a thin film of water very near the organism in which there is a gradient of O_2 between the concentration in the water and the concentration at the surface of the tissue. This depleted boundary layer is formed within the momentum boundary layer (the transition region between the velocity of the bulk water and the no-flow layer right at the surface of the organism) when the rate of O_2 uptake at the surface is much faster than the diffusion of O_2 through the water. The thickness of a depleted, diffusive boundary layer is controlled by the water velocity of the bulk flow, the turbulence within that flow, and the topology of the surface. Presumably, coral and algae have adapted surface features that enhance mass transport to their surfaces, especially in very low-flow environments (Jokiel 1978, Anderson and Charters 1982, D'Elia and Cook 1988, Dennison and Barnes 1988). Dennison and Barnes (1988) showed that net photosynthesis and calcification of coral increase in stirred water environments. They suggested that diffusive boundary layers around coral tissue may be responsible for their observations.

For this study, we designed some initial experiments to determine whether the curvilinear feature of an O_2 uptake curve is a result

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of diffusive boundary layers near the tissue of *Pocillopora damicornis*.

MATERIALS AND METHODS

Oxygen Uptake Kinetics

Assuming that the respiration rate of a coral is constant in an incubation chamber that is open to air, the rate of O_2 uptake into the coral can be described by the following equation:

$$dO_2/dt = b + a(O_2 - O_{2s}) \quad (1a)$$

where O_2 is the concentration of O_2 in the water, O_{2s} is the concentration of O_2 at saturation, and b and a are constants; b represents the rate of respiration by the coral, $a(O_2 - O_{2s})$ is the rate of gas influx or efflux across the air-water interface. When the incubation chamber is closed to the atmosphere, a becomes diminishingly small compared to b, thus Equation 1a can be approximated by:

$$dO_2/dt = b \quad (1b)$$

The respiration rate, b, is constant or a zero-order rate reaction.

There must be some concentration, even if it is very low, where diffusion of O_2 to the surface becomes slower than the rate of dark respiration. At this concentration the rate of O_2 uptake into the coral is governed by the diffusion gradient between the bulk concentration in the water and the concentration immediately at the surface. According to Fick's First law, the flux of O_2 is proportional to the gradient of O_2 across the boundary layer, $F = D(dO_2/dz)$ where D is the diffusion coefficient of O_2 and dz is the thickness of the boundary layer. Under these conditions Equation 1 must be modified so that the rate of O_2 uptake is proportional to O_2 concentration:

$$dO_2/dt = k(O_2) + a(O_2 - O_{2s}) \quad (2a)$$

Again, assuming a is small compared to k, Equation 2 can be approximated by the following first-order equation:

$$dO_2/dt = k(O_2) \quad (2b)$$

Thus, it must be that as O_2 is depleted in a sealed incubation chamber during dark respiration, the rate of uptake will shift from being a constant (or zero-order kinetics) to being proportional to O_2 (or first-order kinetics). Changes in water velocity will affect the thickness of the boundary layer, hence the rate of uptake, because the first-order nature of the uptake is related to the thickness of the boundary layer by Fick's Law. Increasing the thickness of the boundary layer decreases the gradient of O_2 across it (dO_2/dz). The point of transition between the zero-order kinetics and first-order kinetics should be affected by water velocity and turbulence.

In contrast to diffusion limitation, Michaelis-Menton enzyme kinetics also show a transition between a first-order rate reaction and a zero-order rate reaction, but have a characteristic form of a hyperbolic fit, $dO_2/dt = V_m S/K_s + S$, where V_m is the maximum rate when the enzyme is "saturated" and K_s is the concentration when the uptake rate is $1/2\ V_m$. Imposing a diffusive boundary layer, or a resistance to uptake, between the water and a Michaelis-Menton type uptake at the surface of the organism creates a different shape transition between a clearly first-order rate reaction and a constant maximum uptake rate (Smith and Walker 1980).

In the experiments here, we hypothesized that if diffusive boundary layers exist around *P. damicornis*, then water motion should influence V_m and particularly K_s.

Experimental Procedure

Fifteen colonies of *P. damicornis* were collected from the reef flat of Checker Reef, Kaneohe Bay, Oahu, and then kept in an open flowing-water holding tank for 4 days before the experiments. To measure the kinetics of O_2 uptake, colonies were placed in a closed aquarium containing seawater supersaturated with O_2 (Figure 1), which was placed in the dark. The salinity of the incubation water was 34.8‰ and the temperature ranged between 26 and 27°C.

Gas exchange at the air-water interface was virtually eliminated in the aquarium by covering the water surface with a gas-impermeable

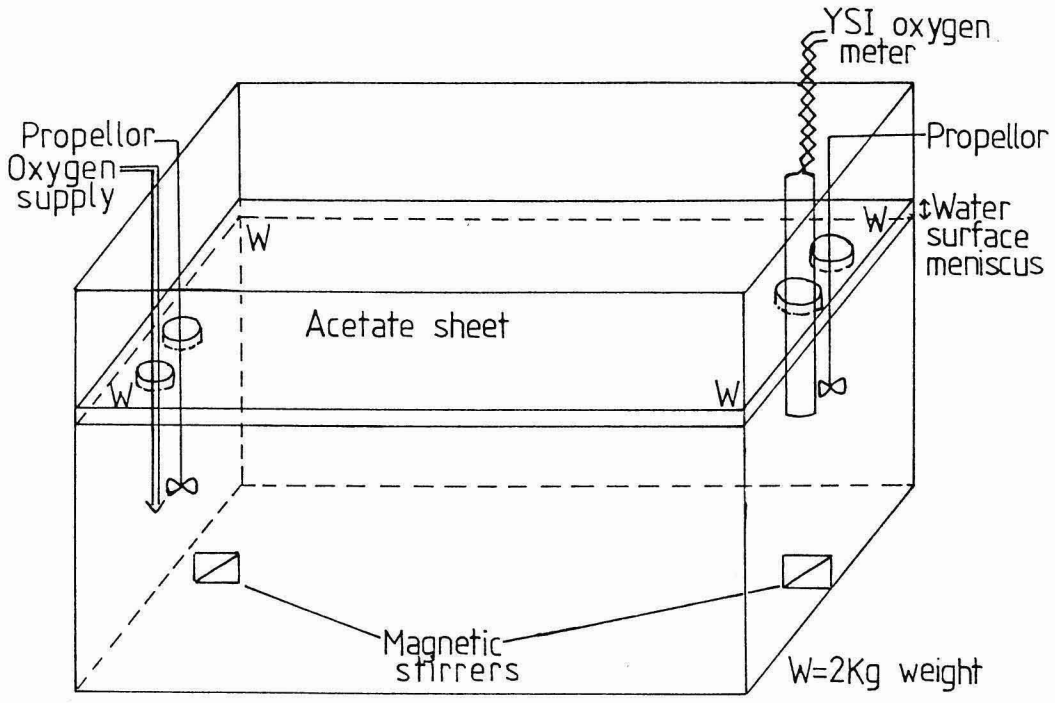

FIGURE 1. A diagram of the experimental aquarium. An acetate sheet covered the surface of the water to eliminate gas diffusion across the air-water interface. Magnetic stirrers and propellors provided water motion within the coral branches.

acetate sheet resting on an acetate rim coated with silicon grease (Figure 1). Weights were placed over the rim to ensure a tight seal, and excess water above the sheet was siphoned off so that the water surface meniscus was within the width of the sheet. No gas bubbles were visible beneath the acetate sheet. Pure, medical-grade O_2 was bubbled through the seawater to supersaturate the water with O_2. Oxygen concentrations were measured with a YSI O_2 meter that was calibrated against standard Winkler titrations (YSI = 1.09, Winkler = 0.145, $r^2 = 0.999$). A magnetic stirrer was placed beneath the oxygen sensor to ensure adequate water motion across the membrane of the sensor. Different levels of water motion were created by rotating propellors suspended in the aquarium (Figure 1). Diffusive boundary layers are thickest at lowest flows; therefore in these experiments, water velocity was kept to a minimum. Water velocity was measured with a hot-film anemometer, which was calibrated in a unidirectional flow in a 10-m flume. At low water velocities, water motion within branches of algae is independent of the bulk water velocity (Anderson and Charters 1982). Thus, to estimate water motion within coral colonies, 12 measurements of water velocity were made at random positions within the branches of coral. The mean value of these 12 measurements was used to quantify water motion. Ten measurements of the rate of O_2 uptake were made at 10 different levels of water velocity on the same 15 colonies. Oxygen concentrations were recorded every 5 min until the concentration reached 0.2 – 0.3 mg O_2 l^{-1}, at which time the experiments were terminated.

A control experiment was performed to determine the rate of gas exchange through the acetate sheet of the incubation aquarium. Oxygen was bubbled into filtered seawater until the concentration was approximately three times saturation. The aquarium did not

contain any coral. Oxygen concentrations were measured with a YSI O_2 meter and Winkler titrations over the following 2 days. The rate coefficient for gas diffusion (a in Equation 1a) was only 1–2% of the rate coefficient for biological uptake (b in Equation 1a), indicating that the aquarium was truly sealed and that gas exchange with the atmosphere was negligible.

The rate of O_2 uptake was calculated by dividing the difference between successive O_2 measurements by the time interval. The O_2 concentration for each rate of O_2 uptake was the mean concentration for that sampling interval. The best estimates of V_m and K_s for a hyperbolic fit were determined by a weighted least squares fit of a linear form of $dO_2/dt = V_m O_2/K_s + O_2$: $(O_{2o} - O_2) + K_s \ln(O_{2o}/O_2) = V_m t$. All O_2 uptake rates were plotted against O_2 concentration.

RESULTS

Plots of O_2 versus time in all 10 experiments became curvilinear at low O_2 concentrations, confirming the statement that rates of O_2 uptake decrease at lower O_2 concentrations. The results of two experiments, a low-flow experiment and a high-flow experiment, are shown in Figure 2.

For all 10 experiments, the rate of O_2 up-

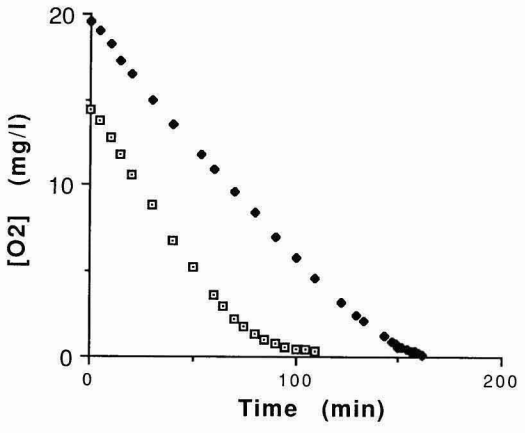

FIGURE 2. Oxygen uptake in low water motion, 0.22 cm s^{-1}, exp. 1, (□), and high water motion, 1.0 cm s^{-1}, exp. 10, (♦). Note the curvilinear nature of the plots at low O_2 concentrations.

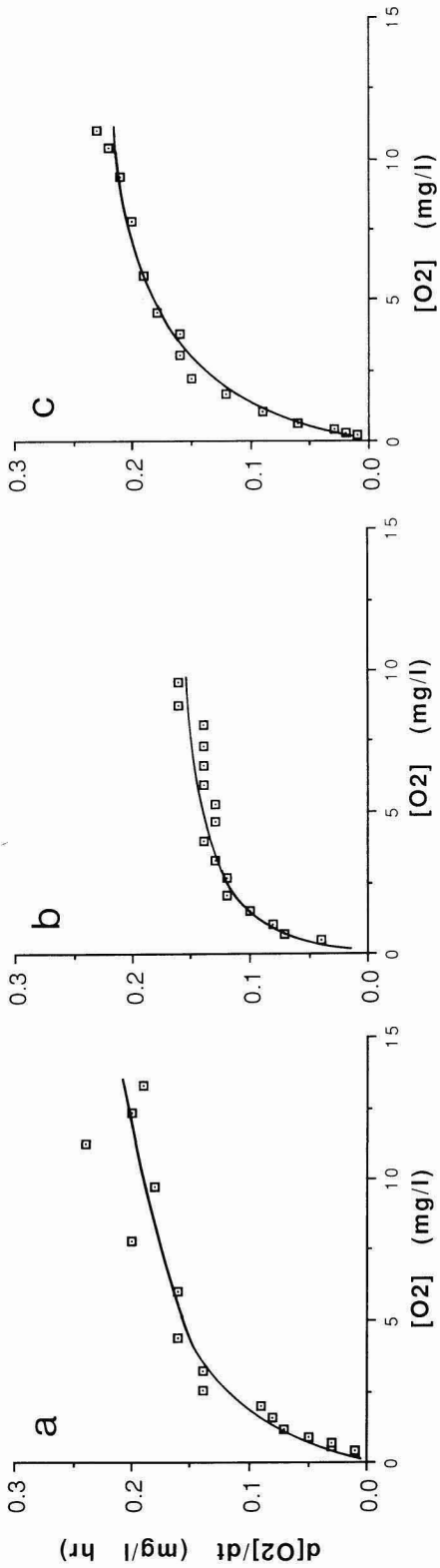

FIGURE 3. Rate of O_2 uptake versus O_2 concentration for three different water velocities: a, 0.22 cm s^{-1}; b, 0.64 cm s^{-1}; and c, 1.0 cm s^{-1}. In each figure, the line through the points is the best-fit hyperbolic curve; the parameters are given in Table 1.

TABLE 1

SUMMARY OF EXPERIMENTAL RESULTS*

Exp.	U	O_{2i}	O_{2f}	V_m	K_s
1	0.22	19.6	0.15	0.24	2.52
2	0.22	15.7	0.27	0.19	1.78
3	0.29	19.0	0.15	0.12	1.11
4	0.46	9.9	0.20	0.18	0.86
5	0.50	15.0	0.20	0.17	1.31
6	0.50	14.8	0.20	0.18	1.51
7	0.53	14.6	0.25	0.16	1.31
8	0.64	10.0	0.30	0.17	0.99
9	1.00	13.0	0.20	0.26	2.40
10	1.00	14.4	0.35	0.16	1.32
Mean	0.54	14.6	0.23	0.18	1.51
SD	0.28	3.2	0.065	0.041	0.56

* U = mean water velocity (cm s^{-1}); O_{2i} and O_{2f} = initial and final concentration (mg O_2 l^{-1}); V_m = maximum rate of uptake (mg O_2 l^{-1} min^{-1}); K_s = half-saturation constant (mg O_2 l^{-1}).

take throughout an experiment was well described using a hyperbolic equation (Figure 3*a,b,c*). Note that at supersaturated concentrations of O_2, the rate of O_2 uptake still increased. V_m varied between 0.12 and 0.27 mg O_2 l^{-1} min^{-1}, with a mean of 0.18; K_s varied between 0.86 and 2.52 mg O_2 l^{-1}, with a mean value of 1.51 (Table 1).

There were no correlations of V_m or K_s with water velocity. Further, the first-order rate coefficients for O_2 uptake for O_2 concentrations below K_s had r^2 near 0.99, yet these first-order rate coefficients were not correlated with water velocity.

DISCUSSION

The results of this study indicate that water velocity in the range of 0.22 – 1.0 cm s^{-1} did not have a major influence on the shape of the O_2 uptake curve, thus on the kinetics of O_2 uptake. The good fit to a hyperbolic equation for enzyme reactions indicates that diffusive boundary layers are not important in determining the rate of O_2 uptake; rather the concentration directly determines the rate of the enzyme reaction. We chose extremely low water velocities to maximize the thickness of any diffusive boundary layers and therefore create the largest changes in O_2 uptake with respect to changes in water velocity. Yet we could not create a large range in water motion within the experimental chamber. Future experiments on the effects of flow on coral metabolism should be done in larger chambers, in which the flow can be controlled over a 20-fold range in velocity.

The results of this study are not inconsistent with any other literature on coral respiration. No study to date has conclusively shown that dark respiration is a function of water velocity. Dennison and Barnes (1988) measured a 25% increase in net photosynthesis for coral in stirred and unstirred water. The change was probably due to changes in photosynthesis, not light respiration. It is also evident that coral respiration in *P. damicornis* is not maximal at concentrations near O_2 saturation. The uptake mechanism is not "saturated" or maximal even at two times O_2 saturation. We suggest that uptake capacity may be extremely efficient to scavenge O_2 from tissue during photosynthesis; that is, the coral may try to get as much O_2 from zooxanthellae as possible.

For ecological studies, measurements of coral respiration must be made at ecologically relevant concentrations, and those concentrations reported, otherwise the measured rate of respiration may be entirely inappropriate.

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