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Measurement of benthic photosynthesis and calcification in flowing-through seawater with stable carbonate chemistry

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Abstract

Estimation of photosynthetic or calcification rates of benthic organisms under stable seawater chemistry is important to fathom their capacity of CO_2 fixation under constant or controlled levels of pCO_2 and acidity of seawater. The flowing-through system, introduced here, can hold large individuals or colonies and maintain the carbonate chemical parameters stable while photosynthetic or calcification rate is measured based on the assimilation pipe inlet and outlet differences in dissolved O_2 concentrations or total alkalinity. The data obtained with this system for macroalgae showed constancy over time under controlled conditions, resulting in higher photosynthetic rates compared with those measured in a closed mode, which caused significant changes in the carbonate system (decreased pCO_2 and DIC and increased pH). When the method was applied to measurements of calcification based on the changes in total alkalinity, reliable data were obtained for both coralline algae and oysters. In addition, it can also be applied to measure respiration of both macrophyte and animals and to test the effects of increased pCO_2 and current speeds when these factors are controlled under either laboratory or field conditions while exposed to natural solar radiation.

Studying the effects of ocean acidification requires strict control of seawater carbonate system (Gattuso et al. 2010). However, traditional methods for measuring photosynthetic or respiratory rates of benthic organisms have relied on methods employing closed bottles or systems, which often cause remarkable changes in seawater carbonate chemistry.

Photosynthetic rate for either micro- or macroalgal species is determined through changes in oxygen concentration or the fixed amount of inorganic carbon measured over incubation periods. Such measurements are usually carried out in closed systems (vials or jars) to detect the changes of O_2 evolution or carbon uptake. Photosynthetic rates obtained in such closed vessels are supposed to be derived from linear changes in O_2 evolution or carbon removal by photosynthesis during the measuring period. Nevertheless, removal of inorganic carbon in the closed system, even with intensive stirring, can lead to remarkable changes in the carbonate chemistry in seawater, and accumulation of O_2 can inhibit photosynthetic processes by affecting photorespiration (a process sensitive to the ratio of O_2 to CO_2) (Wingler et al. 2000) or by triggering additional oxygen radicals that can damage photosynthetic apparatus and light-absorbing molecules. The incubation period with closed systems can often be long enough to alter the seawater carbonate chemistry (such as increased pH and decreased pCO_2) and influence physiological performances. Such bottle effects can be visualized on the recorded changes in O_2 concentration measured with O_2 electrode or gas-inlet mass-spectrometer (Gao et al. 1992a). On the other hand, many species of algae perform CO_2 concentrating mechanisms (CCMs, Giordano et al. 2005; Raven et al. 2011), therefore, cells may adjust their CO_2 or HCO_3^- acquisition strategies in response to changes in the carbonate chemistry. There is a possibility that acclimation to the changes in carbonate chemistry occurs during the period of measurements in view of stimulated activity of some enzymes involved in CCMs, such as extracellular carbonic anhydrase (Wu and Gao 2009). Subsequently, it is important to measure the photosynthetic rate under constant seawater carbonate chemistry. For macroalgal measurements, since thalli are often

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too large to be staffed in small vessels, punched discs are often used instead of a whole plant (Han et al. 2003). Different parts of macroalgae show different photosynthetic activities (Gao and Umezaki 1988) and injured thalli due to cutting could enhance respiration and reduce photosynthetic rate if measured immediately (Gao 1989). On the other hand, calcification of animal calcifiers, such as corals, is usually measured with total alkalinity (TA) anomaly technique in closed vessels or enclosed seawaters (Langdon et al. 2010), which can also result in big changes in carbonate systems due to decreased TA and/or respiration. Consequently, there is a need to measure benthic photosynthesis and calcification in stable carbonate chemistry with a system that can maintain benthic individuals.

Materials and procedures

We developed a seawater flow-through system, which consists of a transparent pipe, flow meter, O_2 monitoring unit, and seawater supplying tank or pump (Fig. 1). This method has been applied previously to monitor the photosynthetic rates of macroalgae under sunlight (Gao and Umezaki 1989; Gao and Xu 2008), but without evaluating its feasibility for environmental studies, such as studies on the effects of increased pCO_2 and UV radiation. Photosynthesis, respiration (nontransparent pipe or dark-covered), and even calcification rates can be estimated on the basis of the O_2 concentration or TA changes, flow rate, and biomass amount of samples.

Samples of benthic plants (such as macroalgae or seagrasses) need to be fixed on a stainless line or a glass stick and placed firmly in the pipe, so they will not get tangled in flowing seawater. At a reasonable flow rate, significant changes of dissolved O_2 concentration in seawater through the pipe could be measured. O_2 concentrations in the inlet and outlet seawater can be monitored simultaneously and continuously with a Clark-type oxygen electrode. Rate of photosynthetic O_2 evolution [P, $\mu molO_2$ g $(FW)^{-1}\cdot h^{-1}$] is determined as follows:

$$P = (A\text{-}B)*F*60*W^{-1} \quad (1),$$

where A and B represent dissolve O_2 concentrations ($\mu mol\ O_2\cdot L^{-1}$) in the outlet and inlet seawater, respectively; F, the flow rate ($L\cdot min^{-1}$) of seawater; W, mass (g) of the samples used. To achieve high CO_2/low pH conditions, an additional department can be connected to the open flow-through system; aeration of CO_2-enrich air can be applied to the water tank from which seawater is supplied to the pipe. If a CO_2-enriching device (such as CO_2 enrichlor CE-100, Wuhan Ruihua Instrument & Equipment) is not available, an airbag containing air of desired CO_2 level can be connected to the air phase of a closed water tank in which the carbonate system of seawater reached equilibrated state with the CO_2 level in the air.

Assessment and discussion

The net photosynthetic rate of a red macroagla, *Gracilaria lemaneiformis,* measured with the flowing-through system were significantly higher, by about 76%, than that measured in the same system that was closed, while little effect was shown in respiration (Fig. 2). Net photosynthetic rate of the corraline alga *Corallina sessilis* maintained at a constant value over a period as long as 5 h when the flowing system was used, but it decreased with time when the thalli was held in the closed system (Fig. 3). Although the net photosynthetic rate of the thalli measured in semi-closed mode (the seawater was aerated at the outlet and recirculated to inlet flow) showed the similar change pattern as that in the open system, but the rates were lower compared to the flow-through open system (Fig. 3).

Because the photosynthetic rate of macroalgae can be measured in flowing through seawater, diurnal physiological performance can easily be followed with changes of solar radiation (Gao and Xu 2008). For studies on effects of ocean acidification, pH-adjusted seawater by bubbling desired levels of CO_2 can be stored in a water tank. It is also applicable to aerate CO_2-enriched air directly into the water tank with a CO_2 enriching device (CO_2 enrichlor CE-100, Wuhan Ruihua Instrument & Equipment). There would be a slight decrease of pCO_2 and a small rise of pH due to photosynthetic removal of inorganic carbon. However, when the flow rate and biomass

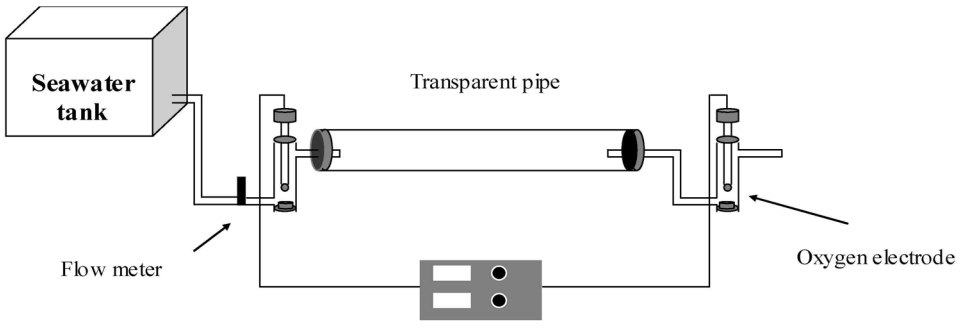

Fig.1. Outline of the flow-through system used for measuring photosynthesis or calcification of benthic organisms under CO_2-enriched/low pH conditions.

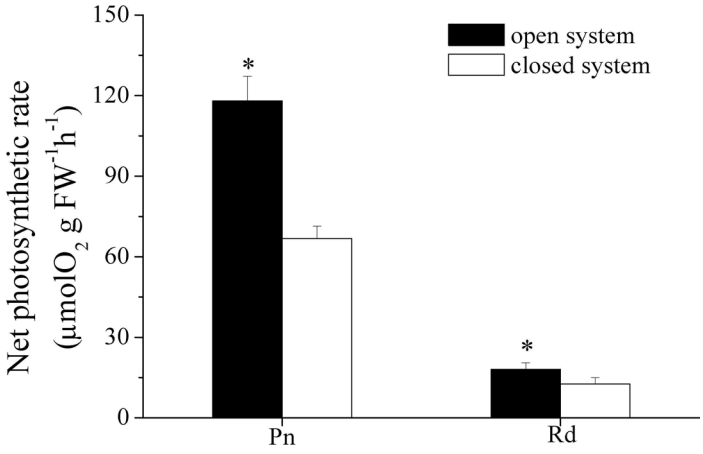

Fig. 2. Net photosynthetic and respiratory rates of *Gracilaria lemaneiformis* measured in open or closed system. The measurement in the open system was carried out with a transparent pipe (4 cm in diameter, 35 cm long, 0.44 L) at 600 μmol photon m^{-2} s^{-1} of PAR and 20°C. It took less than 10 min for the O_2 concentration at the outlet flow to be constant at a flow rate of 0.2 L min^{-1}. The measurement in the closed system was carried out at the same levels of light and temperature. It took about 5 min for the change of O_2 concentration to be constant with the closed system. Clark-type oxygen electrode was used. The densities of biomass for open and closed systems were similar, with the values of 12.5 g L^{-1}. Asterisks represent the significant difference between the open and closed systems.

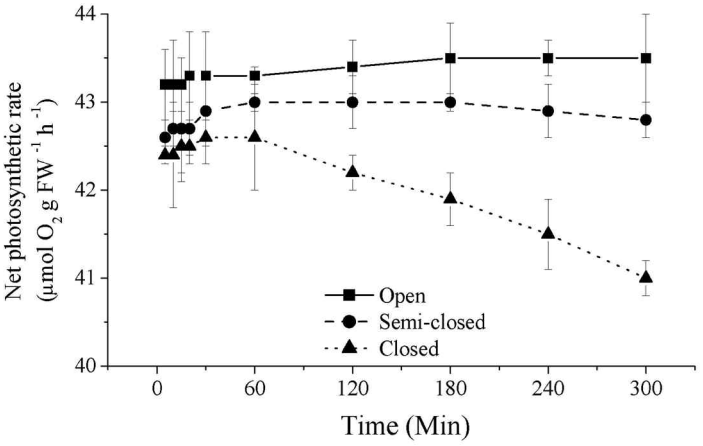

Fig. 3. Changes in photosynthetic O_2 evolution rate of the coralline alga *Corallina sessilis* under the open, semi-closed (circulated flowing seawater that is aerated before running into the tube) or closed (without releasing of dissolved gases) systems over time at 200 μmol photon m^{-2} $s^{-1}$of PAR and 25 ± 1°C with the same assimilation pipe (as mentioned in Fig. 1). It took less than 5 and 15 min in the closed and open (or semi-closed) system, respectively, for the O_2 concentration at the outlet to be constant at a flow rate of 0.6 L min^{-1}.

inside the assimilation tube are maintained constant, these changes are almost constant and insignificant (Table 1), which would not affect comparisons between low CO_2/high pH and high CO_2/low pH conditions. Therefore, one can carry out experiments under the CO_2-enriched/low pH conditions, while minimizing the effects of accumulated O_2 and removed DIC due to photosynthesis.

Table 1. Parameters of the seawater carbonate system under the ambient CO_2 concentration (380 ppmv). Total alkalinity (TA), partial pressure of CO_2 in seawater (pCO_2), pH, salinity, and temperature were measured and used to derive all other parameters according to (Dickson et al. 2007). Data are the means ±SD of 3 measurements. Different superscript letters indicate significant difference between inlet and outlet of the running through system at flow rate of 0.6 L min^{-1} with 1 g (fresh weight) of *Corallina sessilis* in a quartz tube (Φ4 cm, 35 cm long, 0.44 L).

	Inlet	Outlet
DIC (μM)	2036 ± 38[a]	2025 ± 45[a]
HCO_3^- (μM)	1845 ± 33[a]	1839 ± 40[a]
CO_3^{2-} (μM)	178 ± 12[a]	167 ± 20[a]
pCO_2 (μM)	15 ± 2[a]	14 ± 3[a]
TA (μM)	2316 ± 22[a]	2310 ± 45[a]
Ω_c	3.7 ± 0.2[a]	3.7 ± 0.5[a]
pH	8.4 ± 0.1[a]	8.4 ± 0.2[a]

The accuracy of the running-through system for measurements of photosynthetic or respiratory rates as well as calcification depends on the difference of O_2 or TA (or Ca^{2+}) between the inlet and outlet. If the ratio of biomass to the volume within the assimilation pipe is small, one needs to maintain very slow flow rate (<200 mL min^{-1}) to ensure detectable difference between the inlet and outlet. If the pipe is large, then more time is required for the O_2 concentration or TA in the outlet seawater to become constant (turnover rate of the seawater determines the lag phase).

During the measurements either under indoor constant light levels or outdoor changing light conditions, the difference between the inlet and outlet seawater may be too small and bring about relatively large variation. In this case, one can convert the running-through system into a circulating open mode, by circulating the same volume of seawater but still maintaining the system open by bubbling the open water tank to which the water is circulated and from which the seawater flows to the inlet (Fig. 3). Although nutrients-limitation may occur for long-term incubations, such a circulated open system could result good results when examining changes in total alkalinity or calcium ions. Estimation of rates can be done according to Fig. 4A and the following equation:

$$R = (C1 - C2)/(T2 - T1)/B \quad (2),$$

where R represent rate of calcification normalized to per unit of time as well as biomass (Fig. 4A); C1 and C2, the levels of TA at time T1 and T2, respectively; B, the biomass used. Because the change in the concentration may not be linear over time, it is important to make the segment of time as short as possible. One can examine the rate at different concentrations of the substrate (such as Ca^{2+}), so that it can be used for

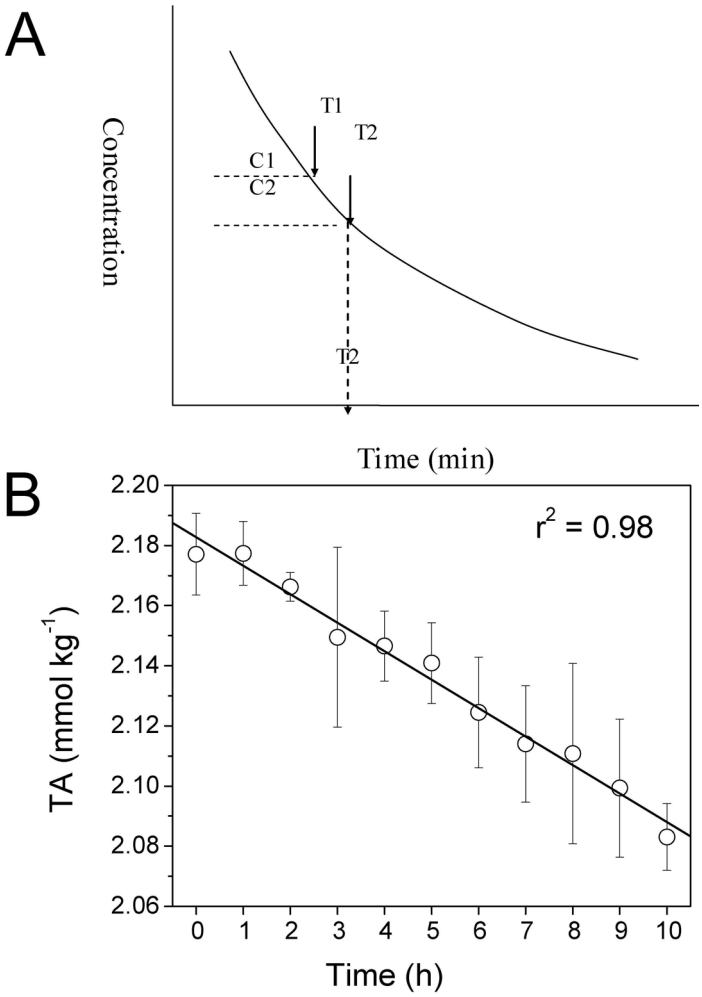

Fig. 4. A) Illustration of the method for calculation of benthic calcification rates in a circulated open system or a closed system in which changes of TA (or calcium ion concentration) is monitored. B) Changes in seawater TA over time when the oyster *Crassostrea angulata* was incubated in running-circulated open system (aeration was applied to the outlet seawater before it runs into the tube). Fifteen oysters were placed in the tubes for each measurement, and the total weigh was about 460 g (fresh weight with shells). The error bars represent the standard deviations of triplicate measurements.

establishment of relationship of the rate with substrate concentration. When the oyster *Crassostrea angulata* was maintained in the circulating open system, TA at the outlet decreased linearly with time (Fig.4B), whereas the pCO_2 and O_2 concentrations remained stable. Net clarification rates (mmol $CaCO_3$ g FW^{-1} h^{-1}) were estimated using the equation: $G = -\Delta TA/2$, where ΔTA is the variation of TA during the incubations in meq g FW^{-1} h^{-1} (Smith and Key 1975). The calcification rate of *C. angulata* was 0.11 ± 0.03 µmol $CaCO_3$ g FW^{-1} h^{-1}, which is comparable with the rates of *C. gigas* reported by Gazeau et al. 2007.

On the other hand, one can convert the open system into a closed system without any leaking gases from the seawater and circulate (not going back to the water tank) the seawater at a constant flow rate. In this case, the rate of photosynthesis can be estimated according to the changes in O_2 concentration and the exact volume of the system, including the pipe and connecting tubes. However, such closed systems can also result in bottle effects to affect carbonate chemistry and result in significant decrease in photosynthetic rate in the same way as traditional methods if operated for long period. Nevertheless, the better part of the closed system in contrast to the bottles is that it can hold large samples in flowing seawater.

Comments and recommendations

Because current speeds affect the thickness of the boundary layers around the benthic organisms, such as macroalgae, significant changes in photosynthetic rates were observed under different current speeds (Gao et al. 1992b). Therefore, when the flow-through system is used, data about current speed, size of the assimilation pipe, and flow rate should be reported in addition to the measured photosynthetic rates. On the other hand, when applied under solar radiation, the photosynthetic rates depend on the levels of light, making analysis of data difficult. However, this is the most important way to obtain ecologically relevant data. Data obtained during a daytime cycle can be plotted against levels of solar photosynthetically active radiation (PAR) in both morning and afternoon, so one can visualize the differences between morning and afternoon at the same levels of PAR or compare differential responses during early morning, noontime, or late afternoon periods (Gao and Xu 2008). To examine the effects of solar UV radiation, quartz tubes must be used to allow UV irradiances to penetrate.

The following recommendations need to be considered in application of this method:

1. If two different electrodes are used at inlet and outlet sides, responses of the electrodes must be checked to see if they are identical. Sometimes, the electrodes, even from the same maker, show quite different responses to changes in O_2 concentration. It is recommended to use one electrode to measure both sides.
2. Make sure that the samples are not self-shaded, and always mention the biomass density in the pipe when reporting data.
3. It takes several min for the outlet O_2 concentration or TA to reach a constant level due to the time required for turnover of the seawater. Different volumes of the pipe and different flow rate affect this lag phase. It requires less than 5 min if a pipe of less than 500 mL is used at a flow rate of 200 mL per min. Photosynthetic rate has little effects on the lag phase.
4. Make sure the difference of O_2 concentration or TA between the outlet and inlet is significant enough to reduce the measurement errors. The optimal biomass to the assimilation tube volume is suggested for the tested species in Table 2.
5. Pipes made of different materials have different light transmission features. Glass, polycarbonate, and acryl are opaque

Table 2. Change in dissolved O_2, total dissolved inorganic carbon (DIC), and total alkalinity (TA) of seawater in the running through system with designed biomass to tube volume ratio and flow rate.

Species	Biomass to tube volume ratio (g L^{-1})	Flow rate (L min^{-1})	Change in O_2 (%)	Change in DIC (%)	Change in TA
Gracilaria lemaneiformis (red macroalga)	14	0.2	25%	3% (decrease)	nd
Corallina sessilis (coralline alga)	30	0.2	21%	3% (decrease)	4%
Crassostrea angulate (Oyster)*	508	0.2	nd	nd	5%

*indicates a running circulating open system for the animal.

for UVB, glass can transmit a part of UVA wavelength band, and polycarbonate material transmits much less PAR compared to glass. UV-transparent materials (such as quartz) are preferable even one does not consider to distinguish the effects of UV radiation.

6. When the running-through open system or running through circulating mode are applied outdoor under solar radiation, solar radiation levels must be monitored simultaneously to analyze the relationship of PAR with the rate. In most areas, data of solar radiation can be obtained from local meteorological stations.
7. Due to the high sensitivity of dissolved O_2 to temperature, the running-through tube must be placed in a temperature-controlled water bath or chamber.
8. The electrodes (pH or oxygen) need to be regularly calibrated when seawater temperature changes or when applied under outdoor conditions.

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