

Review of: "Effects of experimental CO2 enrichment on the PSII photochemical efficiency of *Symbiodinium* sp. in *Acropora millepora*"

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Potential competing interests: No potential competing interests to declare.

Here's my review of the paper. I have provided some questions and suggestions for each part.

Abstract

Elaborate on what is meant by the experiment being "inconclusive." Was it due to variability in the data, limitations in the experimental design, or another factor?

Include a brief explanation of the significance of measuring PSII photochemical efficiency and why it's important for understanding the effects of CO2 enrichment.

Highlight any notable findings, even if they were not statistically significant, to provide a clearer summary of the experiment's outcomes.

Introduction

The introduction mentions past experiments and their findings. How does this study aim to build on or challenge these previous findings?

What are the specific biochemical mechanisms through which CO2 enrichment might affect the PSII photochemical efficiency in *Symbiodinium* sp.?

How do the experimental conditions (e.g., light levels, temperature) reflect natural reef environments? Are there any limitations in the experimental design that might affect the applicability of the results to natural settings?

Provide a more detailed review of the conflicting findings in previous studies regarding the effects of CO2 enrichment on coral symbiosis.

Clearly state the hypotheses being tested. This will help readers understand the specific aims and expected outcomes of the study.

M & M

Experimental Conditions and Setup:

We assume that they hosted the same *Symbiodinium* clade! How can you assume? *Symbiodinium* clade type is A, but you have to clarify it based on molecular techniques. What is the exact clade/s associated with the *Acropora* here? This is a very important part. Please see the paper: Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts.

How were the three tanks differentiated during the experiment? Were there any physical or environmental differences between them aside from CO₂ levels?

Could the text clarify how the PAM fluorometer and LED lighting were synchronized in terms of their operations during the light cycle simulation?

CO₂ Enrichment and pH Control:

What specific methods or equipment were used to control CO₂ levels and pH in the seawater? How was the CO₂-enriched air injected into the system and monitored?

How was pH stability ensured during the gradual increase and maintenance of pCO₂ levels in the experimental tanks?

Data Collection and Analysis:

Regarding the statistical analysis, what specific fluorescence parameters (e.g., F_v/F_m , $\Delta F/F_m'$) were used to evaluate the effects of CO₂ enrichment on photosynthetic efficiency?

Can you provide more detail on the frequency and duration of the saturation pulse and induction/recovery analyses? How were these data points used to assess PSII efficiency under varying CO₂ conditions?

Seawater Chemistry and Environmental Controls:

What measures were taken to ensure consistent seawater parameters (e.g., salinity, temperature) across all tanks throughout the experiment?

Were there any unexpected challenges or environmental fluctuations (aside from the COVID-19 lockdown) that impacted data collection or experimental outcomes?

Data Handling and Interpretation:

How were missing data points handled in the statistical analysis, particularly those attributed to the pandemic lockdown and transport issues?

Could the paper elaborate on the methods used to calibrate and validate the fluorescence measurements obtained from the PAM fluorometer?

Results and Discussion sections:

CO₂ Enrichment and Seawater Chemistry:

Could the text clarify how the derived seawater carbonate chemistry parameters (Fig. 1 and S1 Table 2) were used to confirm the increase in pCO₂ due to CO₂ enrichment?

Were there any unexpected variations or challenges in maintaining these parameters across different experiments?

PSII Photochemical Efficiency and Diurnal Variation:

The diurnal variations in PSII photochemical efficiency (F_v/F_m and $\Delta F/F_m'$) are noted to exhibit distinct patterns (Fig. 2). Could the study elaborate on whether these variations correlate with specific environmental factors beyond light intensity, such as temperature fluctuations or nutrient availability?

Impact of Measurement Accidents:

In Experiment 2, a measurement error significantly affected the PSII photochemical efficiencies (Fig. 3). How did this incident influence the overall conclusions drawn from the experiment? Were there measures taken to mitigate such errors in subsequent experiments?

Effects of CO₂ Enrichment on Coral Physiology:

The paper suggests that CO₂ enrichment may counteract the decline in PSII efficiency observed under ambient conditions (Fig. 3 and Discussion). Could the study discuss potential mechanisms through which CO₂ enrichment might influence these physiological parameters, particularly considering variations observed across different experiments?

Chlororespiration and Photoprotection Mechanisms:

The discussion mentions chlororespiration and its potential role in photoprotection and metabolic adjustments (Discussion, paragraphs 319-333). Could the authors elaborate on how these processes were inferred from the data and their implications for coral health under varying environmental conditions?