Peer Review

Review of: "Effects of experimental CO_2 enrichment on the PSII photochemical efficiency of *Symbiodinium* sp. in *Acropora millepora*"

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This manuscript presents an insightful and methodologically rigorous investigation into how elevated pCO_2 levels influence PSII photochemical efficiency in Symbiodinium sp., the symbiotic algae of Acropora millepora, under stable thermal and light conditions. The relevance of this research is underscored by the pressing need to understand how ocean acidification—one of the most critical consequences of climate change—affects coral health at the photophysiological level. The study is well-contextualized within the broader discourse on coral resilience, offering both precision in design and a thoughtful treatment of biological complexity.

One of the most commendable strengths of the study is its use of pulse-amplitude-modulation (PAM) fluorometry with high temporal resolution. By conducting saturation pulse analyses every 30 minutes over multiple 14-day cycles, the authors achieve an exceptionally granular understanding of diel fluctuations in PSII photochemical parameters. This methodological depth allows for a detailed examination of daily oscillations in $\Delta F/Fm'$ and Fv/Fm, capturing the dynamic behavior of symbiont photosynthesis in an unprecedented manner. Such fine-scale monitoring provides valuable insights that go beyond static assessments and better reflect natural physiological rhythms.

The gradual and realistic manipulation of pCO_2 levels, rising from ~496 to ~1290 μatm over nine days, is another significant asset of the experimental approach. This progressive ramping simulates environmental change in a manner that avoids acute stress responses, better reflecting conditions corals might encounter in the natural world. The authors also maintained stable light (~65 μmol quanta m^{-2} s^{-1})

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and temperature (25 °C) regimes, effectively isolating the variable of interest and enhancing the validity of their interpretations regarding CO_2 effects.

An unplanned but scientifically fruitful outcome of the study was the discovery of physiological heterogeneity along the coral branch, stemming from an accidental repositioning of a PAM measuring head. This led to valuable observations indicating distinct photophysiological behaviors between the basal and apical regions of the coral. The authors wisely embraced this incident as a learning opportunity, offering an important reflection on the spatial complexity of coral physiology that should inform future sampling and experimental design.

Even though the statistical analyses did not confirm a significant effect of CO_2 enrichment on PSII efficiency across all parameters, the transparency and rigor with which the authors report these inconclusive findings is praiseworthy. Rather than overstating outcomes, the manuscript contributes meaningfully to the scientific discourse by presenting variability as a legitimate and informative result. The careful documentation of partial trends—especially the apparent mitigation of photochemical decline in two of the three experiments—lays the groundwork for replication and refinement in future studies.

The clarity and detail of the methodological description further enhance the value of the study. Parameters such as ΔF/Fm', Fv/Fm, Qm, and rETR/PAR slopes are not only accurately presented but also interpreted in the context of diel biological rhythms. While some sections could benefit from added clarity for readers less familiar with PAM techniques, the overall level of detail supports reproducibility and invites constructive engagement from the coral photobiology community.

The presentation of the time series data and the decision to examine photophysiological behavior under both ambient and CO_2-enriched conditions strengthen the manuscript's contribution to the literature on sub-lethal stress responses in coral symbionts. This approach also highlights the importance of temporal context when interpreting photosynthetic performance, encouraging more ecologically relevant assessments of coral resilience.

From a broader perspective, the study aligns well with ongoing efforts to bridge experimental physiology and environmental change scenarios. It offers not only data but also a valuable experimental framework that can be adopted and extended by other researchers in the field. The combination of methodological precision, careful control of variables, and ethical reporting of limitations speaks to the authors' scientific integrity and enhances the manuscript's credibility.

In terms of writing, the manuscript maintains a formal and accurate tone throughout. A slightly more accessible narrative structure in the discussion could improve readability, particularly for multidisciplinary audiences. However, the scientific language remains appropriate for a specialized readership, and the integration of methodological insights with broader ecological implications is well-executed.

In conclusion, this manuscript presents a technically sound and conceptually valuable contribution to coral ecophysiology. Despite the absence of statistically confirmed effects, the study's strengths—especially the high-resolution temporal data, thoughtful experimental design, and openness to complexity—underscore its scientific merit. It provides a meaningful platform for future research and reflects the kind of methodological sophistication and intellectual honesty that are essential for progress in climate-sensitive marine science.

Declarations

Potential competing interests: No potential competing interests to declare.