Ciliary flows in corals ventilate target areas of high photosynthetic oxygen production

Pacherres, Cesar O.; Ahmerkamp, Soeren; Koren, Klaus; Richter, Claudio; Holtappels, Moritz

Published in:
Current Biology

DOI:
10.1016/j.cub.2022.07.071

Publication date:
2022

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY

Citation for published version (APA):
Pacherres, C. O., Ahmerkamp, S., Koren, K., Richter, C., & Holtappels, M. (2022). Ciliary flows in corals ventilate target areas of high photosynthetic oxygen production. Current Biology, 32(19), 4150-4158.e3. https://doi.org/10.1016/j.cub.2022.07.071
Ciliary flows in corals ventilate target areas of high photosynthetic oxygen production

Graphical abstract

Highlights

- Chlorophyll in the coral tissue is higher in the coenosarc and lower in the mouth
- \( O_2 \) in the DBL shows an inverse distribution from that of Chla
- Ciliary vortices at the coral surface carry \( O_2 \) from areas of high to low production
- Cilia-generated vortices may help minimize oxidative stress inside the coral tissue

Authors
Cesar O. Pacherres, Soeren Ahmerkamp, Klaus Koren, Claudio Richter, Moritz Holtappels

Correspondence
cesar.pacherres@bio.ku.dk (C.O.P.), sahmerka@mpi-bremen.de (S.A.)

In brief
Pacherres, Ahmerkamp et al. present simultaneous measurements of flow and \( O_2 \) in the coral boundary layer along with Chla content in the tissue beneath, showing a patchiness in the \( O_2 \) distribution that is shifted away from \( O_2 \)-production zones by ciliary vortices; a mechanism that might help corals mitigate potential oxidative damage and bleaching.
Ciliary flows in corals ventilate target areas of high photosynthetic oxygen production

Cesar O. Pacherres,1,2,3,7,8,10,* Soeren Ahmerkamp,4,5,7,9,* Klaus Koren,6 Claudio Richter,1,2 and Moritz Holtappels1,5

1Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, 27568 Bremerhaven, Germany
2Department of Biology and Chemistry, University of Bremen, 28359 Bremen, Germany
3Marine Biological Section, Department of Biology, University of Copenhagen, 3000 Helsingør, Denmark
4Max Planck Institute for Marine Microbiology, 28359 Bremen, Germany
5MARUM - Center for Marine Environmental Sciences, University of Bremen, 28359 Bremen, Germany
6Center for Water Technology, Section for Microbiology, Department of Biology, Aarhus University, 8000 Aarhus, Denmark
7These authors contributed equally
8Twitter: @pacherres_co
9Twitter: @SoerenAhm
10Lead contact
*Correspondence: cesar.pacherres@bio.ku.dk (C.O.P.), sahmerka@mpi-bremen.de (S.A.)
https://doi.org/10.1016/j.cub.2022.07.071

SUMMARY

Most tropical corals live in symbiosis with Symbiodiniaceae algae whose photosynthetic production of oxygen (O2) may lead to excess O2 in the diffusive boundary layer (DBL) above the coral surface. When flow is low, cilia-induced mixing of the coral DBL is vital to remove excess O2 and prevent oxidative stress that may lead to coral bleaching and mortality. Here, we combined particle image velocimetry using O2-sensitive nanoparticles (sensPIV) with chlorophyll (Chl a)-sensitive hyperspectral imaging to visualize the microscale distribution and dynamics of ciliary flows and O2 in the coral DBL in relation to the distribution of Symbiodiniaceae Chl a in the tissue of the reef building coral, Porites lutea. Curiously, we found an inverse relation between O2 in the DBL and Chl a in the underlying tissue, with patches of high O2 in the DBL above low Chl a in the underlying tissue surrounding the polyp mouth areas and pockets of low O2 concentrations in the DBL above high Chl a in the coenosarc tissue connecting neighboring polyps. The spatial segregation of Chl a and O2 is related to ciliary-induced flows, causing a lateral redistribution of O2 in the DBL. In a 2D transport-reaction model of the coral DBL, we show that the enhanced O2 transport allocates parts of the O2 surplus to areas containing less chl a, which minimizes oxidative stress. Ciliary flows thus confer a spatially complex mass transfer in the coral DBL, which may play an important role in mitigating oxidative stress and bleaching in corals.

INTRODUCTION

Coral reefs are among the most diverse and economically important ecosystems on the planet. Despite their importance as ecosystem engineers, the corals providing the foundation of today’s reefs are threatened by a multitude of anthropogenic changes acting on many spatial and temporal scales. Nutrification of coastal areas in the wake of deforestation, increased terrestrial run-off over the last century, and overfishing, dating back to the origin of human expansion, have caused coral-algal phase shifts. Global effects such as ocean acidification, deoxygenation, and warming have caused severe mass bleaching and mortality of corals contributing to the demise of corals in all reef provinces over the last decades. However, although the importance of corals and their threats can be assessed on a broad scale (colonies and reefs), it is the individual coral polyp (usually mm in size) and its symbionts that are directly responding to anthropogenic disturbances. Hence, our ability to estimate the response of coral reefs to environmental stressors requires a deep understanding of polyp-scale physiology and the manner in which it reacts to disturbances.

Tropical scleractinian corals live in symbiosis with dinoflagellate algae (Symbiodiniaceae), an association that allows them to recycle essential elements and thrive in nutrient-poor waters. Within the coral tissue, symbiont distribution can be highly heterogeneous, with densities dependent on tissue structure, light, and nutrient availability. Among the Symbiodiniaceae, chlorophyll concentrations can be highly variable, since the coral holobiont can adapt to reduced light by increasing the symbiont and/or chlorophyll densities in order to optimize light harvesting. Symbiont and pigment densities, together with the tissue’s optical properties, generate physiological microniches—suggested to play the role of refugia for minor symbiont populations—which might be important for the coral’s response to stress. Therefore, understanding where the symbionts are located along and across the coral tissue becomes highly relevant, although in vivo studies of Symbiodiniaceae distribution at μ-metric resolutions are still scarce.
The relationship between the coral and its Symbiodiniaceae symbionts is highly complex. Although the coral consumes most of the \( \text{O}_2 \) produced by its symbionts, the surplus diffuses out of the tissue into the coral-water interface—the diffusive boundary layer (DBL). Diffusion acts as a bottleneck for solute exchange and indirectly controls the coral’s physiology and stress response. In the coral Favia sp., spatial variations in \( \text{O}_2 \) and \( \text{CO}_2 \) within a single polyp were attributed to coral morphology causing \( \mu \)-metric variations in the thickness of the DBL and differences in the light field. Similarly higher photosynthetic rates were found in the middle parts of the coralite, tentacles, and tissue surrounding the mouth of Galaxea fascicularis, whereas the wall of the coralite and the coenosarc showed lower photosynthetic rates (see also Figure S1 for details on coral structure). This heterogeneity was attributed to differences in symbiont distribution, localized higher photosynthetic activity in response to increased metabolic demand and local supply of solutes through the DBL, but concurrent assessments of symbiont distribution and \( \text{O}_2 \) and \( \text{CO}_2 \) are so far lacking. The response of corals to environmental stress, and subsequent bleaching, has also been partly attributed to the DBL controlling the exchange of \( \text{O}_2 \) and solutes between the coral and the water column. Although \( \text{O}_2 \) exchange in the coral tissue and DBL has long been considered passive by means of diffusion, more recent work has shown the importance of ciliary currents enhancing the effective diffusivity at the coral surface. Ciliary action was shown to lower excessive \( \text{O}_2 \) concentrations at the coral surface serving predominantly as a homeostatic control mechanism in coral stress response.

The heterogeneities in symbiont distribution, photosynthesis, host activity (respiration), and ciliary flows are therefore likely to result in a complex patterning of the \( \text{O}_2 \) microenvironment of the coral, with differential \( \text{O}_2 \) accumulation and/or consumption and contrasting cellular responses to \( \text{O}_2 \) levels. Although \( \text{O}_2 \) is essential for all aerobic organisms, excess \( \text{O}_2 \) can be detrimental for photoautotrophs especially under high temperatures. Since the photosynthetic apparatus of the symbiont—Rubisco Form II—has a higher affinity to \( \text{O}_2 \) over \( \text{CO}_2 \), the excess of the former causes photorespiration and the production of \( \text{O}_2 \) radicals. This not only entails higher metabolic costs but also may cause cellular damage and, ultimately, coral bleaching. Moreover, the documented high spatial variability of photosynthetic activity raises the question of how corals deal with the potential negative impacts of localized hyperoxia.

Therefore, understanding the \( \text{O}_2 \) dynamics along and across the different compartments of the coral, i.e., DBL, tissue, and symbionts, is of paramount importance since it has a direct influence on its health, performance, and ability to respond to its environment. Here, we provide the first simultaneous assessment of the microscale flow field and two-dimensional \( \text{O}_2 \) distribution in the coral boundary layer under controlled ambient illumination and currents, along with a mapping of Symbiodiniaceae Chl concentrations in the underlying coral tissue. We aim to characterize the Chla distribution inside the coral tissue and relate it with the \( \text{O}_2 \) and flow dynamics at the coral surface, examining the role of ciliary flows in the transport of \( \text{O}_2 \) away from the tissue surface.

**RESULTS AND DISCUSSION**

**Chla concentrations in the coral tissue**

Using hyperspectral imaging technology, we analyzed the areal Chla distribution in the tissue of the coral *Porites lutea* at a sub-millimeter scale. We observed higher normalized absorbance of 0.8 in the coenosarc, indicating high Chla concentration (Figure 1B, ROI 1; Figure 1D, yellow areas). In contrast,
normalized absorbance was 0.3 at the mouth openings, indicating low Chl a concentration (Figure 1B, ROI 2; Figure 1D, blue areas). As the polyps of Porites tend to remain partially retracted during the day in order to enhance planar density and photosynthetic performance of its symbionts, it is no surprise that the coenosarc contains higher Chl a concentrations than the area surrounding the mouth. The quantification and distribution of Chl a densities in the thin veneer of tissue covering the complex coral skeleton has only recently been made possible by the application of high-resolution optical techniques requiring extensive sample preparation or the short-term removal of culturing water. Here, images were taken in a flow-through chamber, without disturbing the coral. We expected the spatial heterogeneities in symbiont distribution to be reflected also in the O2 DBL’s landscape, as the differential diffusion of photosynthesis-derived O2 has been shown to lead to a patchy distribution of O2 diffusing to the surface of the coral, with highest values over the coenosarc and lowest values over the polyp mouths.

Flow field and O2 concentrations at the coral surface
In order to relate the heterogeneous Chla distribution inside the tissue with the O2 dynamics at the DBL, we used particle image velocimetry (PIV) using O2-sensitive nanoparticles in an image velocimetry system (sensPIV) (Figure 2A). We observed a heterogeneous O2 distribution along the coral surface. However, areas of high O2 concentrations (hotspots) coincided with areas of low Chla concentration over the mouth openings of the coral polyps, whereas areas of lower O2 concentrations coincided with areas of high Chla concentration over the coenosarc (Figure 2D). The opposed patchiness suggests the superposition of two independent processes: passive diffusion of O2 from the sites of production (symbionts) and active transport of water by ciliary currents, as outlined below.

Under the flow conditions of our experiments (300 and 1,500 μm s⁻¹), cilia on the surface of the coral generated vortical currents (Figure 2B). At high ambient flow speeds, the average height and width of the vortices were 300 ± 55 μm and 1,500 ± 230 μm, respectively. At low ambient flow speeds (300 μm s⁻¹), the corresponding values were 500 ± 50 μm and 1,500 ± 220 μm, respectively (Figure 2C). Within the vortices, the vertical flow velocities reached up to 300 μm s⁻¹ and ~200 μm s⁻¹ (Figure 2B), in line with previous reports. The vortices’ structure was similar to those found in Pacherres et al. with the upward flow of the vortex facing toward the ambient current, whereas the downward flow was located leeward, suggesting cilia beating against the ambient current (Figure S3). The upward flank of the vortices transported water from the coral polyp periphery aloft (red areas, Figure 2B), whereas the downward flank was situated somewhat leeward of the polyp mouth opening (blue areas, Figure 2B).

As a result, the main part of the vortices was situated on top of the mouth opening. In darkness, we found slightly lowered O2 concentrations in the downwelling areas suggesting respiration (Figure 2C).
with similar velocity magnitudes inside the vortex area (Table S1). In the light, we found high O$_2$ concentrations near the coral surface, with highest values over the mouths and lower values in the periphery (Figure 2D).

**Combining O$_2$ at the DBL and tissue Chla**

The inverse relation between O$_2$ and Chla distributions is in conflict with the current paradigm of an exclusively diffusive transport of O$_2$, which would predict a positive relation between Chla and O$_2$. We found the opposite, a redistribution of O$_2$ by cilia-induced vortical currents dominating O$_2$ transport in *Porites*. The vortices were located above the Chla-poor areas, with their upward flow extending into the adjacent Chla-rich areas (Figure 3). This allows O$_2$ to be transported laterally from the main production site into the vortex, which itself extends vertically as a bulged interface into the ambient water with lower O$_2$. As a result, O$_2$ accumulation in the Chla-rich tissue is alleviated, and at the same time, the high O$_2$ concentration in the vortex increases the concentration gradient toward the ambient water and thus the diffusive flux.

Previous microsensor studies of O$_2$ concentrations at the surface of *Favia* sp. found peaks of gross photosynthetic production in the coenosarc and large variations in the O$_2$ concentrations in the water column above the mouth. On the other hand, *Galaxea fascicularis* showed an order of magnitude increase in gross O$_2$ production over the polyp mouth compared with the coenosarc. Although our observations are in line with some of these early studies, it is important to acknowledge that corals exhibit a high plasticity of forms, tissue properties, symbiont distribution, and Chla content (see introduction), all of which influence the diffusion of solutes along, across, and outside of the tissue. Therefore, further research on the linkages between the different coral compartments, using other coral species, will be needed in order to better understand how coral form and functions are related and the way in which they might optimize coral respond to stressors.

**Simulations of the boundary layer dynamics of *P. lutea***

To investigate the relation between tissue Chla and the diffusive and advective fluxes of photosynthetic O$_2$, we simulated the effects of diffusion and ciliary flow along the coral surface by means of a simplified planar two-layer model. The lower layer has a thickness of 100 $\mu$m, representing the tissue of *P. lutea* in which the Symbiodiniaceae are located, and transport is limited to diffusion (dotted line in Figure 4A). The heterogeneously distributed symbionts produce O$_2$ at a constant rate, assuming constant light regime and no oxidative stress. The layer above represents the water column in which the cilia stir the boundary layer under flow conditions. The ciliary flow is driven by a moving boundary with horizontal velocity components that oscillate in both directions along the surface at an amplitude (maximum ciliary flow) that was adjusted to $c_{vel} = 150$ $\mu$m s$^{-1}$, similar to previous measurements. The undisturbed flow velocity in the simulation was set to 300 $\mu$m s$^{-1}$, similar to the measurements.

The interaction of the flow field and the beating of cilia leads to vertical flow speeds of 80–100 $\mu$m s$^{-1}$, which are slightly lower than the maximum vertical speeds from the experiments (up to 300 $\mu$m, Figure 1B) and the velocities seen in the upward zones of the vortices in Shapiro et al. At the coral surface, the opposing horizontal velocities lead to the formation of two stagnation points: at one stagnation point, the currents converge, driving the flow aloft into the boundary layer; at the other, the currents diverge where the downward flow returns to the coral surface and is deflected sideways. The upward and downward flows drive the vortices lining the surface of the coral. The horizontal extent of the vortices is largely determined by the boundary conditions, i.e., the wavelength of the oscillating boundary, which was adjusted to $\delta = 1,200$ $\mu$m, similar to the average size of the calyx of the coral (see details of calyx size in Figure 3B). The specific shape of the vortices leads to two distinct regions within the coral boundary layer, which are determined by the location of the stagnation points along the surface of the coral (Figure 4A, red dots and dashed line). The first region (Figure 4A, inserted panel: R1) is the vortex proper, where streamlines are closed, indicating no advective exchange with the surrounding water. The second region (Figure 4A, inserted panel: R2) is
characterized by a strong flow lining the periphery of the vortex enhancing the concentration gradient and diffusive exchange with the tissue. In the simulations, O\textsubscript{2} produced diffuses from the coral tissue in proportion to the observed Chl\textsubscript{a} distribution (Figure 2D). For simplicity, the heterogeneity of O\textsubscript{2} production is simulated by a sinusoidal curve whose spatial extent was adjusted to match the experimentally obtained results: highest O\textsubscript{2} production was situated outside the vortex structures, whereas lowest O\textsubscript{2} production was situated underneath the vortex (Figure 3). The produced O\textsubscript{2} diffuses through the tissue before reaching the water column. Along the tissue surface, diffusive exchange fluxes are highest where O\textsubscript{2} gradients between tissue and the ambient water are strongest. The strongest O\textsubscript{2} gradients occur where O\textsubscript{2} is produced at a higher rate and ambient water with low O\textsubscript{2} is directed downward at high velocities (Figure 4A, inserted panel: R2). In contrast, O\textsubscript{2} concentrations within the vortices are elevated, fed by the upward flow of O\textsubscript{2}-enriched water from the tissue surface. Although rotating in the vortex, O\textsubscript{2} diffuses across the vortex boundaries into the ambient water so that the downward flow carries less O\textsubscript{2} and can be recharged again (Figure 4A, inserted panel: R1).

The simulated O\textsubscript{2} distribution (Figure 4A) exhibits a similar pattern as the measured O\textsubscript{2} distribution (Figure 4B); five distinct vortices reaching up to 500 \(\mu\text{m}\) into the flow field become visible where O\textsubscript{2} concentrations are supersaturated. With the model, it is now possible to simulate the interplay of diffusive and advective fluxes, thus resolving the paradox of high O\textsubscript{2} concentrations above low O\textsubscript{2}-production sites (Figure 4).

We used the model to test how the location of the vortices above the coral surface affects the O\textsubscript{2} distribution in the DBL and inside the tissue of the coral. O\textsubscript{2} accumulates in the vortices independent of the relative location of vortices and O\textsubscript{2} production sites (Figure 5A). Consequently, the O\textsubscript{2} concentration in the DBL does not reflect the O\textsubscript{2} concentration and production rate in the tissue directly below but is reshaped by the interacting vortical and ambient flow fields. In general, the vortices help to decrease the O\textsubscript{2} concentration in the underlying tissue, as described by Pacherres et al.\textsuperscript{33} At ciliary flow conditions similar to the ones in the experiments, we found the spatially averaged O\textsubscript{2} concentration in the tissue to be reduced by 53%, compared with no ciliary flow conditions. However, the efficiency of this reduction depends on the relative location of vortices and hotspots of O\textsubscript{2} production, which has a particular impact on harmful excess O\textsubscript{2} concentrations in the tissue (Figures 5B and 5C).

In summary, the simulation shows that the observed O\textsubscript{2} and Chl\textsubscript{a} patterns are not directly connected, but the result of the advective flux interacting with the flow field generated by the cilia (Figure 3). The results further indicate that positioning the vortices adjacent to, rather than above, the O\textsubscript{2} production sites selectively enhances the ventilation of O\textsubscript{2}-producing symbiont patches, which helps avoid photosynthetic inhibition.
**Figure 5. Modeled O2 concentration above ambient in which the location of the vortices is phase-shifted relative to the peaks of O2 production**

(A) A phase shift of 180° (upper panel) implies that upward directed velocities in the vortex coincide with the peaks of O2 concentrations, as seen in the experimental results—Figure 4B. A phase shift of 0° (lower panel) implies that the vortex is located above the zones of increased O2 production. Mouth (M) opening and coenosarc (C). See also Video S1. White bar indicates 500 μm.

(B) Modeled O2 concentration above ambient along the entire tissue domain, where the gray bar indicates the section shown in (A).

(C) Percentage of tissue area that is above a critical threshold of 300 μmol L−1 above ambient O2 concentration. The red and gray bars indicate the minimum and maximum, respectively. See also Figure S5 and Table S2.

---

**Implications for the coral’s response to environmental stress**

Ciliary vortices localized adjacent to specific sites of excess O2 production seem beneficial for the coral in the face of oxidative stress, especially under weak currents, which have been found to exacerbate bleaching.31,52 It has been suggested that photosynthetic activity by the symbionts may be inhibited by O2 accumulation through photorespiration and the formation of reactive oxygen species (ROS).40,53,54 Photorespiration occurs when O2 is used as substrate instead of CO2, resulting in the loss of energy,54 whereas ROS are formed through photoreduction of O2 and are believed to directly damage the photosystem II of the chloroplast.37,56 Our results show that the O2 concentration in the coral tissue can not only be modulated by ciliary flows as such but also depend on the relative location of the vortices with respect to areas of O2 production, further reducing photosynthetic inhibition by the reallocation of O2. The generation of this heterogeneous O2 landscape is of particular importance when taking into consideration that the ability of an organism to adapt to environmental changes, e.g., climate change, highly depends on its life history exposure to short-term and short-scale environmental fluctuations.44,57,58 It is known that adjacent colonies of the same coral species exposed to the same water temperatures can present different bleaching responses,59 and more so, different regions within the same colony may show differential bleaching severities.13 Our findings suggest that microscale heterogeneities in both the internal arrangement of symbionts and the external micro-currents generated by the ciliated surface of the coral represent a composite buffer to mitigate oxidative stress. We postulate that this buffer is an important pre-requisite for coral resilience to bleaching in the face of global warming.

Alternatively, localized vortices might as well be redistributing O2 to areas where the tissue has less symbionts (and therefore has less local supply of O2 for the host tissue). During the day, coral symbionts produce an excess of O2 that by far surpasses the metabolic requirements of a healthy coral.60 However, measurements of O2 concentrations inside the gastric cavity of corals have shown extremely low O2 concentrations even during the day.61 Whether the coral utilizes ciliary flows as an external transport mechanism linking zones of O2 production with zones of O2 consumption would require further exploration.

The observed localized transport mechanism is unlikely to be restricted to O2 but should also be effective for other solutes. With regard to the seawater carbonate system, vortex transport of DIC and protons might be of significant importance to coral calcification and should be considered in studies of coral physiology under climate change.62,63 Since coral calcification depends on pH, light, and currents,63,64 vortex-altered proton transport in the DBL33 along with the coral’s capacity to upregulate its inner calcifying fluid65,66 might help explain the sometimes contradicting, species-specific response of corals to ocean acidification. Research is needed to assess potential vortex effects on coral calcification and coral resilience to ocean acidification.

The ability of corals to enhance mass transport in specific areas (here: the coenosarc) (Figure 2C) might have implications not only in the coral’s response to its environment but also in its relationship with bacteria and viruses that inhabit its surface.67,68 The community of prokaryotic and eukaryotic microbes, viruses, and archaea comprising the coral microbiome is extremely diverse69 and has been associated not only with diseases70 but also with the capacity of corals to resist environmental stress by the acquisition of nutrients, defense against pathogens, horizontal gene transfer, etc.71–73 The ability of cilia to generate chemical microenvironments, such as seen here for the O2 hotspots, could provide an advantage to certain coral-associated microbes by enhancing the transport of the...
metabolic compounds necessary for their development. Further research will be needed in order to unveil the consequence of the observed microenvironment creation and the repercussions it might have upon coral-microbe relations and coral health.

Conclusions

Altogether, this study demonstrates that the spatial arrangement of local interactions between ciliary flows and physiological processes (photosynthesis) can give rise to local heterogeneities in the chemical landscape and solute exchange. It extends the coral’s ability to respond to environmental stressors well into the coral’s boundary layer, where the flow field can be shaped according to local needs. A 3D exploration of the coral DBL, taking into consideration the different elements that intervene in its shape and characteristics, can be an important next step to reveal further insights into the complex coral-water interface and better understand the role of the DBL in the coral’s relationship with its environment.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Experimental set-up
  - Hyperspectral imaging
  - SensPIV measurements
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Model formulation
  - Limitations of the model and topography effects

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.07.071.

ACKNOWLEDGMENTS

We thank Esther Lüdtke and Ulrike Holtz for their help with the culturing of the coral colonies, Dr. Arjun Chernu (ZMT) for providing the hyperspectral camera, and Paul Faerber for technical support. This research was conducted in the framework of the Phd project of C.O.P. at the University of Bremen and the Alfred Wegener Institute (AWI). It was supported and financed by FONDECYT, an initiative from the Consejo Nacional de Ciencia, Tecnología e Innovación (CONICYT), Peru, Contrato 086-2016-FONDECYT and the AWI (PoF4.8: Marine and Polar Life). MPI-MM Bremen provided logistic and instrumental support. S.A. acknowledges funding from the Max Planck Society (MPG). K.K. acknowledges funding from the Grundfos Foundation and a Sapere Aude grant from the Independent Research Fund Denmark (IRFD): DFF-8048-00057B.

AUTHOR CONTRIBUTIONS

C.O.P., S.A., C.R., and M.H. designed the experiments for this study. C.O.P. conducted all the experiments. C.O.P. and S.A. analyzed the sensPIV and hyperspectral data and generated the result figures. M.H. and S.A. worked on the model and its figures. K.K. created and supplied the sensPIV particles necessary for the oxygen experiments. C.O.P., S.A., K.K., C.R., and M.H. contributed to the interpretation of the collected data and conceived and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: March 8, 2022
Revised: July 1, 2022
Accepted: July 26, 2022
Published: August 23, 2022

REFERENCES

1. Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S., and Turner, R.K. (2014). Changes in the global value of ecosystem services. Glob. Environ. Change 26, 152–158.
2. Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradley, R.H., Cooke, R., Erlandson, J., Estes, J.A., et al. (2001). Historical overfishing and the recent collapse of coastal ecosystems. Science 293, 629–637.
3. van der Zande, R.M., Achiatis, M., Bender-Champ, D., Kubicek, A., Dove, S., and Hoegh-Guldberg, O. (2020). Paradise lost: end-of-century warming and acidification under business-as-usual emissions have severe consequences for symbiotic corals. Glob. Change Biol. 26, 2203–2219.
4. McCulloch, M., Fallon, S., Wyndham, T., Hendy, E., Lough, J., and Barnes, D. (2003). Coral reef of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature 421, 727–730.
5. Fabricius, K.E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar. Pollut. Bull. 50, 125–146.
6. Richter, C., Roa-Quiaoit, H., Jantzen, C., Al-Zibdah, M., and Kochzies, M. (2008). Collapse of a new living species of giant clam in the Red Sea. Curr. Biol. 18, 1349–1354.
7. Hughes, T.P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265, 1547–1551.
8. Hughes, D.J., Alderdice, R., Cooney, C., Kühl, M., Pemberton, M., Voolstra, C.R., and Suggett, D.J. (2020). Coral reef survival under accelerating ocean deoxygenation. Nat. Clim. Change 10, 296–307.
9. Knowlton, N., Grottoli, A.G., Kleypas, J.A., Obura, D., Corcoran, E., de Goeij, J.M., Felis, T., Harding, S.P., Mayfield, A.B., Miller, M.W., et al. (2021). Rebuilding Coral Reefs: A Decadal Grand Challenge (International Coral Reef Society).
10. Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., et al. (2017). Global warming and recurrent mass bleaching of corals. Nature 543, 373–377.
11. Hoegh-Guldberg, O., and Jones, R. (1999). Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar. Ecol. Prog. Ser. 183, 73–86.
12. De’ath, G., Fabricius, K.E., Sweatman, H., and Puotinen, M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proc. Natl. Acad. Sci. USA 109, 17995–17999.
13. Putnam, H.M., Barott, K.L., Ainsworth, T.D., and Gates, R.D. (2017). Rebuilding Coral Reefs: A Decadal Grand Challenge (International Coral Reef Society).
14. Muscatine, L. (1973). Nutrition of Corals. In Biology and Geology of Coral Reefs, , O.A. Jones, and R. Endean, eds. (Academic Press), pp. 77–115.
15. Muller, E.B., Kooijman, S.A.L.M., Edmundo, P.J., Doyle, F.J., and Nisbet, R.M. (2009). Dynamic energy budgets in syntrophic symbiotic relationships between heterotrophic hosts and photoautotrophic symbionts. J. Theor. Biol. 259, 44–57.

4156 Current Biology 32, 4150–4158, October 10, 2022
16. Muscatine, L., Ferrier-Pagés, C., Blackburn, A., Gates, R.D., Baghdasarian, G., and Alemand, D. (1998). Cell-specific density of symbiotic dinoflagellates in tropical anthozoans. Coral Reefs 17, 329–337.

17. Wangpraseurt, D., Wemtzel, C., Jacques, S.L., Wagner, M., and Kühl, M. (2017). In vivo imaging of coral tissue and skeleton with optical coherence tomography. J. R. Soc. Interface 14, 20161003.

18. Laisise, P.P., Roberson, L., Gu, Y., Qian, C., and Smith, D.J. (2020). Long-term imaging of the photosensitive, reef-building coral Acropora m鲁tica using light-sheet illumination. Sci. Rep. 10, 10369.

19. Dubinsky, Z., Falkowski, P.G., Portal, J.W., Muscatine, L., and Smith, D.C. (1984). Absorption and utilization of radiant energy by light- and shade-adapted colonies of the hermatypic coral Stylophora pistillata. Proc. R. Soc. Lond. B 222, 203–214.

20. Falkowski, P.G., and Dubinsky, Z. (1981). Light-shade adaptation of Stylophora pistillata, a hermatypic coral from the Gulf of Elat. Nature 289, 172–174.

21. Wangpraseurt, D., Larkum, A.W., Ralph, P.J., and Kühl, M. (2012). Light gradients and optical microniches in coral tissues. Front. Microbiol. 3, 316.

22. Al-Horani, F.A., Al-Moghribi, S.M., and de Beer, D. (2003). The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral Galaxea fasciulata. Mar. Biol. 142, 419–426.

23. Shashar, N., Cohen, Y., and Loya, Y. (1993). Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. Biol. Bull. 185, 455–461.

24. Kühl, M., Cohen, Y., Dalsgaard, T., Jørgensen, B.B., and Revsbech, N.P. (2000). A microsensor study of light enhanced Ca$^{2+}$ uptake and photosynthesis in the reef-building coral Porites lutea. Limnol. Oceanogr. 45, 247–256.

25. Lesser, M.P. (1996). Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in ymbiotic dinoflagellates. Limnol. Oceanogr. 41, 271–283.

26. Shapiro, O.H., Fernandez, V.I., Garren, M., Debaillon-Raymundo, L.J. (2021). Going with the flow: how corals in high-flow environments enhance photosynthesis in marine benthic autotrophs by increasing the light available to the phototrophic partner. Cell Rep. Methods 3, 169–178.

27. Jordan, D.B., and Ogren, W.L. (1981). Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase. Nature 291, 513–515.

28. Shapiro, O.H., Fernandez, V.I., Garren, M., Debaillon-Raymundo, L.J. (2021). Going with the flow: how corals in high-flow environments enhance photosynthesis in marine benthic autotrophs by increasing the light available to the phototrophic partner. Cell Rep. Methods 3, 169–178.

29. Al-Horani, F.A., Ferdelman, T., Al-Moghrabi, S.M., and de Beer, D. (2005). Water flow facilitates coral calcification. Coral Reefs 24, 109–118.

30. Nakamura, T., van Woesik, R., and Yamazato, K. (2003). Water flow facilitates coral calcification. Coral Reefs 22, 2527–2531.
in *Symbiodinium* sp., symbiotic dinoflagellates of cnidarians. New Phytol. 204, 81–91.

57. Brown, B., Dunne, R., Goodson, M., and Douglas, A. (2002). Experience shapes the susceptibility of a reef coral to bleaching. Coral Reefs 21, 119–126.

58. Safaie, A., Silbiger, N.J., McClanahan, T.R., Pawlak, G., Barshis, D.J., Hench, J.L., Rogers, J.S., Williams, G.J., and Davis, K.A. (2018). High frequency temperature variability reduces the risk of coral bleaching. Nat. Commun. 9, 1671.

59. Cunning, R., Ritson-Williams, R., and Gates, R.D. (2016). Patterns of bleaching and recovery of *Montipora capitata* in Kane‘ohe Bay, Hawaii, USA. Mar. Ecol. Prog. Ser. 557, 131–139.

60. Al-Horani, F.A., Al-Moghrabi, S.M., and de Beer, D. (2003). Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. J. Exp. Mar. Biol. Ecol. 286, 1–15.

61. Agostini, S., Suzuki, Y., Higuchi, T., Casareto, B.E., Yoshinaga, K., Nakano, Y., and Fujimura, H. (2012). Biological and chemical characteristics of the coral gastric cavity. Coral Reefs 31, 147–156.

62. Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Safaie, A., Silbiger, N.J., McClanahan, T.R., Pawlak, G., Barshis, D.J., Cunning, R., Ritson-Williams, R., and Gates, R.D. (2016). Patterns of bleaching and recovery of *Montipora capitata* in Kane‘ohe Bay, Hawaii, USA. Mar. Ecol. Prog. Ser. 557, 131–139.

63. Comeau, S., Cornwall, C.E., Pupier, C.A., DeCarlo, T.M., Alessi, C., Treherm, R., and McCulloch, M.T. (2019). Flow-driven micro-scale pH variability affects the physiology of corals and coralline algae under ocean acidification. Sci. Rep. 9, 12829.

64. Chan, N.C.S., Wangpraseurt, D., Kühl, M., and Connolly, S.R. (2016). Flow and coral morphology control coral surface pH: implications for the effects of ocean acidification. Front. Mar. Sci. 3, 10.

65. McCulloch, M., Falter, J., Trotter, J., and Montagna, P. (2012). Coral resilience to ocean acidification and global warming through pH up-regulation. Nat. Clim. Change 2, 623–627.

66. McCulloch, M.T., D’Olivo, J.P., Falter, J., Holcomb, M., and Trotter, J.A. (2017). Coral calcification in a changing world and the interactive dynamics of pH and DIC upregulation. Nat. Commun. 8, 15686.

67. Ainsworth, T.D., Fordyce, A.J., and Camp, E.F. (2017). The other microeukaryotes of the coral reef microbiome. Trends Microbiol. 25, 980–991.

68. Webster, N.S., and Reusch, T.B.H. (2017). Microbial contributions to the persistence of coral reefs. ISME J. 11, 2167–2174.

69. Bourne, D.G., Morrow, K.M., and Webster, N.S. (2016). Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu. Rev. Microbiol. 70, 317–340.

70. Sweet, M.J., and Séré, M.G. (2016). Ciliate communities consistently associated with coral diseases. J. Sea Res. 113, 119–131.

71. Gerardo, N.M. (2013). The give and take of host-microbe symbioses. Cell Host Microbe 14, 1–3.

72. Weyl, L., Edwards, R., Rodriguez-Brito, B., Liu, H., and Rohwer, F. (2007). Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ. Microbiol. 9, 2707–2719.

73. van Oppen, M.J.H., Leong, J.-A., and Gates, R.D. (2009). Coral–virus interactions: a double-edged sword? Symbiosis 47, 1–8.

74. Klein, R., Pätzold, J., Wefer, G., and Loya, Y. (1993). Depth-related timing of density band formation in *Porites* spp. corals from the Red Sea inferred from X-ray chronology and stable isotope composition. Mar. Ecol. Prog. Ser. 97, 99–104.

75. Veron, J.E.N. (2000). Corals of the World (Australian Institute of Marine Science).

76. Wall, M., Putchim, L., Schmidt, G.M., Jantzen, C., Khokiattiwong, S., and Richter, C. (2015). Large-amplitude internal waves benefit corals during thermal stress. Proc. Biol. Sci. 282, 20140650.

77. Hackerott, S., Martell, H.A., and Erín-Lopez, J.M. (2021). Coral environmental memory: causes, mechanisms, and consequences for future reefs. Trends Ecol. Evol. 36, 1011–1023.

78. Koren, K., Brodersen, K.E., Jakobsen, S.L., and Kühl, M. (2015). Optical sensor nanoparticles in artificial sediments – a new tool to visualize O₂ dynamics around the rhizome and roots of seagrasses. Environ. Sci. Technol. 49, 2286–2292.

79. Willert, C., Stasicki, B., Kliner, J., and Moessner, S. (2010). Pulsed operation of high-power light emitting diodes for imaging flow velocimetry. Meas. Sci. Technol. 21, 075402.

80. Bartzke, G., Siemann, L., Büssing, R., Nardone, P., Koll, K., Hebbeln, D., and Huhn, K. (2021). Investigating the prevailing hydrodynamics around a cold-water coral colony using a physical and a numerical approach. Front. Mar. Sci. 8, 1375.
STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Experimental models: Organisms/strains** | | |
| *Porites lutea* | Alfred Wegener Institute, Claudio Richter, Pacherres et al. | [https://doi.org/10.1242/jeb.085548](https://doi.org/10.1242/jeb.085548) |
| **Software and algorithms** | | |
| sensPIV processing | Ahmerkamp at al. | [https://doi.org/10.1016/j.crmeth.2022.100216](https://doi.org/10.1016/j.crmeth.2022.100216) |
| Matlab 2018b | Mathworks | [https://de.mathworks.com](https://de.mathworks.com) |
| Comsol Multiphysics v5.6 | Comsol | [https://www.comsol.com](https://www.comsol.com) |
| Python | Phyton | [https://www.python.org/](https://www.python.org/) |
| SensorTrace-PRO | Unisense | N/A |
| Micro-hyperspectral algorithm | This paper | [https://doi.org/10.6084/m9.figshare.20347899.v1](https://doi.org/10.6084/m9.figshare.20347899.v1) |
| **Other** | | |
| RGB cmos camera | FLIR | GS3-U3-51S5C-C |
| Lens array | Optem Fusion | N/A |
| LED pulsing system | iLA_5150 | LPS3 |
| Gear Pump | Ismatec | ISM901B |
| Long-pass filter | Midopt | LP515 |
| Polyester Optical Filters | Lee Felters | O10 Medium Yellow |
| Temperature sensor | PyroScience | Pt100 |
| Oxygen microsensor | Unisense | OX-10 |
| Micromanipulator | Unisense | N/A |
| Fiber optic lamp | Schott | 1500 |
| Lens for hyperspectral system | Optem Fusion | N/A |
| Hyperspectral camera | FLIR | Blackfly |
| Micromanipulator | PyroScience | N/A |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources should be directed and will be fulfilled by the lead contact, Cesar O. Pacherres ([cesar.pacherres@bio.ku.dk](mailto:cesar.pacherres@bio.ku.dk)).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- All data reported in this paper will be shared by the lead contact upon request.
- All original code is publicly available as of the date of publication. DOI is listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Colonies of the massive coral *Porites lutea*, reared at the aquaria facilities of the Alfred Wegener Institute (AWI) were used as fragment source. They were kept in artificial seawater (salinity 32.25 ± 2.89) (Dupla Marine Premium Reef Salt Natural Balance) under conditions mimicking those found at the depth of their origin (15 m), i.e. 25.2 ± 0.17 °C, a 12-h light-dark cycle, light intensities between 75 and 80 μmol quanta m⁻² s⁻¹ (LI-COR LI-192, USA) (see also Figure S4) and a pH of 7.9 ± 0.11 (YSI, USA) (see also Table S3 for extended parameters). Food was provided in the form of freshly hatched Artemia nauplii every second day. Before the start of the experiments,
The stock solution contained 2 mg mL\(^{-1}\) which was further diluted by a factor of 200. Illumination was achieved using a LED pulsing system (LPS3, iLA_5150, Germany) connected to LED light sheet optics similar to the one described by Willert et al.\(^{79}\) The light sheet was approx. 1 mm thick and intensity reached 4500 mol quanta m\(^{-2}\) s\(^{-1}\) (LI-COR LI-192, USA). In order to resolve the O\(_2\) and flow field around the coral fragment, we used O\(_2\) sensitive nanoparticles,\(^{78}\) which have proven ideal for mapping O\(_2\) concentrations at relevant scales.\(^{50}\)

The stock solution contained 2 mg mL\(^{-1}\) which was further diluted by a factor of 200. Illumination was achieved using a LED pulsing system (LPS3, iLA_5150, Germany) connected to LED light sheet optics similar to the one described by Willert et al.\(^{79}\) The light sheet was approx. 1 mm thick and intensity reached 4500 mol quanta m\(^{-2}\) s\(^{-1}\) (LI-COR LI-192, USA) at a wavelength of around 468 nm. For each experiment one hundred images were captured under light and dark conditions (180 and 0 mol quanta m\(^{-2}\) s\(^{-1}\) respectively). To resolve the O\(_2\) and flow field around the coral fragment, we used O\(_2\) sensitive nanoparticles,\(^{78}\) which have proven ideal for mapping O\(_2\) concentrations at relevant scales.\(^{50}\)
during 50 ms illumination of the camera chip. The recorded images were post-processed using custom-built Matlab (MathWorks, R2018b) algorithms to obtain the O$_2$ concentration signal and a map of the particle movement inside the chamber.

Nanoparticle readings were later compared and calibrated with O$_2$ profiles, performed before each experiment, using an electrochemical sensor of 10 μm tip diameter (Unisense, Denmark). The profile was recorded 3 min before the images were captured. Values were recorded using the SensorTrace-PRO software (Unisense, Denmark). For each experiment, microelectrodes were 2-point calibrated in O$_2$-free (bubbling pre-filtered seawater with $N_2$ gas for 10 min) and air-saturated FASW of known salinity and temperature. The tip of the sensor was carefully placed at the surface of the coral. A micromanipulator (Unisense, Denmark) was programmed to move the sensor up in 20 μm steps. The range of the vertical profile was 1000 μm. At each step, dissolved O$_2$ was measured one time with a sampling interval of 2 s.

To directly relate the O$_2$ production of the coral colony with the Chl$\alpha$ concentration inside the coral tissue we carefully pinpoint the area of the coral observed through the camera and later extracted the Chl$\alpha$ concentrations from the same area out of the hyperspectral images.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Model formulation**

The cilia beating of corals generates a complex flow pattern, which is reproduced using numerical simulations which were performed in Comsol (Comsol Multiphysics, V5.6). The flow field is given by the Stokes equations:

$$0 = - \nabla p + \mu \nabla^2 \mathbf{u}$$

(Equation 1)

Where $\mathbf{u}$ is the velocity vector, $p$ the pressure, $\mu$ dynamic viscosity and $\nabla$ the gradient-operator. The continuity equation for incompressible fluids reads:

$$\nabla \cdot \mathbf{u} = 0$$

(Equation 2)

The O$_2$ distribution was calculated solving the stationary advection-diffusion equations:

$$0 = D \nabla^2 C - \mathbf{u} \cdot \nabla C + R_H$$

(Equation 3)

$R_H$ is the volumetric O$_2$ production rate (see below), $D$ is the diffusion coefficient for O$_2$ in sea water at 20°C: 2.1 10$^{-9}$ m$^2$ s$^{-1}$. According to the model proposed by Shapiro, the cilia-induced currents were simulated assuming oscillating horizontal velocity components at the coral surface (slip boundary condition):

$$u_x = c_{vel} \cdot \sin\left(\frac{2\pi}{d} x\right)$$

(Equation 4)

where $x$ is the horizontal distance, $c_{vel}$ the maximum ciliate beating velocity and $d$ is the characteristic length scale of the vortices, see text for values and explanation. Volumetric rates of O$_2$ production in the tissue were simulated based on the assumption that the clustering of chlorophyll a at certain areas of the tissue follows a sinus curve:

$$R_H = R_C \left(1 + \sin\left(\frac{2\pi}{d} x + m\pi\right)\right)$$

(Equation 5)

Where $R_C$ is the production rate, $m$ is the parameter for the relative phase shift. The mathematical model allowed us to simulate O$_2$ concentrations inside the DBL and in the tissue in the presence of a heterogeneous O$_2$ production rate by the coral.

**Limitations of the model and topography effects**

We applied a simplified 2D transport-reaction model in order to investigate the effect of the ciliary flow on the O$_2$ concentration inside the tissue and within the coral boundary layer. The model approach does not consider 3D effects or changes in the topography of the coral, which might become substantial when investigating the turbulent flow field along an entire coral colony see for example Bartzke et al. However, on the scales of the investigated coral fragments, the topographical features where an order of magnitude smaller (21 – 123 μm) than the observed vortices (typically around 1 mm) and were not a substantial factor within the experiments. It remains a task for future studies to investigate how O$_2$ is re-distributed along an entire coral colony and how topography and ciliary flow act in tandem for coral ventilation.

Further, in the modelling approach we investigated the effect of the ciliary flow by comparing the tissue volume below and above a specific O$_2$-threshold of 300 μmol L$^{-1}$ (Figure S5). This O$_2$-threshold was inferred from an experiment in which a microsensor was placed directly above the coral tissue and the light intensity was increased until O$_2$ production was affected (see Figure S5). However, to test the sensitivity of the obtained model results, we tested varying thresholds between 200 μmol L$^{-1}$ and 400 μmol L$^{-1}$. Overall, the pattern of maxima and minima along the different phase shifts persisted. Only the difference between the maximum and minimum varied between 6-19 % (compared to 18 % for 300 μmol L$^{-1}$, see Table S2).