Light microenvironment and single-cell gradients of carbon fixation in tissues of symbiont-bearing corals

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23 **Abstract**

24 **Recent coral optics studies have revealed the presence of steep light gradients and**
25 **optical microniches in tissues of symbiont-bearing corals. Yet, it is unknown whether**
26 **such resource stratification allows for physiological differences of *Symbiodinium* within**
27 **coral tissues. Using a combination of stable isotope labelling and nanoscale secondary**
28 **ion mass spectrometry (NanoSIMS), we investigated *in hospite* carbon fixation of**
29 **individual *Symbiodinium* as a function of the local O_2 and light microenvironment**
30 **within the coral host determined with microsensors. We found that net carbon fixation**
31 **rates of individual *Symbiodinium* cells differed on average about 6-fold between upper**
32 **and lower tissue layers of single coral polyps, whereas the light and O_2**
33 **microenvironments differed approximately 15-and 2.5-fold, respectively, indicating**
34 **differences in light utilisation efficiency along the light microgradient within the coral**
35 **tissue. Our study suggests that the structure of coral tissues might be conceptually**
36 **similar to photosynthetic biofilms, where steep physico-chemical gradients define form**
37 **and function of the local microbial community.**

38 The quantity and quality of solar radiation is arguably the most important
39 environmental resource that affects the structure and function of photosynthetic communities
40 in both terrestrial and aquatic environments. Sunlight is of key importance for symbiont-
41 bearing corals, driving the symbiotic interaction between the coral animal and its
42 photosynthetic microalgae of the genus *Symbiodinium* (Roth, 2014). Light attenuation
43 through the water mass and over the reef matrix has a fundamental role in structuring
44 morphology, function and distribution of corals and their symbiotic algae with depth
45 (Falkowski *et al.*, 1990). Recent studies on the optical properties of corals have shown that
46 light is also a highly stratified resource at the level of individual coral polyps and tissue layers
47 (Wangpraseurt *et al.*, 2014). Steep light gradients exist within the polyp tissues of some

corals and light can attenuate by more than an order of magnitude within tissues, *i.e.*, comparable to the attenuation that can occur in open oceanic waters between the surface and >25 m of water depth (Kirk, 1994; Wangpraseurt *et al.*, 2012). In this study, we investigated whether such light gradients within coral tissues are correlated with a stratification of *Symbiodinium* physiology *in hospite*.

We used fiber-optic and electrochemical microsensors together with stable isotopic labelling and nanoscale secondary ion mass spectrometry (NanoSIMS) to estimate single-cell carbon fixation rates across light gradients within coral tissues. We collected several fragments of *Favites sp.* from the Heron Island reef flat (152° 69'E, 20° 299'S), Great Barrier Reef, Australia. Fragments were cultured under a downwelling photon irradiance (400-700 nm) of ~100 µmol photons m^{-2} s^{-1} (12/12 h cycle), in aerated seawater (25°C, salinity 33). Photosynthesis-irradiance curves for the investigated corals were determined with an imaging pulse amplitude modulated fluorometer (I-PAM, Walz GmbH, Germany; Ralph *et al.*, 2005). Values for saturating irradiance, E_{max}, and irradiance at onset of saturation, E_k, were ~350 µmol photons m^{-2} s^{-1} and ~160 µmol photons m^{-2} s^{-1}, respectively (data not shown). These values are typical for healthy corals kept under moderate irradiance (Ralph *et al.*, 2005). To ensure incubations at irradiance levels where photosynthesis and irradiance correlated linearly, *i.e.*, on the linearly increasing part of the P vs I curve, all experiments were performed at ~80 µmol photons m^{-2} s^{-1} (12/12 h cycle). Microsensor measurements of scalar irradiance (tip size ~ 60 µm; Lassen *et al.*, 1992) and O_2 concentration (OX-50, tip size 50 µm, Unisense A/S Aarhus) were performed within the polyp and coenosarc tissues of corals as described previously (Figure 1A, B; Wangpraseurt *et al.*, 2012). After microsensor measurements, corals were incubated with ^{13}C-bicarbonate (Supplementary Text S1). NanoSIMS imaging was then applied on coral tissue sections, as described by Pernice *et al.* (2014) to quantify the assimilation of dissolved inorganic carbon into individual

Symbiodinium cells across polyp (oral and aboral) and coenosarc tissues of corals. Briefly, corals were incubated in small aquaria with 2 mM $NaH^{13}CO_3$ in artificial seawater (recipe adapted from Harrison *et al.*, 1980). After 24 hours of isotopic incubation, coral fragments were sampled, chemically fixed and processed for NanoSIMS analyses (see Kopp *et al.*, 2013; Pernice *et al*., 2012; Pernice *et al*., 2014; and Supplementary Text S1, Figure S1).

Our combined approach of using NanoSIMS and microsensors within the tissue of corals provides, to the best of our knowledge, the first evidence for physiological differences of individual *Symbiodinium* cells *in hospite* in relation to the local microenvironmental conditions across different coral tissue layers, *i.e.*, oral vs aboral parts of polyp and coenosarc. Quantitative analysis based on tissue sections from different coral tissue layers showed that mean incorporation of ^{13}C-bicarbonate by individual *Symbiodinium* cells was up to 6.5-fold higher in the upper oral polyp and coenosarc tissues compared to the lowermost layer of polyp tissues (δ^{13}C: 1609 ± 147‰, n = 25 for *Symbiodinium* cells in upper oral polyp tissue; 1696 ± 205‰, n = 33 for *Symbiodinium* cells in coenosarc tissue and 246 ± 82‰, n = 17 for *Symbiodinium* cells in the lowest aboral layer of polyp tissue). Although the sample sizes in this study are small and the ^{13}C signal is heterogeneous within individual *Symbiodinium* cells (because of carbon fixation hotspots in specific compartments; Supplementary Figure S2; Kopp *et al.*, 2015), the magnitude of the difference in mean ^{13}C incorporation between the aboral part of the polyp and the 2 other parts of coral tissue was clear and statistically significant (one-way ANOVA $F_{2,75} = 15.91$; $p < 0.0001$; 6.5-fold increase in polyp oral vs aboral polyp tissue, Fischer LSD $p < 0.0001$; 6.9-fold increase in coenosarc vs aboral polyp tissue Fischer LSD $p < 0.0001$; and no significant difference between oral polyp vs coenosarc tissue, Fischer LSD $p = 0.718$; Figure 1C, D, E, and F; Supplementary Table S1). The internal microenvironment within the corresponding polyp tissues was highly stratified with respect to light and O_2 (Figure 1G, H). Scalar irradiance

98 decreased about 15-fold from the surface to the bottom of the polyp tissues. Gradients of O_2
99 were less steep but still significant, with an approximate reduction in O_2 concentration by
100 about 2.5 times (Figure 1; Supplementary Table S2, S3; ANOVA F_1=16,4; p = 0.006).

101 These results suggest that coral tissues are vertically stratified systems that affect the
102 physiological activity of their symbionts along a fine-scale microenvironmental gradient. The
103 presence and role of microscale heterogeneity has hitherto largely been ignored in the field of
104 coral symbiosis research, while much is known for other photosynthetic tissues. For instance,
105 for terrestrial plant leaves and for aquatic photosynthetic biofilms, it is known that the
106 photosynthetic unit can adapt to microenvironmental light gradients, where
107 chloroplasts/phototrophs harboured in low-light niches show increased photosynthetic
108 quantum efficiencies at low light levels (Al-Najjar *et al*., 2012; Terashima and Hikosaka,
109 1995). While the steady state O_2 concentration values reported here are a function of the
110 different metabolic processes of the coral holobiont (i.e. *Symbiodinium* photosynthesis and
111 the combined respiration by the coral host, *Symbiodinium* and microbes), the NanoSIMS
112 approach allowed us to separate ^{13}C fixation of *Symbiodinium* from the host metabolic
113 activity. Our study provides the first experimental evidence from carbon fixation
114 measurements that *Symbiodinium* cells can adapt to optical microniches in coral tissues. The
115 15-fold reduction in irradiance with depth in the coral tissue led only to an approximate 6.5-
116 fold reduction in net carbon fixation suggesting enhanced light harvesting efficiency or a
117 reduced P/R ratio for *Symbiodinium* harboured in aboral tissues. While such enhanced
118 efficiency under low light often reflects adaptation of the photosynthetic apparatus (e.g. an
119 increase in light harvesting complexes (Walters, 2005) and reduced cell respiration (Givnish,
120 1988) it might additionally be the result of physiologically distinct populations or clades of
121 *Symbiodinium*. Several studies have revealed remarkable genetic and physiological diversity
122 among different *Symbiodinium* clades (Loram *et al*., 2007; Stat *et al*., 2008; Baker *et al*.,

123 2013, Pernice *et al.*, 2014). Although *Favites sp.* corals from Southern Great Barrier Reef are
124 generally reported in association with one specific *Symbiodinium type* (clade *C3*; Tonk *et al.*,
125 2013), *Symbiodinium* diversity within the microenvironment of these common corals could
126 have been overlooked and such physiological diversity could further provide selective
127 advantage to different genotypes in microenvironments within coral tissue. Coral tissues
128 might thus exhibit similar characteristics to photosynthetic biofilms where steep physico-
129 chemical microgradients give rise to different pheno- and ecotypes of phototrophs along
130 those gradients (Musat *et al*., 2008; Ward *et al.*, 1998).

131 These first experiments were performed under sub-saturating irradiance of ~80 µmol
132 photons m^{-2} s^{-1}. Earlier studies showed that the local scalar irradiance in upper vs deeper
133 tissue layers relates to the incident photon irradiance in a linear fashion such that at stressful
134 incident irradiance levels of e.g. 2000 µmol photons m^{-2} s^{-1}, light levels in the lowermost
135 polyp tissue layers are ~200 µmol photons m^{-2} s^{-1} (Wangpraseurt *et al.*, 2012), still
136 representing optimal conditions for photosynthesis. We thus consider it likely that excess
137 irradiance triggering photoinhibition in oral tissues is unlikely to cause photoinhibition of
138 *Symbiodinium* in aboral polyp tissues. The internal light field is species-specific and in some
139 thin-tissued, branching corals such as *Pocillopora damicornis*, intra-tissue light attenuation is
140 not very pronounced (Szabó *et al*., 2014, Wangpraseurt *et al.*, 2012). The ability to harbor
141 *Symbiodinium* cells in low-light niches might be an important resilience factor for thick-
142 tissued corals, such as massive faviids, during and after coral bleaching. Our study gives first
143 insights to the functional diversity of *Symbiodinium* along microscale gradients in coral tissue
144 and underscores the importance of considering such heterogeneity in studies linking symbiont
145 diversity and coral physiology responses to environmental stress factors.

146

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The authors declare no conflict of interest.

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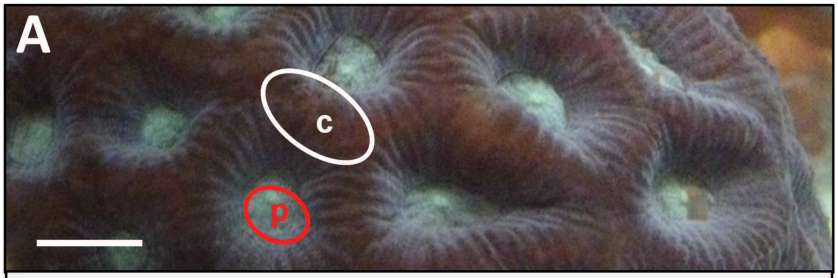
Titles and legends to figures

Figure 1

Internal microenvironment and single-cell ^{13}C assimilation by *Symbiodinium* cells within *Favites sp.* (A) Representative measurement locations indicating connecting tissue (c, coenosarc; white circle) and polyp tissue (p; red circle). Scale bar is 0.5 cm. (B) Schematic diagram of the vertical arrangement of the polyp tissue structure (not drawn to scale). The coral tissue consists of oral and aboral gastrodermal tissues that contain photosymbiont cells (approx 10 µm in diam.). The two tissue layers are separated by a flexible gastrodermal cavity and the entire mean polyp tissue thickness was 1150 µm (±385 SD, *n*=8) as determined by microsensor profiles. The NanoSIMS images (C-E) show the $^{13}C/^{12}C$ isotopic ratio for *Symbiodinium* cells in coenosarc tissue (C), the upper oral polyp tissue (D), and in the lowest layer of aboral polyp tissue (E). Scale bars are 10 µm. The color scale of the NanoSIMS images is in Hue Saturation Intensity (HSI) ranging from 220 in blue (which corresponds to natural $^{13}C/^{12}C$ isotopic ratio of 0.0110) to 1000 in red (which corresponds to $^{13}C/^{12}C$ isotopic ratio of 0.05, ~4.5 times above the natural $^{13}C/^{12}C$ isotopic ratio). Quantification of ^{13}C enrichment of individual *Symbiodinium* cells was obtained by selecting Regions of Interest (ROIs) that were defined in Open_MIMS (http://nrims.harvard.edu/software/openmims) by drawing the contours of the *Symbiodinium* cells directly on the NanoSIMS images. (F) Mean enrichment measured in *Symbiodinium* cells by NanoSIMS, in coenosarc tissue (in white, *n*=33), in upper oral polyp tissue (in grey, *n*=25), in the lowest layer of polyp tissue (in turquoise, *n*=17), and in the control treatment (*n*=20). Bars in the histograms indicate the standard error of the mean enrichment quantified for the different whole *Symbiodinium* cells for each tissue category. Microsensor measurements of (G) scalar irradiance and (H) O_2 performed along depth gradients within the polyp tissue (mean ±SD, *n*=4). Measurements were averaged for the first 100 µm from the tissue surface (oral) and the last 100 µm from the skeleton (aboral). The oral and aboral depth was defined through gentle touching of the microsensor tip at the surface of the coral tissue and skeleton, respectively.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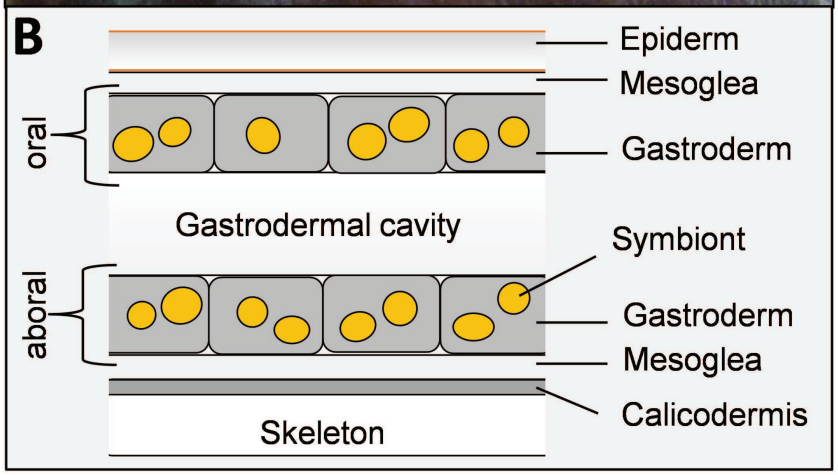
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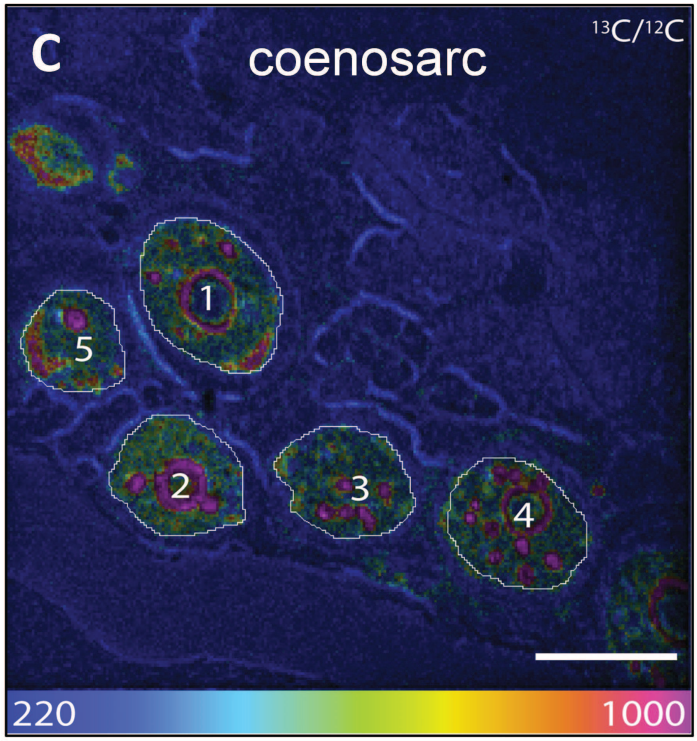
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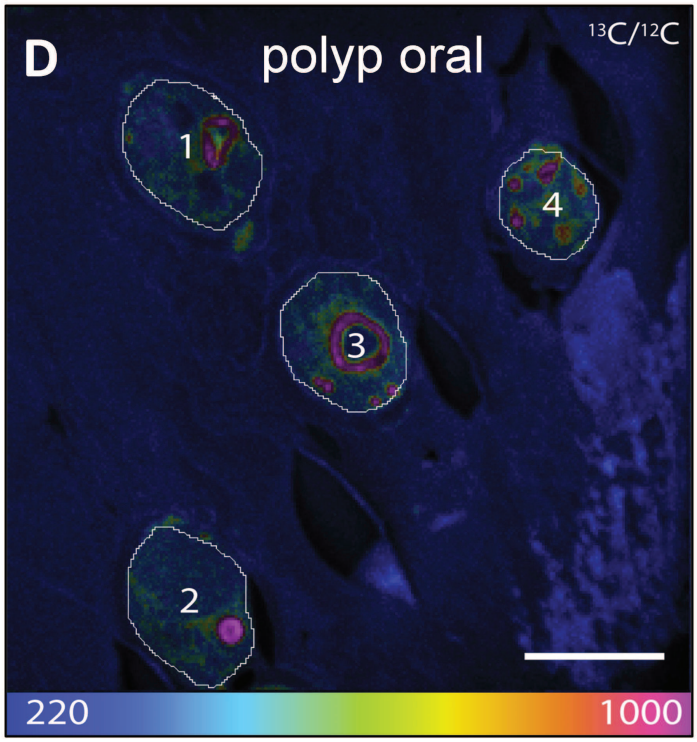
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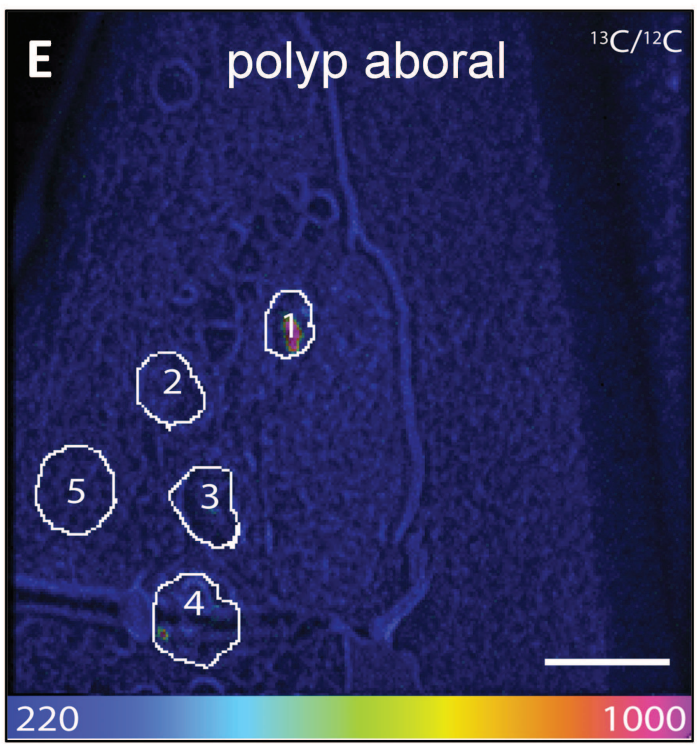
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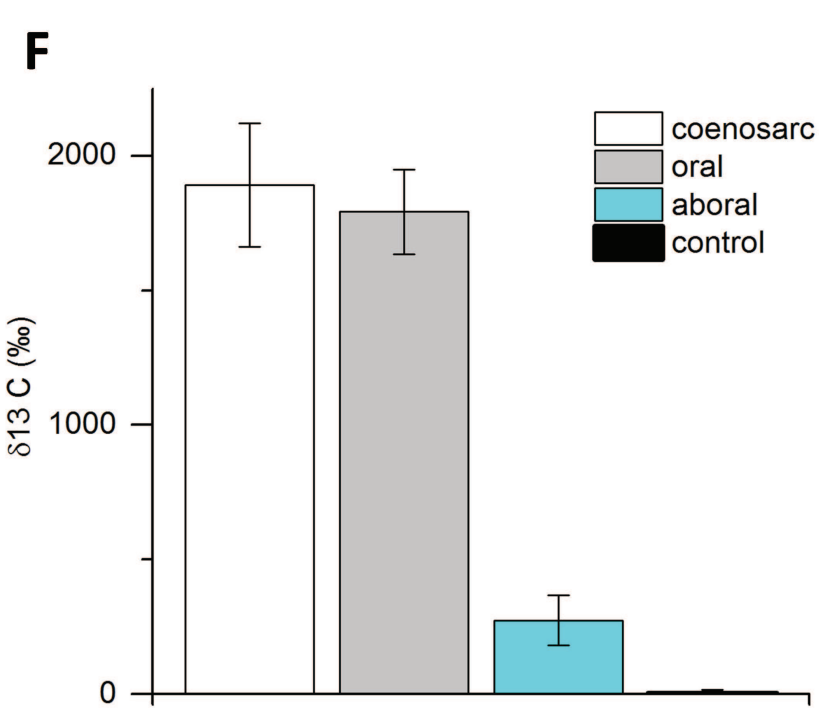
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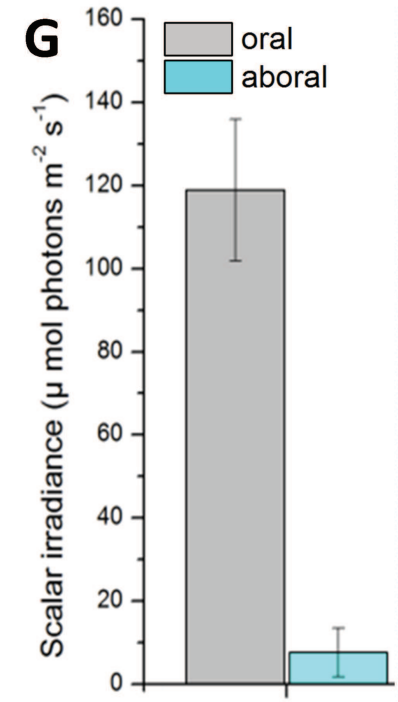
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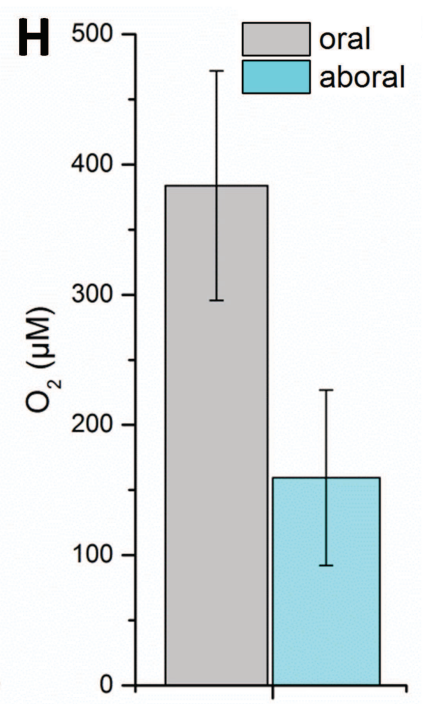
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