The influence of photosynthesis on host intracellular pH in scleractinian corals

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INTRODUCTION
Photosynthetic symbioses in Cnidaria are major contributors to the structural and trophic foundation of shallow water coral reef ecosystems at tropical and subtropical latitudes. Reef-building corals form a symbiosis with photosynthetic dinoflagellates belonging to the genus Symbiodinium, which reside intracellularly in host endodermal cells (Muscatine, 1990; Trench, 1993). This endosymbiosis provides corals with access to a valuable source of photosynthetically fixed carbon that is used for host respiration and other essential processes (Muscatine, 1990; Venn et al., 2008; Yellowlees et al., 2008).

The relationship between corals and their symbiotic dinoflagellates is sensitive to changes in the marine environment associated with climate change (Hoegh-Guldberg, 1999; Hoegh-Guldberg et al., 2007). Elevations in seawater temperature and ocean acidification can exert physiological stress on both coral and dinoflagellate partners (Lesser, 2007; Anthony et al., 2008; Crawley et al., 2010). Much research on coral biology is currently directed towards improving our understanding of how and why corals are sensitive to environmental change, but the field is currently impeded as this previous study was built on a single time point measurement of all organisms and most organisms seek to minimise variations in pH by a system of intracellular buffers and membrane transporters in order to maintain steady-state metabolism (Busa and Nuccitelli, 1984; Casey et al., 2010).

When changes in pH do occur, they are frequently linked with transitions such as changes in rates of cell metabolism and events such as cell activation and division (Roos and Boron, 1981; Buda, 1986; Casey et al., 2010). In algae, pH also significantly increases on exposure to light because of the activity of photosynthesis (Smith and Raven, 1979; Kurkdjian and Guern, 1989). For example, differences of 0.4–0.5 pH units have been observed between photosynthesising and non-photosynthesising cells of the giant single-celled algae Chaetosmorpha darwinii (Raven and Smith, 1980) and the dinoflagellate Prorocentrum micans. The single previous study performed on cnidarian pH suggests that coral pH may also be responsive to light (Venn et al., 2009). It was observed that pH in coral cells containing dinoflagellate symbionts exposed to light have a higher pH than those kept in dark conditions. However, for a more complete understanding of the interactions between host pH and photosynthesis, further research into pH dynamics is required as this previous study was built on a single time point measurement with a fixed light intensity.

Important advances in the understanding of intracellular pH regulation in many organisms including corals have been facilitated by the use of pH-sensitive intracellular dyes (Dubbin et al., 1993; Lemasters et al., 1999). Certain dyes [such as carboxyseminaphthodiazfluor-1 (SNARF-1)] emit fluorescence at two wavelengths, which can be calibrated to the concentration of
ions in the cell (e.g. H⁺). Used together with confocal microscopy, this approach allows the monitoring of dynamic pH changes in living cells. In the case of coral cells, the use of confocal microscopy has proven advantageous for cells containing algal symbionts, where high spatial resolution is required to analyse the coral cell cytoplasm, which is stretched tightly around intracellular symbionts (Venn et al., 2009).

The present study investigated the hypothesis that changes in the pH of corals are shaped by the photosynthetic activity of coral symbionts. This involved: (1) testing whether changes in coral pH could be blocked in the presence of a photosynthetic inhibitor; (2) characterising the dynamics of coral pH during light exposure; (3) determining whether pH values vary on exposure to a range of irradiance levels lying within the corals’ photosynthesis-irradiance (PI) response curve; and (4) examining the rate of pH recovery under darkness. To achieve this, we analysed pH in cells isolated from the reef coral Stylophora pistillata (Esper 1797) using confocal microscopy and the pH-sensitive intracellular probe SNARF-1. We monitored pH in symbiont-containing and symbiont-free cells under controlled conditions of constant seawater pH and oxygen levels in flowing seawater to focus on the effect of intracellular mechanisms.

**MATERIALS AND METHODS**

### Coral culture and preparation of cells

*S. pistillata* colonies were maintained at the Centre Scientifique de Monaco in aquaria supplied with flowing Mediterranean seawater (salinity 38.2) with a 2% h⁻¹ exchange rate, at 25±0.5°C. Irradiance levels were provided at 275 μmol photons m⁻² s⁻¹ photosynthetically active radiation (PAR) on a 12h:12h light:dark cycle. Corals were fed four times a week with frozen shrimp, krill and live Artemia salina nauplii. Cells were isolated from branches of *S. pistillata* colonies immediately before each experiment by gentle brushing of the tissue with a soft bristle-tooth brush into 50 ml filtered seawater (FSW). The resulting cell suspension was centrifuged once (350 g, 4 min) and the pellet of cells was resuspended in FSW as described previously (Venn et al., 2009). In the case of experiments on the photosynthetic response of coral cells to irradiance (PI response), preparations of isolated cells were adjusted to a density of 2.5×10⁶ cells ml⁻¹ and a 3 ml aliquot was taken for oxygen electrode analysis. A second aliquot (5 ml) was stored frozen (−20°C) for quantification of chlorophyll (chl). For pH experiments, cell preparations were adjusted to a density of 2.5×10⁵ cells ml⁻¹ with FSW. Viability staining using AnnexinV-conjugate (Invitrogen, Grand Island, NY, USA) and Sytox-green (Invitrogen) confirmed that cells remained viable during experiments, as in a previous study (Venn et al., 2009).

### Analysis of the photosynthetic response of cell preparations to irradiance by oxygen electrode

Cell preparations were transferred to an open perfusion chamber (POC cell cultivation system, PECON, Erbach, Germany) and mixed with 2 ml of the cell permeant acetoxymethyl ester acetate of SNARF-1 (SNARF-1 AM) (Invitrogen) in FSW (10 μmol l⁻¹ SNARF-1 AM, 0.01% pluronic F-127 and 0.1% DMSO 0.01%). Cell preparations were then dark-incubated for 30 min at 25°C to load cells with SNARF-1 AM and washed by 5 min perfusion with FSW in the dark to remove residual traces of the dye.
SNARF-1 fluorescence was measured by confocal microscope (Leica SP5, Buffalo Grove, IL, USA) and calibrated to pH (NBS scale) using methods published previously (Venn et al., 2009). Briefly, cells were excited at 543 nm and SNARF-1 fluorescence emission was captured in two channels at 585 and 640±10 nm whilst simultaneously monitoring in transmission. In cells containing symbiont, the use of 543 nm as the excitation wavelength minimised chlorophyll autofluorescence, as 543 nm lies outside of absorption spectra of chl a and in the low region of absorption of the peridinin–chlorophyll–protein complex (Frank and Cogdell, 1996).

pHi image analysis was performed using LAF-AS software (Leica) using digital regions of interest (ROI) to confine fluorescence analysis to the coral cell cytoplasm, avoiding dinoflagellate symbionts and their autofluorescent inclusion bodies, which appear with extended exposure time (Kazandjian et al., 2008) (Fig. 2). The 585/640 nm fluorescence intensity ratio (r) was calculated after subtracting background fluorescence recorded in a second ROI in the surrounding cell medium. r was related to pH by the following equation:

$$\text{pH} = \text{pK}_a - \log \left( r - r_B / r_A - r \times F_{B(02)} / F_{A(02)} \right),$$

where pK$_a$ is the logarithmic acid dissociation constant, F is fluorescence intensity measured at 640 nm (l2) and the subscripts A and B represent the values at the acidic and basic end points of the calibration, respectively. Intracellular calibration of pHi with SNARF-1 was performed for each experiment in vivo by ratiometric analysis of SNARF-1 fluorescence in cells exposed to buffers ranging from pHi 6 to pHi 8.5 containing the ionophore nigericin (Venn et al., 2009).

The fluorescence ratio of SNARF-1 was sensitive to differences in illumination caused by the halogen lamp used for photosynthesis and pHi experiments in the light. These changes were not related to photodegradation, but rather to the weak excitation of the dye by the wider spectral range of illumination added by the lamp. As such, it was necessary to perform in vivo calibrations for each light level used in the investigation.

**Experiments to determine the influence of photosynthesis on pH**

In all pH experiments, cell preparations were perfused at a rate of 50 μmol h$^{-1}$ and temperature maintained at 25°C by a thermostatic microscope stage insert (PECON). Illumination was provided by the same irradiance halogen lamp used for the PI response experiments.

Three sets of experiments were performed to investigate the influence of photosynthesis on pH. The first set of experiments compared the response of coral cell pH in the presence and absence of the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Sigma-Aldrich, St Louis, MO, USA). Experiments were carried out over 30 min, during which cell preparations were perfused with a seawater solution containing 100 μmol l$^{-1}$ DCMU and 0.1% acetone (DCMU stock solutions were prepared in acetone) or a control seawater solution containing 0.1% acetone. pH measurements were made on symbiont-containing and symbiont-free cells in the dark or under 300 μmol photons m$^{-2}$ s$^{-1}$ irradiance in the presence and absence of DCMU. Measurements were taken at 1, 5, 10, 15, 20, 25 and 30 min. Separate experiments conducted by oxygen electrode analysis (as described above) confirmed that 100 μmol l$^{-1}$ DCMU was sufficient to block photosynthetic evolution of oxygen by coral cell preparations (data not shown).

The second set of experiments examined the influence of light intensity on pH dynamics of coral cells. We examined the pH dynamics of coral cells containing symbionts on exposure to various light levels; experiments were conducted over 30 min at each light level (dark, 50, 100, 200, 250, 300, 400, 500 and 800 μmol photons m$^{-2}$ s$^{-1}$). Measurements were taken at 1, 5, 10, 15, 20, 25 and 30 min. Values at 0 min were obtained using a separate calibration, with cells analysed immediately after SNARF-1 loading and washing with no light exposure, and are presented with the time course data.

In the third set of experiments, we investigated pH dynamics on return to darkness after exposure to light. Cells were exposed to 400 μmol photons m$^{-2}$ s$^{-1}$ for 20 min before measurements were taken for 35 min at 0 μmol photons m$^{-2}$ s$^{-1}$.

In all three sets of experiments, five cells were measured at each time point and experiments were conducted three times for each cell type, light level and treatment. Preliminary tests were performed to see whether oxygen levels or pH values shifted in the seawater...
in the perfusion chamber during the course of the experiments under the range of light levels used. Oxygen levels were monitored by placing a needle type oxygen microsensor (PreSens) in the seawater within the perfusion chamber and values were recorded with TX3v602 software (PreSens). Seawater pH was monitored by adding SNARF-1 to the perfused seawater and measuring the fluorescence ratiometrically as described previously (Venn et al., 2011). pH data were analysed using SPSS statistical software by repeated-measures ANOVA or one-way ANOVA after establishing that data conformed to a normal distribution and homogeneity of variance.

**RESULTS**

**PI response of isolated cell preparations**

Prior to performing analysis of pH in coral endoderm cells, we characterised the PI relationship of isolated cell preparations (Fig. 1). Generation of a PI curve established that the maximum rate of photosynthesis was reached at approximately 300 μmol photons m⁻² s⁻¹ PAR and remained stable up to 1000 μmol photons m⁻² s⁻¹.

**Establishing the link between coral cell pH and symbiont photosynthesis**

Coral cell pH was analysed using ratiometric analysis of the pH-sensitive dye SNARF-1 AM in cells with and without symbionts (Fig. 2). In symbiont-containing cells, the low pH symbiosome was visible around the dinoflagellate as described previously (Venn et al., 2009). When symbiont-containing cells were exposed to light in the absence and presence of DCMU, pH significantly increased in control (non DCMU treated) cells from pH 7.03±0.03 to pH 7.37±0.03 over 20 min (repeated-measures ANOVA, F(6,8)=17.372, P<0.001), while pH in DCMU-treated cells remained unchanged (pH 7.04±0.03; repeated-measures ANOVA, F(6,8)=0.557, P>0.05; Fig. 3A). pH also remained unchanged in symbiont-containing cells exposed to dark conditions (with and without DCMU) (Fig. 3A) and in symbiont-free cells in the light and dark (with and without DCMU) (Fig. 3B). Separate experiments that monitored external seawater [O₂] and pH by oxygen and pH electrode showed the renewal seawater in the perfusion chamber was sufficient to keep seawater [O₂] and pH stable throughout the 30 min exposure to 300 μmol photons m⁻² s⁻¹ (supplementary material Fig.S1) and the full range of irradiances used in subsequent experiments (not shown).

**Impact of irradiance level and duration on pH in coral cells containing symbionts**

pH was measured in coral cells containing dinoflagellate symbionts exposed to a range of light levels (0 to 800 μmol) for 30 min (Fig. 4). pH increased over the course of the experiment at all light levels, but remained stable in cells kept in the dark. pH did not increase immediately on exposure to lower irradiances. At irradiances of 50 to 250 μmol photons m⁻² s⁻¹, a lag phase of 10 min occurred before significant increases in pH were measured (Fig. 4, Table 1). At 300 μmol photons m⁻² s⁻¹, this lag phase shortened to 5 min. For irradiances of 400 μmol photons m⁻² s⁻¹ and above, values of pH measured were higher than dark values at 1 min, suggesting that pH increases occurred with the first minute of light exposure. Following 20 min of light exposure, pH values reached a plateau at all irradiances, with successively higher values of pH associated with higher irradiances. The highest pH values (ranging from pH 7.4 to 7.46) were measured at 300 μmol photons m⁻² s⁻¹ (supersaturating irradiances) (Fig. 4, Table 1).

**Recovery of pH in the dark**

Having established that host pH reaches a plateau at pH 7.4–7.46 after 20 min of supersaturating irradiance, we investigated whether pH declined if cells were returned to dark conditions. After increasing to values of pH 7.36 on exposure to 400 μmol photons m⁻² s⁻¹, pH significantly declined in the dark to 7.08±0.04 over 35 min (repeated-measures ANOVA, F(8,10)=2.850, P<0.05; Fig. 5). This value was similar to initial values (prior to light exposure) and pH values in symbiont-free cells.

**DISCUSSION**

The results of the present study demonstrate that the photosynthetic activity of coral symbionts drives changes in coral host pH in an irradiance intensity-dependent manner. To our knowledge this is
Table 1. pH (mean ± s.e.m.) in symbiont-containing cells isolated from Stylophora pistillata exposed to different light intensities for 30 min

<table>
<thead>
<tr>
<th>Light (μmol photons m⁻² s⁻¹)</th>
<th>1 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>7.26±0.08</td>
<td>7.31±0.05</td>
<td>7.35±0.04</td>
<td>7.37±0.06</td>
<td>7.4±0.04</td>
<td>7.4±0.04</td>
<td>7.46±0.07</td>
<td>F₆,₇₂=4.18; P=0.001</td>
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<tr>
<td>500</td>
<td>7.22±0.02</td>
<td>7.3±0.03</td>
<td>7.38±0.06</td>
<td>7.45±0.03</td>
<td>7.4±0.05</td>
<td>7.42±0.05</td>
<td>7.44±0.04</td>
<td>F₆,₅₄=3.776; P=0.003</td>
</tr>
<tr>
<td>400</td>
<td>7.17±0.05</td>
<td>7.27±0.08</td>
<td>7.34±0.07</td>
<td>7.39±0.08</td>
<td>7.43±0.08</td>
<td>7.4±0.08</td>
<td>7.44±0.05</td>
<td>F₆,₅₄=3.140; P=0.010</td>
</tr>
<tr>
<td>300</td>
<td>7.07±0.1</td>
<td>7.15±0.11</td>
<td>7.3±0.07</td>
<td>7.41±0.03</td>
<td>7.42±0.03</td>
<td>7.4±0.03</td>
<td>7.4±0.05</td>
<td>F₆,₄₈=5.500; P&lt;0.001</td>
</tr>
<tr>
<td>250</td>
<td>7.06±0.05</td>
<td>7.05±0.06</td>
<td>7.13±0.04</td>
<td>7.28±0.09</td>
<td>7.31±0.09</td>
<td>7.28±0.08</td>
<td>7.3±0.08</td>
<td>F₆,₅₄=4.812; P=0.001</td>
</tr>
<tr>
<td>200</td>
<td>7.07±0.06</td>
<td>7.06±0.08</td>
<td>7.1±0.08</td>
<td>7.24±0.06</td>
<td>7.21±0.04</td>
<td>7.23±0.06</td>
<td>7.2±0.06</td>
<td>F₆,₅₄=2.532; P=0.042</td>
</tr>
<tr>
<td>100</td>
<td>7.04±0.06</td>
<td>7.05±0.03</td>
<td>7.13±0.08</td>
<td>7.15±0.07</td>
<td>7.16±0.01</td>
<td>7.15±0.11</td>
<td>7.15±0.12</td>
<td>F₆,₅₄=2.868; P=0.015</td>
</tr>
<tr>
<td>50</td>
<td>7.02±0.08</td>
<td>7.05±0.12</td>
<td>7.07±0.09</td>
<td>7.15±0.08</td>
<td>7.08±0.07</td>
<td>7.13±0.08</td>
<td>7.14±0.1</td>
<td>F₆,₄₈=2.985; P=0.034</td>
</tr>
<tr>
<td>0</td>
<td>7.07±0.02</td>
<td>7.06±0.03</td>
<td>7.09±0.03</td>
<td>7.07±0.03</td>
<td>7.09±0.03</td>
<td>7.07±0.05</td>
<td>7.05±0.05</td>
<td>F₆,₁₁₄=0.237; P=0.05</td>
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</table>

*Post hoc* analysis was performed using paired-sample *t*-tests, followed by repeated-measures ANOVA.

Superscripted numbers (corresponding to time points) indicate mean pH values that are significantly different to pH at other time points.

Fig. 5. pH (mean ± s.e.m.) in symbiont-containing cells isolated from S. pistillata exposed to 20 min of 400 μmol photons m⁻² s⁻¹ irradiance (white) followed by 35 min in the dark (grey).

The first characterisation of the role of algal photosynthesis in altering pH in the cytoplasm of an animal host. In the first part of the investigation we tested whether changes in coral pH could be blocked in the presence of a photosynthetic inhibitor. After determining the value of irradiance for maximal rate of photosynthesis, we conducted experiments with the photosynthetic inhibitor DCMU. This inhibitor has been widely used in experiments on coral symbionts (Jones, 2004), free-living algae and plants (Garrigue et al., 1992) as it blocks photosystem II by binding to plastoquinone, inhibiting the light reactions of photosynthesis. Our experiments showed that while control symbiont-containing cells exposed to a saturating irradiance of 300 μmol photons m⁻² s⁻¹ displayed a significant increase of 0.3 pH units in pH, symbiont-containing cells treated with DCMU exhibited no changes in pH over a 30 min period. As the pH of DCMU-treated cells was not distinguishable from the pH of cells kept in the dark or cells that did not contain symbionts in the light or dark, it is likely that DCMU did not alter coral cell pH regulation by mechanisms other than inhibiting photosynthesis.

One possible way that photosynthesis may have induced an increase in pH of host cells is through modifications of the surrounding seawater in the perfusion chamber during the time course of each experiment, such as an increase in pH resulting from CO₂ removal from seawater by the cells. Indeed, the acid/base balance of most organisms may be affected by changes in the pH of the surrounding environment (Boron, 2004). We ruled this possibility out by perfusing cell preparations with FSW at a rate that kept pH and O₂ levels stable in the perfusion chamber in all treatments. Thus our data point to a role of symbiont photosynthesis in modifying the pH of host cytoplasm by intracellular mechanisms rather than changes in the pH of the surrounding medium. One likely candidate mechanism is the photosynthetic consumption of intracellular CO₂. Depletion of CO₂ from the cytoplasm will cause the conversion of HCO₃⁻ to CO₂ + H₂O, which would contribute to alkalisation of the cell (Allemend et al., 1998). Conversely, the observed recovery in pH occurring in darkness after pre-exposure to irradiance may be due to net production of CO₂. CO₂ is produced by both host and symbiont respiration in the dark, and combined with H₂O is converted back to HCO₃⁻ and H⁺, which acidifies the cell.

In the following stages of the study we investigated the dynamics of pH on exposure to a range of light levels. Several observations taken from these experiments provide insight into the mechanisms underlying how photosynthesis may drive pH changes in corals. Concerning the dynamics of pH, the important result is that the presence of a lag phase is dependent on the light intensity. Indeed, there is a 10 min time lag between the beginning of the exposure to light and when increases in pH occur for irradiances that ranged from 0 to 250 μmol photons m⁻² s⁻¹. This time lag is absent for higher irradiances (300−800 μmol photons m⁻² s⁻¹) as increases in pH occur earlier in the time course. The existence of the time lag could be interpreted as evidence for the gradual depletion of a CO₂ pool that built up within dinoflagellate cells and the symbiosome membrane complex during dark loading with SNARF-1 AM, delaying the eventual consumption of CO₂ from host cells. In this scenario, the depletion of the dinoflagellate CO₂ pool occurs more rapidly at higher irradiances (when rates of photosynthesis are higher) and thus removal of CO₂ from the host cell and the resulting increases in host pH occur at an earlier point in the time course.

Concerning the influence of irradiance levels on pH values, the primary observation is that pH values plateau after 20 min at all light intensities and the value of pH at the plateau increases with increasing light intensity. Maximum pH values (pH 7.4−7.46) were obtained at the maximum rate of photosynthesis and supersaturating light levels. As pH increases do not surpass values of 7.46, it is likely that after a 20 min delay, regulatory mechanisms intervene to prevent pH increasing indefinitely in the cell. Membrane-bound transporters involved in the regulation of pH, well known in many organisms but yet to be characterised in corals, are likely to be involved in this process (Casey et al., 2010). These may include extruders of OH⁻ and bicarbonate-linked transport mechanisms, which are highly important for pH regulation (Russell and Boron, 1976; Furla et al., 2000; Bonar and Casey, 2008) and potentially...
also important for dissolved inorganic carbon transport and CO2-concentrating mechanisms in corals (Al-Moghrabi et al., 1996; Allemand et al., 1998; Brownlee, 2009). Similarly, we presume that regulation by membrane-bound transporters, particularly acid extruders, contributes to the stable values of pH obtained in darkness (pH 7.05). Additionally, as CO2 regularly traverses cell membranes and if the partial pressure of CO2 of cnidarians cells is estimated to be higher than that of the surrounding seawater (Venn et al., 2009), respiratory CO2 may also leak out of the cell.

In our study, external variation in seawater pH was deliberately controlled in order to identify intracellularly driven pH variations. However, one important avenue of future research will be to characterise the interaction of photosynthesis-driven changes in coral pH with changes in extracellular pH in both the coelenteron lumen and the external seawater pH. Previous studies on pH at the coral’s surface have characterised pH variation in the diffusive boundary layer (Kühl et al., 1995; De Beer et al., 2000; Al-Horani et al., 2003). For example, working with microsensors and the coral Galaxea fascicularis, Al Horani and coworkers observed variations of diffusive boundary layer (DBL) pH ranging from pH 7.6 in the dark to 8.5 in the light (Al-Horani et al., 2003). In the coelenteron, variations of 0.6 pH units have been measured between light and dark conditions (Al-Horani et al., 2003). These data from the coelenteron may be of particular relevance when considering our results in the context of the intact organism, because coral symbionts reside in the endoderm cell layer lining the coelenteron, rather than directly facing the surrounding seawater. The mechanistic basis underlying these changes in coelenteron pH may be linked to regulation of pH at the maximum and minimum pH values observed under constant conditions in the present study. Under irradiance, membrane-bound transporters that prevent intracellular pH rising above pH 7.46, such as extrusion of OH− or uptake of H+, may drive alkalinisation of the coelenteron (Furla et al., 2000). Equally, in the dark, regulatory mechanisms that prevent decreases in pH below 7.05 may involve extrusion of H+, which reduces pH in the coelenteron.

Together with knowledge on pH in the DBL and the coelenteron, understanding changes in pH will eventually lead towards a better understanding of ion gradients and ion transport (particularly H+ and dissolved inorganic carbon) across coral tissues. Such an understanding is imperative for a better grasp of the physiological response of corals to environmental changes such as ocean acidification (reduced seawater pH driven by ocean uptake of anthropogenic CO2). A recently published hypothesis by Jokiel illustrates this point. Jokiel argues that reduced rates of calcification in corals exposed to ocean acidification arise through a decrease in the proton gradient across the DBL, impeding the efflux of protons generated by the calcification reaction (Jokiel, 2011). It is proposed that photosynthesis may alleviate this unfavourable gradient by increasing pH in the DBL during daylight hours. Allemand et al. have argued that increases in pH of the coelenteron may also provide a more favourable gradient for the movement of protons from away the site of calcification (Allemand et al., 2004). However, much future research is still required to address gaps in our understanding of how protons and other ions are transported across coral tissues. Indeed, the relative contributions of paracellular and transcellular flux of ions in corals are still a matter of debate (Tambutté et al., 2011).

In conclusion, the present study provides fundamental information about how symbiont photosynthesis changes coral host pH. Such information provides a basis for future studies into acid–base regulation, which are essential for a better understanding of coral biology in general. In eukaryotes, shifts in pH of 0.1 pH units and greater are usually associated with changes in primary metabolic processes such as respiration rate, protein synthesis, cell division and cell cycle progression (Busa and Nuccitelli, 1984; Madhus, 1988; Kurkdjian and Guern, 1989; Boussouf and Gaillard, 2000; Denker et al., 2000; Putney and Barber, 2003). It follows therefore that the relatively large light-dependent shifts in pH observed in the present study have the potential to contribute to many physiological processes, including diel patterns of coral metabolism and growth. Physiological tuning of coral metabolism to light has been studied for several years. The expression of genes and proteins linked to cellular redox states, division rates of both endoderm cells and symbionts, and rates of calcification all show diel periodicity (Willerson et al., 1983; Fitt, 2000; Levy et al., 2006; Levy et al., 2011; Tambutté et al., 2011). The potential role of light-driven pH variation in contributing to regulation of coral cell physiology is worthy of future research.

LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>chl</td>
<td>chlorophyll</td>
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<tr>
<td>DBL</td>
<td>diffusive boundary layer</td>
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<tr>
<td>DCMU</td>
<td>3-(3,4-dichlorophenyl)-1,1-dimethyleurea</td>
</tr>
<tr>
<td>F</td>
<td>fluorescence intensity</td>
</tr>
<tr>
<td>FSW</td>
<td>filtered seawater</td>
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<tr>
<td>I</td>
<td>irradiance</td>
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<tr>
<td>I0</td>
<td>irradiance at which the initial slope (α) intersects Pmax</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetically active radiation</td>
</tr>
<tr>
<td>Pmax</td>
<td>gross maximum photosynthetic rate</td>
</tr>
<tr>
<td>pHi</td>
<td>intracellular pH</td>
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<td>PI</td>
<td>photosynthesis–irradiance</td>
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<td>Pnet</td>
<td>net photosynthetic rate</td>
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<tr>
<td>r</td>
<td>fluorescence intensity ratio</td>
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<tr>
<td>R</td>
<td>respiration rate in the dark</td>
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<tr>
<td>ROI</td>
<td>regions of interest</td>
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<tr>
<td>SNARF-1</td>
<td>carbosymesinaphthodafiuor-1</td>
</tr>
<tr>
<td>SNARF-1 AM</td>
<td>cell permeant acetoxymethyl ester acetate of SNARF-1</td>
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<td>λ2</td>
<td>640 nm</td>
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</table>

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AUTHOR CONTRIBUTIONS


COMPETING INTERESTS

No competing interests declared.

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REFERENCES


Fig. S1. Mean (±s.e.m.) (A) O₂ concentration (µmol kg⁻¹) and (B) pH in seawater of the perfusion chamber containing isolated cells with and without symbionts from S. pistillata. Data shown represent an example at 300 µmol photons m⁻² s⁻¹ irradiance. Data are not shown for other treatments (e.g. dark, DCMU, etc.).