

RESEARCH ARTICLE

Symbiont photosynthesis in giant clams is promoted by V-type H⁺-ATPase from host cells

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ABSTRACT

Giant clams (genus *Tridacna*) are the largest living bivalves and, like reef-building corals, host symbiotic dinoflagellate algae (*Symbiodinium*) that significantly contribute to their energy budget. In turn, *Symbiodinium* rely on the host to supply inorganic carbon (C_i) for photosynthesis. In corals, host 'proton pump' vacuolar-type H⁺-ATPase (VHA) is part of a carbon-concentrating mechanism (CCM) that promotes *Symbiodinium* photosynthesis. Here, we report that VHA in the small giant clam (*Tridacna maxima*) similarly promotes *Symbiodinium* photosynthesis. VHA was abundantly expressed in the apical membrane of epithelial cells of *T. maxima*'s siphonal mantle tubule system, which harbors *Symbiodinium*. Furthermore, application of the highly specific pharmacological VHA inhibitors bafilomycin A1 and concanamycin A significantly reduced photosynthetic O₂ production by ~40%. Together with our observation that exposure to light increased holobiont aerobic metabolism ~5-fold, and earlier estimates that translocated fixed carbon exceeds metabolic demand, we conclude that VHA activity in the siphonal mantle confers strong energetic benefits to the host clam through increased supply of C_i to algal symbionts and subsequent photosynthetic activity. The convergent role of VHA in promoting *Symbiodinium* photosynthesis in the giant clam siphonal mantle tubule system and coral symbiosome suggests that VHA-driven CCM is a common exaptation in marine photosymbioses that deserves further investigation in other taxa.

KEY WORDS: *Tridacna*, *Symbiodinium*, Vacuolar proton ATPase, Carbon-concentrating mechanism, Metabolism, Symbiosis

INTRODUCTION

Photosymbiosis, a partnership in which a host organism harbors photosynthetic microbial or algal cells, provides competitive advantages to hosts through increased energy availability, and to symbionts through translocation of nutrients, particularly dissolved organic nitrogen (Roth, 2014). This arrangement can be particularly advantageous in nutrient-poor environments such as tropical coral reefs, where multiple independent photosymbioses have emerged between dinoflagellate algae of the genus *Symbiodinium* and a diverse array of hosts including scleractinian corals, sea anemones (Trench, 1987) and also mollusks (Yonge, 1975) such as the tridacnid 'giant clams'.

Tridacnid clams are the largest living bivalves and are found throughout the tropical Indo-Pacific, where they live in close association with reef-building corals (Knop, 1996; Yonge, 1975). All described tridacnid species (Huelsenken et al., 2013; Othman et al., 2010) exhibit symbiotic partnerships with *Symbiodinium* hosted extracellularly in modified extensions of the digestive system called zooxanthellae tubules or 'Z-tubules'. These tubules extend upwards from the stomach into the light-exposed tissue of the siphonal mantle where they are ultimately arranged roughly perpendicular to incoming solar radiation (Fig. 1) (Holt et al., 2014; Knop, 1996; Norton and Jones, 1992). Despite the fact that endosymbiotic *Symbiodinium* are intracellular in corals and extracellular in clams, the algal symbionts provide similar benefits to the hosts: a source of photosynthetic reduced carbon that can exceed respiratory demand in both coral (~150%) (Muscatine et al., 1984) and *Tridacna* (up to 400%) (Klumpp and Griffiths, 1994).

In corals, *Symbiodinium* photosynthesis has recently been shown to be stimulated by vacuolar-type H⁺-ATPase (VHA) from host cells (Barott et al., 2015). VHA, an enzyme found in all eukaryotes, utilizes energy from ATP hydrolysis to transport H⁺ across biological membranes (Stevens and Forgac, 1997; Tresguerres, 2016). In corals, VHA acidifies the symbiosomal compartment where *Symbiodinium* resides, and pharmacological VHA inhibition impairs net symbiont photosynthetic O₂ production by up to 80% (Barott et al., 2015). Those results indicate VHA is part of a host-controlled carbon-concentrating mechanism (CCM) that drives the speciation of inorganic carbon (C_i) into CO₂, which is essential because dinoflagellate ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo), the terminal enzyme in carbon fixation, has a low affinity for CO₂ over O₂ (Leggat et al., 2002; Rowan et al., 1996).

Tridacnid clams have also been hypothesized to utilize CCMs to promote *Symbiodinium* photosynthesis (Leggat et al., 1999; Yellowlees et al., 1993). The evidence includes the high abundance of carbonic anhydrases (CAs) in the siphonal mantle (Yellowlees et al., 1993), as well as a recent paper reporting the presence of VHA subunit A (VHA_A) in epithelial cells of *Symbiodinium*-containing Z-tubules (Ip et al., 2018). Furthermore, freshly isolated *Symbiodinium* sustain much greater photosynthetic rates in the presence of CO₂ versus HCO₃⁻ (Leggat et al., 1999; Yellowlees et al., 1993), which is consistent with a CCM controlled by the clam. However, functional evidence for this potential CCM is thus far lacking.

Here, we observed that VHA subunit B (VHA_B) is abundantly expressed in the apical membrane of Z-tubule cells of the giant clam *Tridacna maxima*, and experimentally confirmed VHA participates in a CCM that promotes photosynthesis by *Symbiodinium*.

MATERIALS AND METHODS

Organism acquisition and husbandry

Clams were collected (under ordinance no. 88-184/AT of the French Polynesian Ministère de l'Économie, des Finances, du Travail et de l'Emploi) following all requirements laid out by the Plan de Gestion

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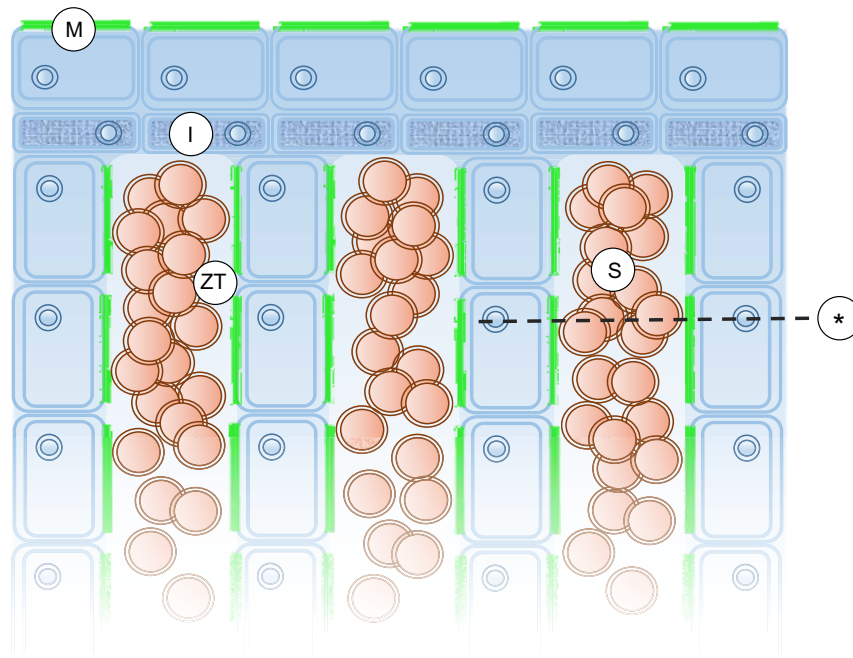


Fig. 1. Diagram of *Tridacna maxima* siphonal mantle tissue. Arrangement of zooxanthellae tubules (ZT), *Symbiodinium* (S), mantle margin cells (M) and light-refractive iridocytes (I). Vacuolar-type H⁺-ATPase (VHA) localization in the apical membrane of epithelial siphonal mantle cells is shown in green. The dotted line (*) indicates the cross-section of a ZT displayed in Fig. 6.

de l'Espace Maritime (PGEM) in French Polynesia and were maintained and studied in ways commensurate with all pertinent University of California guidelines.

Metabolic rate and VHA immunodetection experiments were conducted on juvenile *Tridacna maxima* (Röding 1798) clams ($n=18$; shell length 4.7 ± 0.1 cm, mean \pm s.e.m.) purchased from Oceans, Reefs & Aquariums[®] Aquaculture company (Fort Pierce, FL, USA) and held in a 288 l, recirculating seawater aquarium in Tiburon, CA, USA. Seawater was constantly aerated and maintained at conditions resembling those of a tropical coral reef ($27.2\pm 0.4^\circ\text{C}$, 34.6 ± 0.4 PSU, pH 8.10 ± 0.04 ; mean \pm s.d.) on a 2 h dusk:8 h light:4 h dusk:10 h dark photoperiod. Mean irradiance during the light cycle was 234.65 ± 2.45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (mean \pm s.e.m.). Clams were held for 25 days prior to experimentation.

Photosynthetic activity experiments were conducted on adult *T. maxima* collected from fringing reefs around the island of Mo'orea, French Polynesia. Clams were held in an outdoor flow-through seawater system supplied with water from the adjacent fringing reef ($26.9\pm 1^\circ\text{C}$, 35.96 ± 0.2 PSU, pH 8.13 ± 0.09 ; mean \pm s.d.). Typical $[\text{NH}_4^+]$ on the reef is ~ 14 $\mu\text{mol l}^{-1}$ (<http://observatoire.criobe.pf/wiki/tiki-index.php>), and P_{CO_2} in an adjacent recirculating system was 406 ± 4 μatm (Evensen and Edmunds, 2017). Clams were kept for 14 days prior to experimentation. Experiments involving concanamycin A were conducted during October 2015 ($n=4$ clams; shell length 17.2 ± 3.0 cm; mean \pm s.e.m.), and experiments involving bafilomycin A1 were conducted during October 2016 ($n=3$ clams; shell length 15.0 ± 1.5 cm; mean \pm s.e.m.). In all cases, tissue samples were collected from live clams by quick insertion of a plastic wedge between the valves (to prevent closing) followed by slicing of the adductor muscle and subsequent removal of tissues of interest with a surgical scalpel. All vivisections were conducted during the light cycle and tissues were immediately processed as described below.

Anti-VHA antibodies and western blot analysis

VHA within *T. maxima* tissues was quantified and visualized using custom-made rabbit polyclonal antibodies against an epitope in the

VHA_B subunit, AREEVPGRRGFPY. This epitope is 100% conserved throughout evolution from cnidarians to mammals, including mollusks. These antibodies specifically recognize VHA_B by western blot and immunohistochemistry in diverse taxa including coral (Barott et al., 2015), worms (Tresguerres et al., 2013), mussels (Thomsen et al., 2016), hagfish (Clifford et al., 2015), sharks (Roa et al., 2014) and giant clams (Hill et al., 2018).

To procure tissue samples for western blots, juvenile *T. maxima* ($n=3$) were vivisected and gill, siphonal and byssal mantle samples were frozen in liquid nitrogen and ground to a fine powder with a pestle in a ceramic mortar. For each clam, 0.1 g of tissue was combined with 500 μl of ice-cold S22 buffer (450 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 58 mmol l^{-1} MgCl₂, 10 mmol l^{-1} CaCl₂, 100 mmol l^{-1} Hepes, pH 7.8) containing a protease inhibitor cocktail (catalog no. P8340, Sigma-Aldrich, St Louis, MO, USA) and a phosphatase inhibitor mix (PhosStop, Roche Applied Science, Penzberg, Germany), and homogenized in a glass homogenizer. After a low-speed centrifugation (100 g for 2 min) to remove tissue debris, *Symbiodinium* cells were pelleted by centrifugation (500 g for 10 min at 4°C). The resulting supernatant was centrifuged (2100 g for 30 min at 4°C), and the 'crude homogenate' supernatant fraction was removed from pelleted membranes. *Symbiodinium* and membrane pellets were each resuspended in 100 μl of ice-cold homogenization buffer.

Protein concentration was determined in triplicate by Bradford assay (Bio-Rad, Hercules, CA, USA). Western blotting was conducted as previously described (Roa et al., 2014). In brief, 57–74 μg of total protein was separated on a polyacrylamide mini gel (60 V 15 min, 200 V 45 min) and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). PVDF membranes were then incubated in blocking buffer [Tris-buffered saline-Tween (TBS-T) with 5% non-fat powdered milk] at room temperature for 1 h before incubation in the primary antibody (anti-VHA_B, 30 $\mu\text{g ml}^{-1}$ in blocking buffer) at 4°C overnight. PVDF membranes were washed three times in TBS-T and incubated in secondary antibody (horseradish peroxidase-conjugated goat

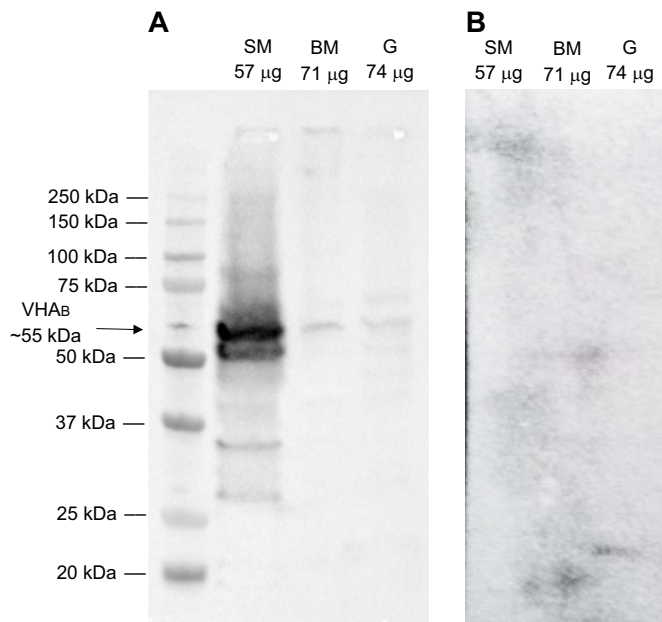


Fig. 2. VHA in *T. maxima* tissues. (A) Western blotting using anti-VHA_B antibodies and (B) peptide pre-absorption control. SM, siphonal mantle; BM, byssal mantle; G, gill. The amount of total protein loaded in each well is indicated.

anti-rabbit antibodies, Bio-Rad, 1:10,000) at room temperature for 1 h. Bands were visualized through addition of ECL Prime Western Blotting Detection Reagent (GE Healthcare, Waukesha, WI, USA) and imaged and analyzed in a Bio-Rad Universal III Hood using ImageQuant software (Bio-Rad). PVDF membranes incubated in blocking buffer with anti-VHA antibodies and 1000-fold excess (mol:mol) blocking peptide served as the peptide pre-absorption control (Fig. 2).

VHA immunolocalization

Siphonal mantle tissue samples containing symbiotic algae (~1 cm×1 cm×0.5 mm thick) and gill (~1 cm×1 cm×0.5 mm) were vivisectioned from juvenile clams ($n=6$) and immersed in fixative (0.1 mol l⁻¹ sodium cacodylate buffer, 3% paraformaldehyde and 0.35% glutaraldehyde; Electron Microscopy Sciences, Hatfield, PA, USA) for 6 h at 4°C. Tissue samples were then transferred to 50%

ethanol for 6 h, and stored in 70% ethanol, followed by dehydration in 95% ethanol (10 min), 100% ethanol (10 min) and xylene (3×10 min) prior to paraffin embedding (55°C, 3×10 min). The next day, tissue samples were sectioned at 12 µm using a rotary microtome and three consecutive sections were attached on glass slides and placed on a slide warmer (overnight at 37°C). Paraffin was removed by xylene (3×10 min), and tissue sections were serially rehydrated in 100% ethanol (10 min), 70% ethanol (10 min) and 0.2% Triton X-100 TBS-T in phosphate-buffered saline (PBS) (10 min). After blocking (0.2% Triton X-100, 2% normal goat serum and 0.02% keyhole limpet hemocyanin in PBS, pH 7.8, 1 h), tissue sections were incubated with anti-VHA_B antibodies (3 µg ml⁻¹ in blocking buffer, overnight at 4°C). Tissue sections were then washed three times in PBS, incubated with the secondary antibody (1:500, horseradish peroxidase-conjugated goat anti-rabbit antibodies, Bio-Rad; Alexa 488, Invitrogen, Grand Island, NY, USA; 1 h at room temperature) and stained with Hoescht 33342 (Invitrogen, Grand Island, NY, USA) at 5 µg ml⁻¹ for 5 min to visualize nuclei. Controls were prepared as above except sections were incubated with anti-VHA_B antibodies and 1000-fold excess (mol:mol) blocking peptide.

Immunofluorescence was detected with an epifluorescence and structured illumination microscope (Zeiss AxioObserver Z1 with Apotome2, Oberkochen, Germany). Digital images were adjusted for brightness and contrast only using Zeiss Axiovision software.

O₂ consumption and production rates

Photosynthetic activity and aerobic metabolism were estimated by closed-cell respirometry at 27±1°C (mean±s.d.). O₂ consumption rate was measured in the dark on freshly vivisectioned ~2 mm×2 mm sections of siphonal mantle tissue immersed in seawater containing 1% DMSO in sealed glass vials (750 µl volume) using a PreSens OxyDish Reader (Loligo 1421-01, Regensburg, Germany). Similar glass vials without tissue served as controls to correct for background O₂ consumption. The concentration of dissolved O₂ was measured every 10 s for 5 min, after which recording was stopped and tissue samples were removed from respirometry cells, gently patted dry, and weighed. O₂ consumption rate was calculated from the raw data by linear regression, normalized by mass and corrected with no-tissue control blanks. For a subset of tissue samples, O₂ consumption rates were also measured post-light exposure to examine phototrophic effects on aerobic metabolism. These samples were prepared as above, but were incubated in bright

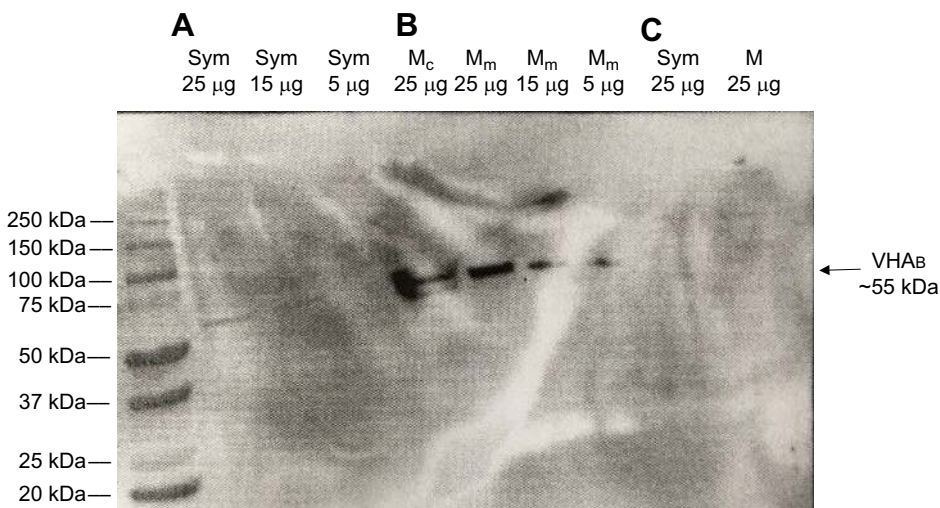


Fig. 3. VHA in *Symbiodinium*. Western blotting using anti-VHA_B antibodies in (A) *Symbiodinium* and (B) siphonal mantle membrane fractions. (C) Pre-absorption control. The relatively high background is due to the increased exposure time necessary to visualize the very faint band present in *Symbiodinium*. The amount of total protein loaded in each well is indicated. Sym, *Symbiodinium*; M_c, siphonal mantle cytoplasm-associated fraction; M_m, siphonal mantle membrane-associated fraction; M, siphonal mantle fraction.

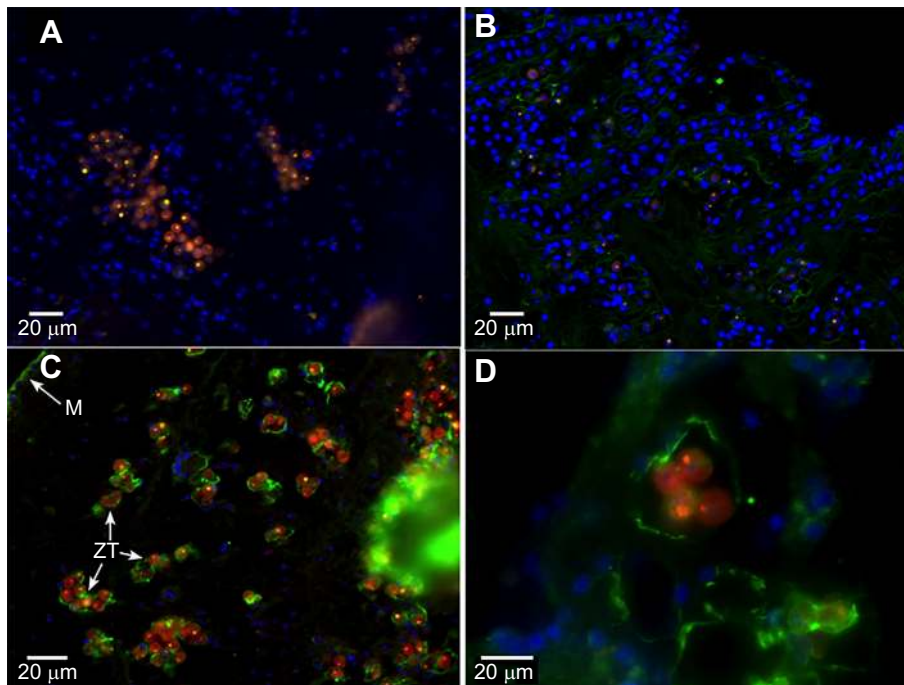


Fig. 4. VHA is localized to zooxanthellae tubules in the giant clam *T. maxima*.

(A–C) Negative control (omission of primary antibody; A) in siphonal mantle, and corresponding anti-VHA_B antibody staining in byssal (B) and siphonal mantle (C). (D) High-magnification image of the zooxanthellae tubule system. VHA is in green, nuclear DAPI staining is in blue and chlorophyll autofluorescence is in red. ZT, zooxanthellae tubules; M, shell–mantle margin.

light supplied by a 28 W fluorescent light source (Coralife model 5300, 10 K/420 nm actinic bulbs) with tissue exposed to an average irradiance of $234.65 \pm 0.775 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (mean \pm s.e.m.; PAR scalar irradiance sensor, Biospherical Instruments, Model QSL2101, San Diego, CA, USA) for 20 min prior to immediately measuring dissolved O_2 concentrations in the dark.

In order to assess the effect of VHA inhibition on photosynthetic productivity, a second series of experiments was conducted in the presence of the highly specific VHA inhibitors bafilomycin A1 and concanamycin A (Dröse and Altendorf, 1983; Huss and Wiczorek, 2009). Tissue samples were vivisected in triplicate from each of three individual clams (total $n=9$) and incubated in sealed vials containing seawater and 1% DMSO under constant illumination (same conditions as described for measurement of phototrophic effects) for 10 min before recording began at 30 s sampling intervals for a further 10 min to establish baseline O_2 production rate. Media was then replaced with either seawater containing 1% DMSO or seawater containing the VHA inhibitor bafilomycin A1 ($1 \mu\text{mol l}^{-1}$ solution in DMSO, final concentration 1%). After a 15 min incubation period, recording resumed at 30 s sampling intervals for 10 min. In total, inhibition experiments took ~ 45 min from vivisection to the end of data collection and all tissue samples were dried and weighed at the conclusion of the experiment.

The effect of pharmacological VHA inhibition on gross O_2 production rates under field-relevant illumination conditions was determined using end-point respirometry. Siphonal mantle tissues from freshly vivisected adult clams (four samples from four different clams, total $n=16$) were placed in replicate 1.5 ml microcentrifuge tubes containing seawater with either 1% DMSO or $1 \mu\text{mol l}^{-1}$ concanamycin A1 in DMSO (final concentration 1%). Half of the replicate tubes were wrapped with aluminium foil to block light and allow for measurement of respiration rates ($n=8$), while the other half were left unwrapped ($n=8$). Tubes containing no clam tissue samples were used to correct for background O_2 production as described previously. All samples were transferred to outdoor holding tanks at 27°C and experiments were performed

under ambient solar irradiance ($\sim 14:00$ h– $15:30$ h). Tissues in the photosynthetic treatments were continuously exposed to $\sim 1980 \pm 40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ as measured with a Mastech Digital Lux Meter (MS6612, Brea, CA, USA) for 25 min prior to measurement of end-point concentration. After 25 min of exposure, microcentrifuge tubes were mixed by gently rocking and seawater samples were immediately pipetted into the glass vials of the OxyDish reader. The concentration of dissolved O_2 was measured for 3 min in each vial and averaged to give final end-point O_2 concentrations for each treatment. Tissues were dried and weighed, and gross O_2 production rates were calculated as the mass-normalized rate of O_2 production in the light-exposed treatments plus the mass-normalized O_2 consumption rates of the dark treatment for each tissue.

Statistical analyses

Photosynthetic rates were estimated from raw data by linear regression using the linear model (lm) function of the stats package in the statistical software program R (v. 3.2.2). Rates post-addition of the inhibitor bafilomycin A1 were then normalized against initial rates for each treatment to generate relative O_2 production data. End-point respirometry data were similarly normalized against mean O_2 production in the DMSO control treatment to give relative rates. All data were then tested for normality using the shapiro.test and test_normality functions of the stats and LambertW packages in R. Homogeneity of variance across treatments was confirmed using the var.test function of the LambertW package. Subsequent hypothesis testing was performed using paired, two-tailed t -tests (with significance threshold $P < 0.05$), comparing photosynthetic rates post-addition of inhibitor with those measured before within the same tissue sample. All data are given as means with s.e.m.

Because of their status as protected species, collection of giant clams from the wild was strictly limited to specimens > 12 cm in shell length, which were rare on easily accessible reefs. As a result, only three/four individuals were available for bafilomycin/concanamycin inhibition experiments, respectively.

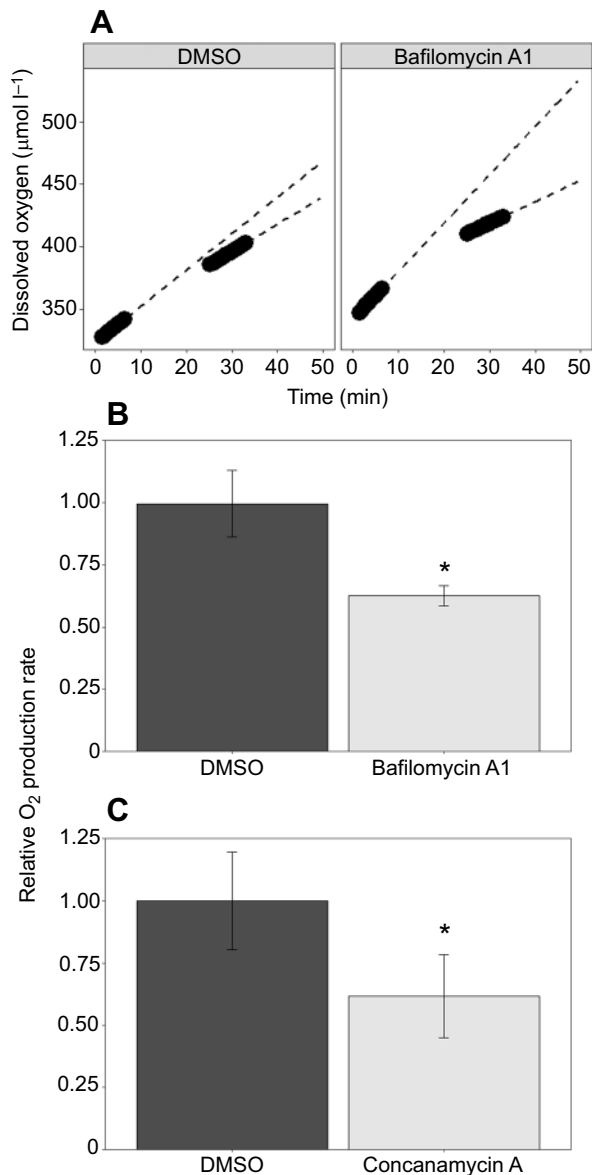


Fig. 5. VHA inhibition reduces net photosynthetic O₂ production in *T. maxima*. (A) Representative O₂ production traces from *T. maxima* before and after addition of DMSO (control; left) and 1 μmol l⁻¹ bafilomycin A1 (right). Breaks in the data correspond to pauses in data acquisition for treatment addition and dashed lines indicate initial photosynthetic production trajectories. (B,C) Relative net O₂ production rates in the presence of DMSO (control) or the VHA inhibitors bafilomycin A1 (B; *n*=9) and concanamycin A (C; *n*=8). Data are plotted as the ratio of the mean rate of O₂ production (mean±s.e.m.) post-acquisition pause to the initial rate and asterisks denote a significant difference from control.

However, the two VHA inhibitors impeded O₂ production to the same degree, so our conclusions are supported by results from independent samples from seven clams under pharmacological VHA inhibition.

RESULTS

VHA is highly abundant in *T. maxima* siphonal mantle tubules

Western blot analyses revealed very high VHA abundance in siphonal mantle, and much lower abundance in *Symbiodinium* and clam byssal mantle and gill (Figs 2 and 3). Immunolabeling in siphonal mantle showed VHA is highly expressed in the apical

membrane of epithelial Z-tubule cells which contain *Symbiodinium* (Fig. 4), similar to VHA_B in *T. squamosa* (Ip et al., 2018).

Clam VHA promotes *Symbiodinium* photosynthesis

To explore whether VHA promotes *Symbiodinium* photosynthesis, we tested the effect of pharmacological VHA inhibition on net O₂ production by isolated siphonal mantle tissue. Under artificial light (~235 μmol photons m⁻² s⁻¹), VHA inhibition with bafilomycin A1 significantly reduced net O₂ production by 37±14% (Fig. 5A,B). Because light intensity in coral reefs is typically much greater, we next tested the effects of VHA inhibition with concanamycin A on siphonal mantle samples exposed to natural tropical sunlight (~2000 μmol photons m⁻² s⁻¹). In these trials, gross O₂ production rates were also significantly reduced, this time by 38±26% (Fig. 5C). Given that VHA abundance in Z-tubule cells is much greater than in *Symbiodinium* [where it is not visible by immunofluorescence (Fig. 4) and is barely detectable by western blotting (Fig. 3)], and that VHA inhibition does not affect O₂ production in *Symbiodinium* isolated from coral (Barott et al., 2015), we conclude that the VHA responsible for stimulating O₂ production in our experiments belongs to host clam cells.

Upregulation of aerobic respiration rate in the light

Finally, we investigated whether *Symbiodinium* photosynthetic activity stimulates aerobic respiration in *T. maxima* siphonal mantle biopsies. Indeed, mass-normalized O₂ consumption rates increased from 22±9 nmol O₂ g⁻¹ min⁻¹ in the dark to 107±21 nmol O₂ g⁻¹ min⁻¹ after 20 min of light exposure.

DISCUSSION

Here, we report that VHA is present in the apical membrane of epithelial Z-tubule cells, and that downregulation of VHA activity using two specific VHA inhibitors significantly reduces photosynthetic O₂ production by *Symbiodinium*. This indicates that host VHA secretes H⁺ into the lumen of the Z-tubules and promotes *Symbiodinium* photosynthesis by elevating CO₂ concentration and overcoming the limitations of *Symbiodinium* RuBisCo, which has low CO₂ affinity. Thus, similar to scleractinian corals (Barott et al., 2015), VHA activity is part of a host-controlled CCM.

A model for this CCM in the tridacnid clam–*Symbiodinium* symbiosis is shown in Fig. 6. As recently proposed (Ip et al., 2017, 2018), CA-2-like proteins present in epithelial Z-tubule cells likely catalyze the hydration of CO₂ produced in host clam cells into H⁺ and HCO₃⁻. H⁺ is then actively transported by VHA into the Z-tubule lumen, resulting in tubular acidification as well as the generation of an electromotive force for HCO₃⁻ secretion. Within the tubule lumen, host-derived extracellular CAs could then dehydrate HCO₃⁻ and H⁺ back to CO₂, which then diffuses into *Symbiodinium* to be fixed into organic carbon compounds during photosynthesis. Additionally, HCO₃⁻ present in the Z-tubule lumen derived from external seawater might also be dehydrated into CO₂ and taken up by *Symbiodinium* (Ip et al., 2018).

Several aspects of this CCM in coral and tridacnid clams remain unknown and will be explored through future research. For example, *Symbiodinium* expresses a P-type H⁺-ATPase (Bertucci et al., 2010; Mies et al., 2017a,b) that is not sensitive to VHA inhibitors (An et al., 2001; Maeshima et al., 1999; Obermeyer et al., 2008; Palmgren, 1998) and might also contribute to the CCM. Supplementary work is also needed to characterize as-yet unidentified HCO₃⁻ transporter(s) in clam host cells that are required (working together with VHA) to deliver C_i to *Symbiodinium*. In addition, other CCMs should be

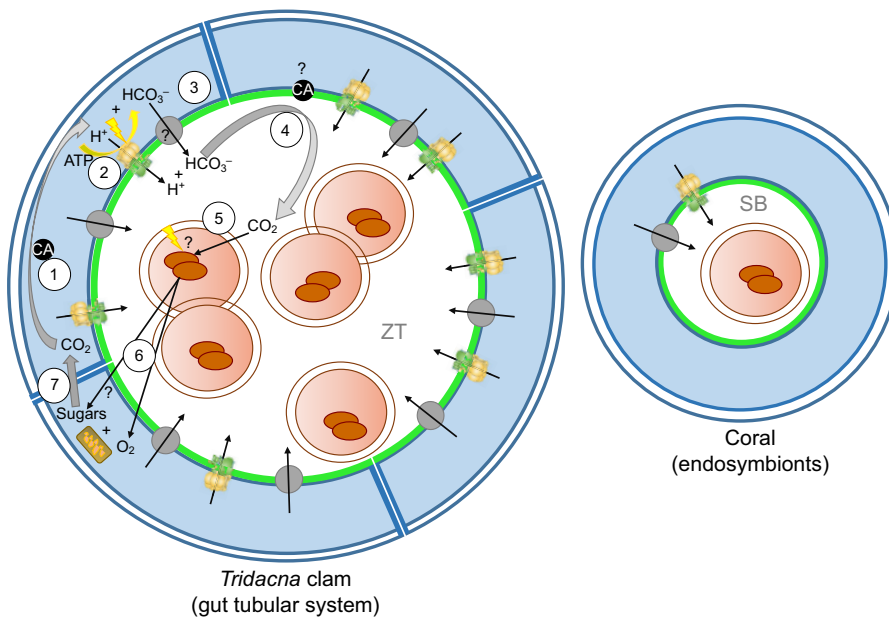


Fig. 6. Model of zooxanthellae tubule system in *T. maxima* as compared with the endosymbiotic arrangement in corals. (Left) In giant clams, *Symbiodinium* are hosted extracellularly in digestive tubules (ZT) bounded by host clam cells (blue) that express VHA in apical membranes (green). (Right) In corals, algae are housed intracellularly in a membrane-bound symbiosome (SB) containing VHA. In both taxa, metabolic CO_2 is converted by host carbonic anhydrase (CA) to H^+ and HCO_3^- (1). H^+ is then actively transported into the tubule lumen by clam VHA (green/yellow structure; 2). HCO_3^- follows via an unknown bicarbonate transport mechanism (gray circle; 3), whereupon putative host-derived CA catalyzes the reconversion of both substrates into CO_2 (4). CO_2 diffuses into *Symbiodinium* cells (5) where it is transported by unknown mechanisms to algal chloroplasts. Algal photosynthesis drives the production of O_2 , which diffuses into host tissues, and sugars (6), which are translocated by unknown mechanisms to host mitochondria where they fuel oxidative catabolism and production of metabolic CO_2 (7) forming a link to the conversion of CO_2 to H^+ and HCO_3^- by CA (1).

present within *Symbiodinium* to drive CO_2 across the various lipid membranes that separate *Symbiodinium* cytoplasm and RuBisCo, which is located in the chloroplast stroma and pyrenoid (Jenks and Gibbs, 2000). Regulation of additional aspects of the symbiosis by host VHA activity such as NH_4^+ and/or PO_3^- supply, or *Symbiodinium* cell division (reviewed in Tresguerres et al., 2017) is another intriguing possibility for future study. Finally, we observed a 5-fold increase in *T. maxima* aerobic metabolic rate as a result of *Symbiodinium* photosynthetic activity. Whether this upregulation was due to the increased O_2 concentration, the increased availability of carbohydrates translocated from *Symbiodinium* to the giant clam host, or a combination of the two remains to be explored.

Conclusions

Tridacnid clam host cells have a VHA-dependent CCM that significantly promotes photosynthesis by extracellular endosymbiotic *Symbiodinium* living in the Z-tubule lumen. In turn, *Symbiodinium* photosynthetic activity allows tridacnid clams to maintain high aerobic respiration rates. This VHA-dependent CCM is a case of convergent evolution with scleractinian corals, and deserves further investigation to determine whether it has evolved in other photosymbioses.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.J.A., M.T.; Methodology: E.J.A., J.N.R., J.H.S., M.T.; Formal analysis: E.J.A.; Investigation: E.J.A., J.N.R.; Resources: E.J.A., M.T.; Data curation: E.J.A.; Writing - original draft: E.J.A.; Writing - review & editing: E.J.A., J.N.R., J.H.S., M.T.; Visualization: E.J.A.; Supervision: J.H.S., M.T.; Project administration: J.H.S.; Funding acquisition: E.J.A., J.H.S., M.T.

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