

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

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Summary Statement

Vacuolar-type H⁺-ATPase (VHA) plays a convergent role in promoting *Symbiodinium* photosynthesis in giant clams and corals suggesting VHA as a common exaptation for carbon concentration in marine photosymbioses.

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 O_2 production by ~40%. Together with our observation that exposure to light increased holobiont aerobic metabolism ~five-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 VHA-driven CCM is a common exaptation in marine photosymbioses that deserves further investigation in other taxa.

1. 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 with molluscs (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 than can exceed respiratory demand in both coral (~150%) (Muscatine et al., 1984) and *Tridacna* (up to 400%) (Klumpp and Griffith, 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 coral, 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 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 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 high abundance of carbonic anhydrases (CAs) in the siphonal mantle (Yellowlees et al., 1993), as well a recent paper reporting 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 compared to 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 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*.

2. Materials and methods

(a) Organism acquisition and husbandry

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

Photosynthetic activity experiments were conducted on adult *T. maxima* collected (under ordinance n° 88-184/AT of the French Polynesian Ministère de l'économie, des finances, du travail et de l'emploi) 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 ± 1 °C, 35.96 ± 0.2 PSU, $\text{pH} = 8.13 \pm 0.09$; $\bar{x} \pm \text{SD}$). Typical $[\text{NH}_4^+]$ on the reef is ~ 14 μM (<http://observatoire.criobe.pf/wiki/tiki-index.php>), and pCO_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; $\bar{x} \pm \text{SE}$), and experiments involving bafilomycin A1 were conducted during October 2016 ($n = 3$ clams; shell length 15.0 ± 1.5 cm; $\bar{x} \pm \text{SE}$). 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.

(b) Anti-VHA Antibodies and Western blot analysis

VHA within *T. maxima* tissues was quantified and visualized using rabbit polyclonal antibodies against an epitope in the VHA_B subunit, AREEVPGRRGFPGY. This epitope is 100% conserved throughout evolution from cnidarians to mammals, including molluscs. 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), mussel (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 μ L of ice-cold S22 buffer (450 mM NaCl, 10 mM KCL, 58 mM MgCl₂, 10 mM CaCl₂, 100 mM Hepes, pH 7.8) containing a protease inhibitor cocktail (catalog no. P8340, SigmaAldrich, 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 μ L 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 were separated on 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 (TBS-T with 5% non-fat powdered milk) at room temperature for 1 h before incubation in the primary antibody (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 (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 BioRad Universal III Hood using ImageQuant software (BioRad). PVDF membranes incubated in blocking buffer with anti-VHA antibodies and 1000-fold excess (mol:mol) blocking peptide served as peptide pre-absorption control (Fig. 2).

(c) VHA Immunolocalization

Siphonal mantle tissue samples containing symbiotic algae (~1 cm x 1 cm x 0.5 mm thick) and gill (~1 cm x 1 cm x 0.5 mm) were vivisected from juvenile clams (n=6) and immersed in fixative (0.1M sodium cacodylate buffer, 3% paraformaldehyde and 0.35% glutaraldehyde; Electron Microscopy Sciences, Hatfield, PA, USA) for 6 hours 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 x 10 min) prior to paraffin embedding (55 °C, 3 x 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 x 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 phosphate buffered saline, pH 7.8, 1 h), tissue sections were incubated with anti-VH_{AB} antibodies (3 $\mu\text{g mL}^{-1}$ in blocking buffer) (overnight at 4 °C). Tissue sections were then washed three times in PBS, incubated with the secondary antibody (1:500, goat anti-rabbit Alexa 555) (1h at room temperature), and stained with Hoescht 33342 (Invitrogen, Grand Island, NY, USA) at 5 $\mu\text{g mL}^{-1}$ for 5 min to visualize nuclei. Controls were prepared as above except sections were incubated with anti-VH_{AB} antibodies and 1000-fold excess (mol:mol) blocking peptide.

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

(d) O₂ consumption and production rates

Photosynthetic activity and aerobic metabolism were estimated by closed-cell respirometry at 27 ± 1 °C. O₂ consumption rate was measured in the dark on freshly vivisected ~ 2 mm x 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). Similar glass vials without tissue served as controls to correct for background O₂ consumption. 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 light supplied by a 28W fluorescent light source (Coralife model 5300, 10K/420nm actinic bulbs) with tissue exposed to an average irradiance of $234.65 \pm 0.775 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (PAR scalar irradiance sensor, Biospherical Instruments, Model QSL2101) for 20 min prior to immediately measuring dissolved O₂ 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 Wieczorek, 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 O₂ production rate. Media was then replaced with either seawater containing 1% DMSO, or seawater containing the VHA inhibitor bafilomycin A1 (1 μM 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 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 O₂ 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 μM concanamycin A1 in DMSO (final concentration 1%). Half of the replicate

tubes were wrapped with aluminum 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 O₂ production as described previously. All samples were transferred to outdoor holding tanks at 27 °C and experiments were performed under ambient solar irradiance (~14:00 – 15:30 h). Tissues in the photosynthetic treatments were continuously exposed to $\sim 1,980 \pm 40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ as measured with a Mastech Digital Lux Meter (MS6612) 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. Concentration of dissolved O₂ was measured for 3 min in each vial and averaged to give final end-point O₂ concentrations for each treatment. Tissues were dried and weighed, and gross O₂ production rates were calculated as the mass-normalized rate of O₂ production in the light-exposed treatments plus the mass-normalized O₂ consumption rates of the dark treatment for each tissue.

(e) 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 O₂ production data. End point respirometry data were similarly normalized against mean O₂ 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 to those measured prior within the same tissue sample. All data are given as mean 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. However, both VHA inhibitors impeded O_2 production to the same degree, so our conclusions are supported by results from independent samples from seven clams under pharmacological VHA inhibition.

3. Results and discussion

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

Western blot analyses revealed very high VHA_B abundance in *T. maxima* siphonal mantle, and much lower abundance in *Symbiodinium*, clam byssal mantle, and gill (Fig. 2-3). Immunolabeling revealed VHA_B was abundantly present in cells in the siphonal mantle (Fig. 4), similar to VHA_B in *T. squamosa* (Ip et al., 2018). Specifically, VHA was present in the apical membrane of epithelial Z-tubule cells, indicating VHA secretes H^+ into the lumen where *Symbiodinium* are present.

Clam VHA promotes *Symbiodinium* photosynthesis

To explore whether VHA promotes *Symbiodinium* photosynthesis, we tested the effect of pharmacological VHA inhibition on net O_2 production by isolated siphonal mantle tissue. Under artificial illumination ($\sim 235 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), VHA inhibition with bafilomycin A1 significantly reduced net O_2 production by 37 ± 14 % (Paired, two-tailed t-Test, $p < 0.05$; 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 ($\sim 2,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). In these trials, gross O_2 production rates were also significantly reduced, this time by $38 \pm 26 \%$ (Paired, two-tailed t-Test, $p < 0.05$; Fig. 5C). Given that VHA abundance in Z-tubule cells is much greater than in *Symbiodinium*, where it is not visible by immunofluorescence (Figure 4) and barely detectable by Western Blotting (Figure 3), and that VHA inhibition does not affect O_2 production in *Symbiodinium* isolated from coral (Barott et al., 2015), we conclude the VHA responsible for stimulating O_2 production in our experiments belongs to host clam cells. This indicates that, similar to scleractinian corals (Barott et al., 2015), VHA activity is part of a host-controlled CCM that allows *Symbiodinium* to photosynthesize despite having RuBisCo with a low affinity for CO_2 .

A model CCM in the tridacnid clam-*Symbiodinium* symbiosis is shown in Figure 6. As recently proposed (Ip et al., 2017; Ip et al., 2018), CA-2 like proteins present in epithelial Z-tubule cells likely catalyze the hydration of CO_2 produced in host clam cells into H^+ and HCO_3^- . H^+ is then actively transported by VHA into the Z-tubule lumen, resulting in tubular acidification as well as in the generation of an electromotive force for HCO_3^- secretion. Within the tubule lumen, host-derived extracellular CAs could then dehydrate HCO_3^- and H^+ back to CO_2 , which then diffuses into *Symbiodinium* to be fixed into organic carbon compounds during photosynthesis. Additionally, HCO_3^- present in the Z-tubule lumen derived from external seawater might also be dehydrated into CO_2 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; Mies et al., 2017b) that is not sensitive to VHA inhibitors (An et al., 2001; Maeshima et al., 1999; Obermeyer, G. Kriechbaumer et al., 2008; Palmgrem, 1998) and might contribute to the CCM. Yet unidentified HCO₃⁻ transporter(s) in host cells would also be required (working together with VHA) to deliver C_i to *Symbiodinium* whereas other CCMs should be present to drive CO₂ across the various lipid membranes that separate *Symbiodinium* cytoplasm and RuBisCo, which is located in the chloroplast stroma and pyrenoid (Jenks and Gibbs, 2000). In addition, regulation of additional aspects of the symbiosis by host VHA activity such as NH₄⁺ and PO₄³⁻ supply, or *Symbiodinium* cell division (reviewed in Tresguerres et al., 2017) are other intriguing possibilities for future study.

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. To what degree this five-fold increase in aerobic metabolic rate stems from the increased O₂ concentration, or from increased availability of carbohydrates translocated from *Symbiodinium* to the giant clam host remains to be explored.

4. 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 if it has evolved in other photosymbioses.

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Data accessibility: Results of Western Blots and additional sets of IHC images for gill tissue have been uploaded as part of the electronic supplementary material.

Ethics: Clams were collected following all requirements laid out by the Plan de Gestion de l'Espace Maritime (PGEM) in French Polynesia and were maintained and studied in ways commensurate with all pertinent University of California guidelines.

Authors' contributions: E.A. designed this study, collected specimens and generated most data. E.A. and M.T. composed this paper. E.A., J.R. and M.T. carried out the analysis of VHA. J.S. and M.T. advised on experimental design and interpretation. All authors critically read and edited the manuscript.

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Figures

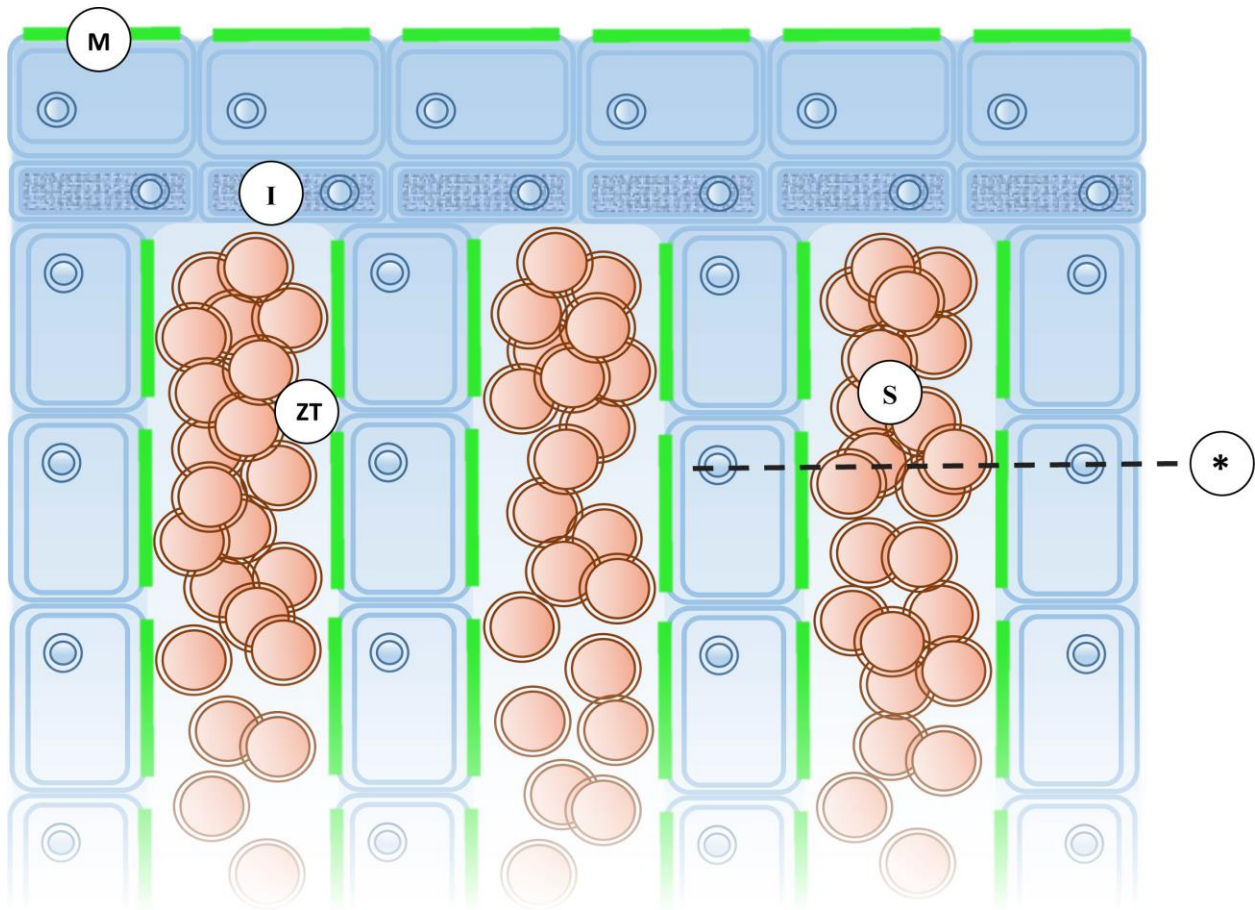


Figure 1. Diagram of *Tridacna maxima* siphonal mantle tissue. Arrangement of zooxanthellae tubules (ZT), *Symbiodinium* (S), mantle margin cells (M), and light-refractive iridocytes (I). VHA localization in the apical membrane of epithelial siphonal mantle cells is shown in green. The dotted line (*)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

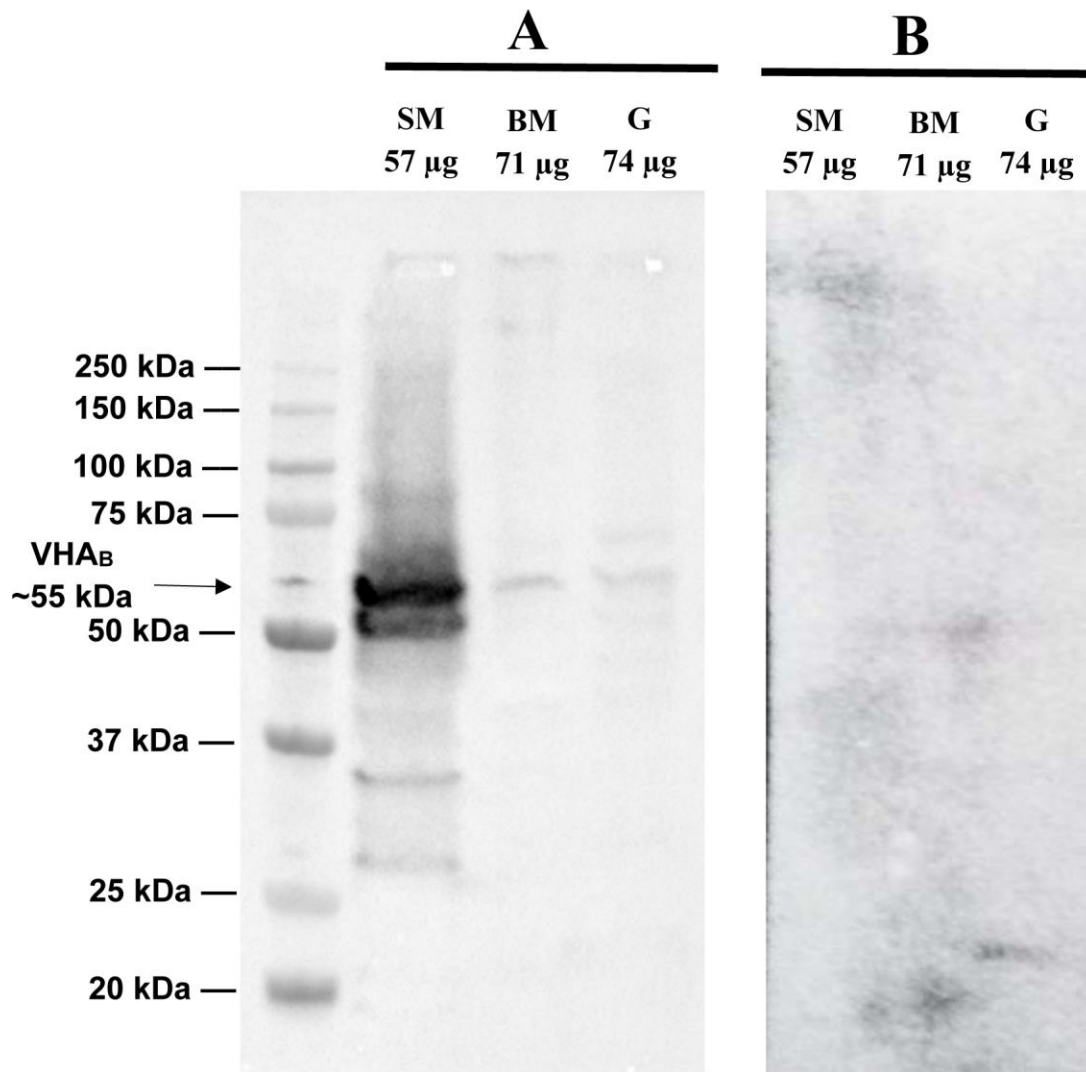


Figure 2. Vacuolar-type H⁺-ATPase in *T. maxima* tissues. (A) Western blotting using anti-VHAB antibodies and (B) peptide preabsorption control. SM, siphonal mantle; BM, byssal mantle; G, gill. The numbers indicate the amount of total protein loaded in each well.

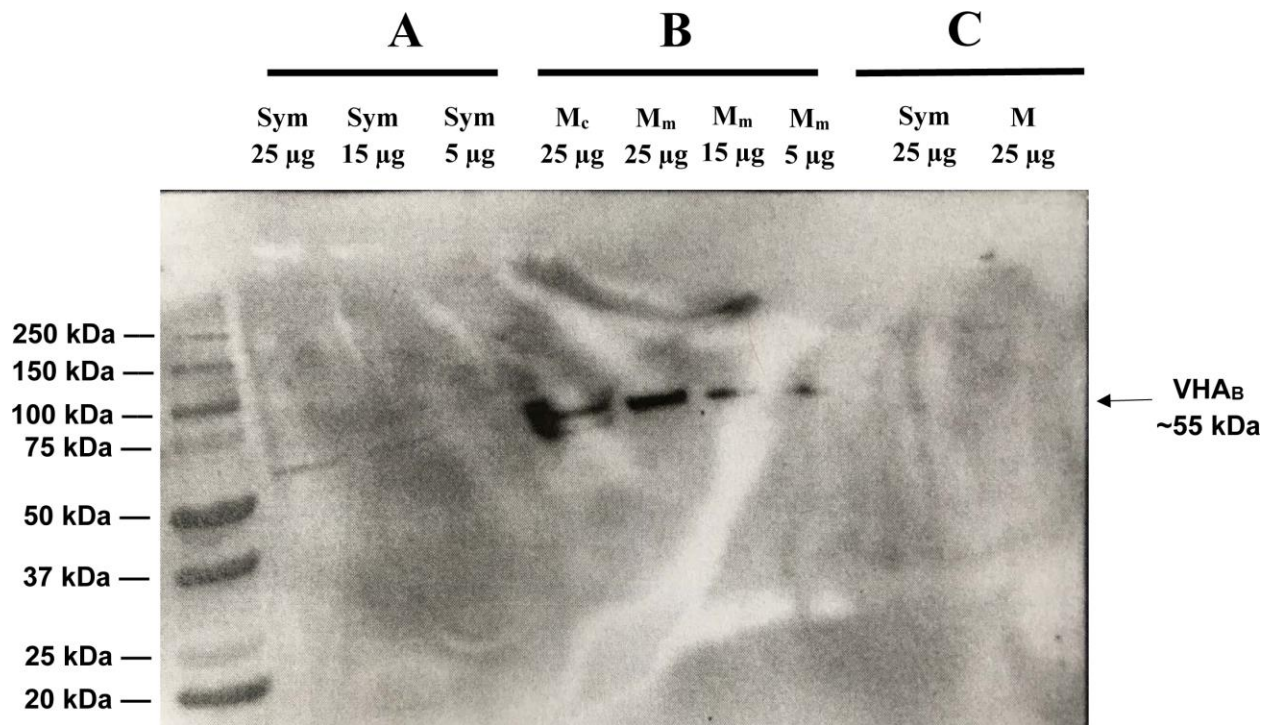


Figure 3. Vacuolar-type H⁺-ATPase in *Symbiodinium*. Western blotting using anti-VHA_B antibodies in (A) *Symbiodinium*, and (B) siphonal mantle membrane fractions. (C) preabsorption control. The relatively high background is due to the increased exposure time necessary to visualize the very faint band present in *Symbiodinium*. The numbers indicate the amount of total protein loaded in each well. *Sym*, *Symbiodinium*; M_c, siphonal mantle cytoplasm associated fraction; M_m, siphonal mantle membrane-associated fraction.

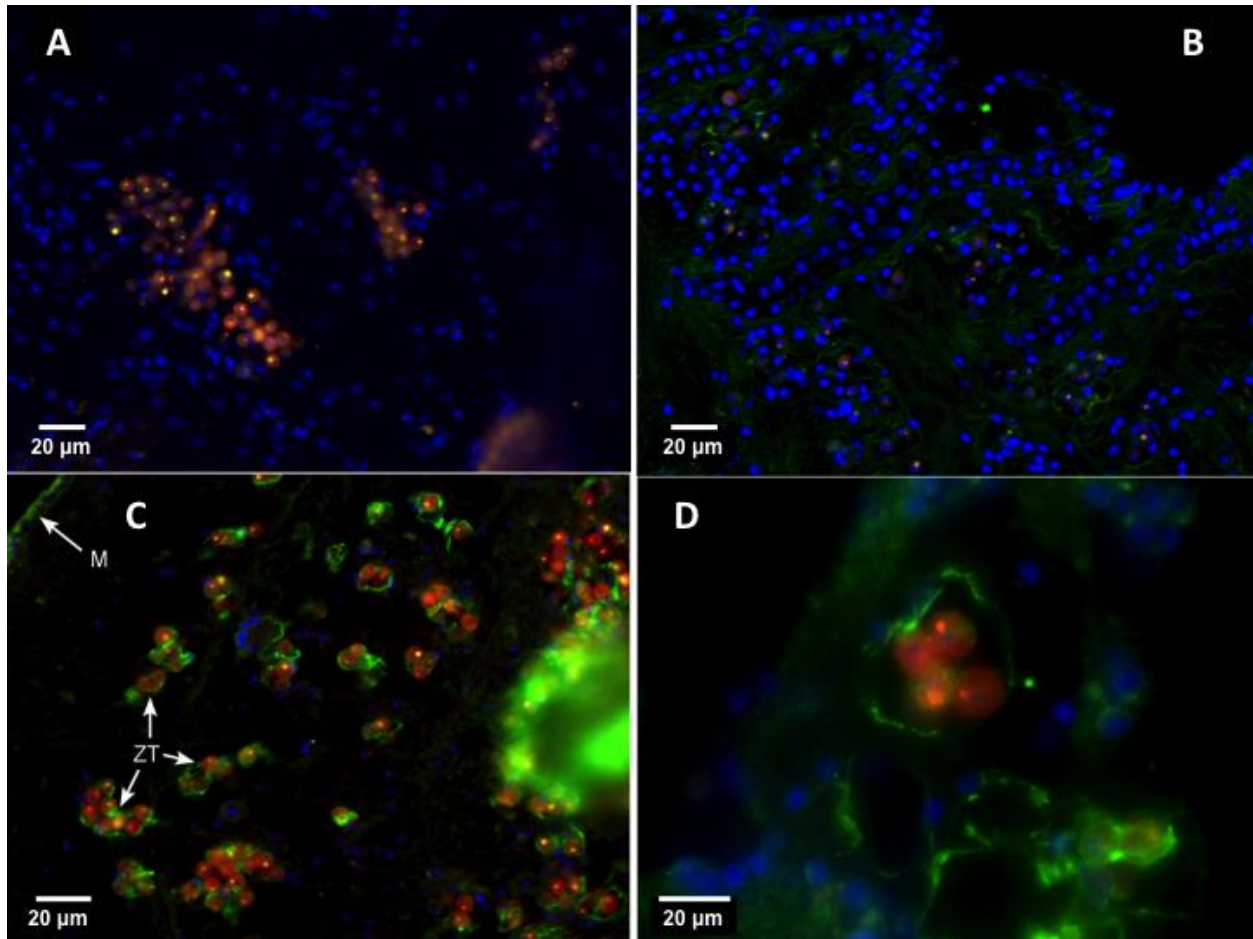


Figure 4. Vacuolar-type H^+ -ATPase is localized to *Zooxanthallae* tubules in the giant clam *T. maxima*. Negative control omission of primary antibody (A) in siphonal mantle, and corresponding anti-VHAB antibody staining in byssal (B) and siphonal mantle (C). High magnification image of the zooxanthellae tubule system shown in (D). VHA in green, nuclear DAPI staining in blue, and chlorophyll autofluorescence in red. ZT, zooxanthellae tubules; M, shell-mantle margin, AM, apical margin of zooxanthellae tube cell.

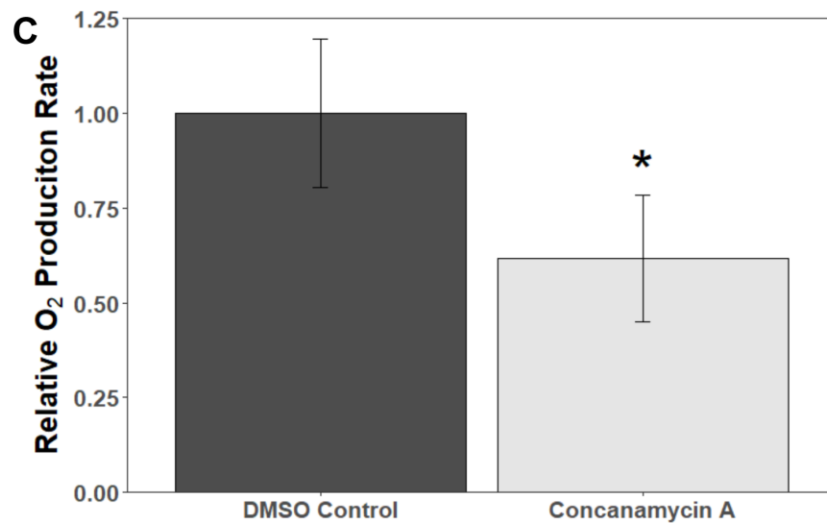
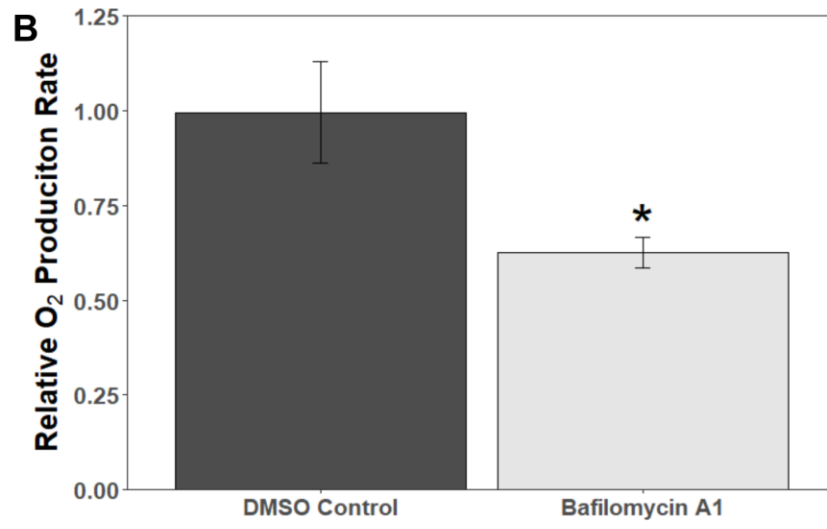
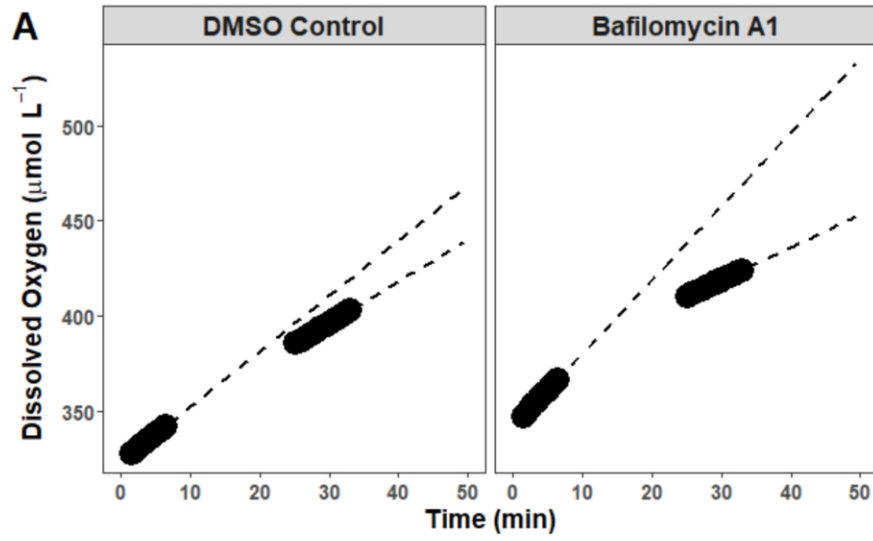


Figure 5. VHA-inhibition reduces net photosynthetic O₂ production in *T. maxima*. (A) Representative O₂ production traces from *T. maxima* before and after addition of DMSO (left) and 1 μM bafilomycin A1 (right). Breaks in data correspond to pauses in data acquisition for treatment addition and dotted lines indicate initial photosynthetic production trajectories. (B) Relative net O₂ production rates in the presence of DMSO or bafilomycin A1 (n = 9) or (C) concanamycin A (n = 8). Data are plotted as the ratio of mean rate of O₂ production ($\bar{x} \pm \text{SE}$) post acquisition pause to the initial rate and asterisk denotes significant difference from control.

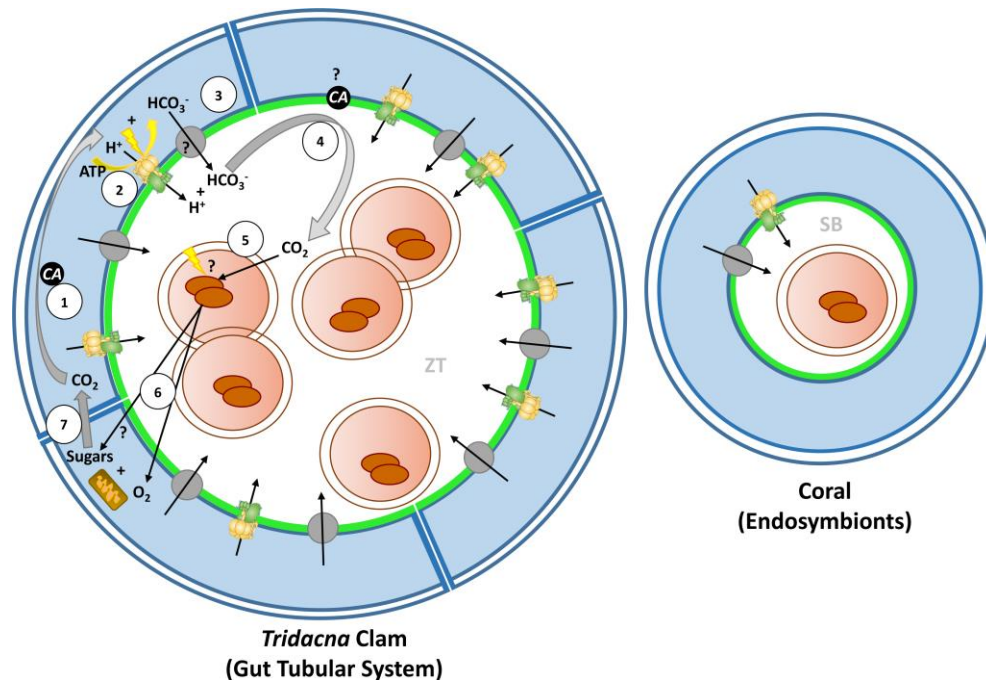


Figure 6. Model of zooxanthellae tubule system in *T. maxima* as compared to endosymbiotic arrangement in corals. (Left) In giant clams, *Symbiodinium* are hosted extracellularly in digestive tubules (ZT) bounded by host clam cells (blue) that express VHA in apical membranes (green). (Right) In corals, algae are housed intracellularly in a membrane-bound symbiosome (SB) containing VHA. In both taxa, metabolic CO₂ is converted by host carbonic anhydrase (CA) to H⁺ and HCO₃⁻ (1). H⁺ is then actively transported into the tubule lumen by clam VHA (green/yellow icon; 2). HCO₃⁻ follows via an unknown bicarbonate transport mechanism (grey circle; 3), whereupon putative host-derived CA catalyzes the reconversion of both substrates into CO₂ (4). CO₂ diffuses into *Symbiodinium* cells (5) where it is transported by unknown mechanisms to algal chloroplasts. Algal photosynthesis drives the production of O₂, 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 CO₂ (7) forming a link to (1).