The effect of ocean acidification (OA) on the photosymbiosis of the two scleractinian coral species *Porites lutea* and *Seriatopora hystrix* - a light exclusion experiment

Die Auswirkung von Ozeanversauerung auf die Photosymbiose der beiden Steinkorallen *Porites lutea* und *Seriatopora hystrix* - ein Lichtausschluss-Experiment

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Abstract

Since pre-industrial times, atmospheric CO_2 concentrations have risen from 280 ppm to > 400 ppm causing a drop in surface ocean pH by 0.1 pH units, which corresponds to a ~ 30% increase in acidity. Ocean acidification (OA) is expected to negatively affect calcifying organisms like scleractinian corals.

Most hermatypic, or reef building, corals live in photosymbiosis with small, single-celled algae (zooxanthellae) of the phylum *Symbiodinium*. The coral provides metabolic nutrients to the algae and benefits from its translocated photosynthetic energy. The algae are assumed to ease the negative effects of OA as they are able to fix excess CO_2 during photosynthesis.

The aim of this bachelor thesis was to analyze the role of the symbiotic algae on the physiological status of the scleractinian corals *Porites lutea* and *Seriatopora hystrix* under the events of OA and additional light deprivation. Coral fragments were collected from a volcanic carbon dioxide vent site within the coral triangle in Papua New Guinea, with seawater pCO_2 values similar to those predicted for 2100 (pH 7.8). Corals from the adjoining reef with normal values of pCO_2 (pH 8.1) served as controls.

Pigment composition and content in the zooxanthellae of the sampled corals were analyzed (via HPLC), as well as biomass and protein content of both coral host and symbiont. The results confirmed former studies in which *Porites lutea* did hardly suffer from OA or even benefit. Pigment concentrations were clearly elevated at the vent compared to the control site and symbiont protein concentrations started to increase at the vent site at the end of the study. *Seriatopora hystrix* instead was significantly affected by OA. Pigment concentrations stayed unchanged but protein concentrations clearly decreased under the influence of OA, whereas biomass concentrations increased. But as biomass build up is a rather tedious process, these findings might not be related to the experiment.

Under the additive stress of light exclusion, both corals were expected to suffer most due to the lack of supporting effects from the symbiosis. Surprisingly, *Porites lutea* was unaffected. Pigment concentrations decreased during darkness but there was no difference between vent and control site. In contrast, *Seriatopora hystrix* was clearly afflicted with both OA and light deprivation. Pigment concentrations declined at both sites but to lower values at the vent site. Interestingly, protein concentrations declined as well at the vent site whereas biomass concentrations were higher compared to those of light control until the mid of the experiment.

Zusammenfassung

Seit der Industrialisierung ist die CO_2-Konzentration in der Atmosphäre von 280 ppm auf > 400 ppm angestiegen. Dieser Anstieg hat im Meer zu einem Abfall des pH-Werts um 0.1 pH-Einheiten geführt und damit zu einer Zunahme der Versauerung um ~ 30%. Daraus resultiert eine geringere Sättigung von Kalzit und Aragonit, was sich negativ auf den Kalkbildungsprozess von Organismen wie Steinkorallen auswirkt.

Die meisten hermatypischen (riffbildenden) Korallen leben in einer Symbiose mit kleinen einzelligen Algen (Zooxanthellen), die zum Stamm *Symbiodinium* gehören. In dieser Symbiose fungiert die Koralle als Wirt und bietet den Algen Stoffwechselprodukte, welche in der Photosynthese der Algen wiederverwertet werden. Im Gegenzug dazu profitiert die Koralle von energiereichen Stoffen, welche von den Zooxanthellen durch Photosynthese gewonnen wurden. Es wird angenommen, dass die Algen den negativen Effekt der Ozeanversauerung lindern können, indem sie das überschüssige CO_2 durch Photosynthese fixieren.

Das Ziel dieser Bachelorarbeit war es, die Rolle der symbiotischen Algen auf den physiologischen Zustand der beiden Steinkorallen *Porites lutea* und *Seriatopora hystrix* unter dem Einfluss von Ozeanversauerung und zusätzlichem Lichtausschluss zu untersuchen. Die Korallenproben stammen aus dem „Korallendreieck“ bei Papua Neu Guinea, wo aufgrund von vulkanischer Aktivität in einigen Bereichen CO_2 aus dem Meeresboden strömt. Der CO_2-Partialdruck (pCO_2) entspricht an diesen Stellen in etwa den Werten, die im Jahr 2100 für die Ozeane vorhergesagt werden (pH 7.8). Die Kontrollkolonien wurden einem benachbarten Riff mit normalen pCO_2-Werten (pH 8.1) entnommen.

Die Pigmentzusammensetzung und -konzentration in den Zooxanthellen wurden mittels HPLC bestimmt. Zudem wurde der Biomasse- und Proteingehalt von Koralle und Symbiont ermittelt. Die Ergebnisse früherer Studien, dass *Porites lutea* kaum durch den Einfluss von Ozeanversauerung beeinträchtigt wird bzw. sogar davon profitierte, konnten hiermit bestätigt werden. Algen in Korallen, die der Ozeanversauerung ausgesetzt waren, wiesen deutlich höhere Pigmentkonzentrationen auf. Gegen Ende des Experiments war auch der Proteingehalt dieser Algen im Vergleich zu den Kontrollkolonien deutlich angestiegen. Im Gegensatz dazu zeigte *Seriatopora hystrix* unter diesen Bedingungen eine deutliche Beeinträchtigung. Im Vergleich zur Kontrollstelle blieben die Pigmentkonzentrationen in den Algen, welche der Ozeanversauerung ausgesetzt waren, zwar unverändert, der Proteingehalt jedoch war von Anfang an deutlich

geringer. Im Gegensatz dazu stieg die Biomassekonzentration an. Da es sich bei dem Aufbau von Biomasse jedoch um einen langwierigen Prozess handelt, ist es möglich, dass diese Entwicklung nicht auf den Versuch zurückzuführen ist.

Weiterhin wurde erwartet, dass zusätzlicher Lichtausschluss die Korallen am meisten schwächen würde, da sie unter diesen Bedingungen keine weitere Unterstützung von ihrem Symbionten erhalten können. Überraschenderweise blieb *Porites lutea* jedoch unbeeinträchtigt und die Pigmentkonzentrationen sanken unter Ozeanversauerung in gleichem Maß sanken wie an der Kontrollstelle. Protein- und Biomassegehalt blieben an beiden Standorten unverändert. Im Gegensatz dazu wurde *Seriatopora hystrix* durch die Kombination von Ozeanversauerung und Lichtentzug deutlich geschwächt. Zwar sanken die Pigmentkonzentrationen ebenfalls an beiden Standorten im Verlauf des Experiments ab, waren jedoch unter Ozeanversauerung gegen Ende der Untersuchung deutlich geringer als in den Kontrollkolonien. Auch der Proteingehalt sank im Vergleich zur Kontrolle deutlich ab, während die Biomassekonzentrationen bis zur Mitte des Experiments höher waren.

List of abbreviations

Ø	diameter
%	per cent
Ω	saturation state
$^{1}O_2$	single state oxygen
A	absorption
ATP	adenosine triphosphate
°C	degree Celsius
Ca^{2+}	calcium ion
$CaCO_3$	calcium carbonate
cf.	*confer* = compare
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
DEP	diatoxanthin epoxidase
DIN	dissolved inorganic nitrogen
DME	daily metabolic energy
E	east
e. g.	*exempli gratia* = for example
G1	gap phase 1
G2	gap phase 2
g	gravity acceleration
H^+	hydrogen ion
H_2O	water

List of abbreviations

HCO_3^-	bicarbonate ion
HPLC	High Pressure Liquid Chromatography
i. e.	*id est* = that is
LHC	light harvesting complex
M	mitotic phase
NADPH	nicotinamide adenine dinucleotide phosphate
NH_3	ammonia
OA	ocean acidification
p	partial pressure
PCP	peridinin-chlorophyll protein
pH	$-\log [H^+]$
PO_4^{3-}	phosphate
ppm	parts per million
RC	reaction centre
rpm	rounds per minute
S	south
S	synthesis phase
spp.	*species pluralis* = species

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1. Introduction

About 600.000 km^2 of the surface of the earth are inhabited by coral reefs (Smith, 1978). Coral reefs are the most diverse marine ecosystems per unit area (Osborne, 2000) and are mainly found in shallow tropical and subtropical waters (reviewed in Johnston, 1980; Osborne, 2000). They require a minimum temperature of 18 °C (Johnston, 1980; Birkeland, 1997; Osborne, 2000) and are restricted to a maximum of 36 °C (Birkeland, 1997). Coral reefs also rely on clear and clean water with sufficient light intensities and moderate salinities (Birkeland, 1997; Osborne, 2000). Each year, the reefs absorb about 700 billion kilograms of carbon. This is one of the reasons why they are of high importance for the welfare of our planet (Birkeland, 1997).

1.1 Basics about corals

Corals belong to the class Anthozoa within the phylum Cnidaria (Grobben, 1908). They can appear in huge colonies, but the single units are polyps (Dana, 1853), which can be very small (1-2 mm diameter) (Goreau et al., 1979; Birkeland 1997) or quite large (20 cm diameter) (Goreau et al., 1979). The polyps are radially symmetrical (Osborne, 2000), sessile and hollow invertebrates, divided into compartments by lamellae and with a mouth-like opening on top surrounded by one or more rings of tentacles (Dana, 1853). They consist of two layers of cells (ectoderm and endoderm) separated by a cell-free, jellylike layer, the mesoglea (Birkeland 1997; Osborne, 2000). Single polyps are connected by a so-called “coenosarc”, a tubular tissue, building large colonies (Dana, 1853). Corals deposit calcium carbonate in form of aragonite (Dana, 1853; Sheppard et al., 2009) and can be massive and “dome-shaped” or appear as branchy and bushy morphotypes (Dana, 1853).

Hermatypic, or reef building corals contain small, single-celled dinoflagellates (zooxanthellae) (Boschma, 1925; Goreau, 1959). Living in symbiosis with the algae, the corals benefit from additional nutrients resulting from photosynthesis (Sargent & Austin, 1949; Muscatine & Carnichiari, 1969; Johnston, 1980; Osborne, 2000). In shallow water, the corals fully rely on the algae and the energy and carbon they receive from the symbionts. At greater depths or in less clear water, there is not enough light for the zooxanthellae to produce as much energy as needed (Barnes & Taylor, 1973), wherefore the corals are forced to capture zooplankton from surrounding water (Dana, 1853; Birkeland, 1997; Osborne, 2000) by using

their tentacles equipped with stinging or hook-like cells (nematocysts) (Goreau et al., 1979; Sheppard et al., 2009).

1.2 Coral symbiosis and the related physiology

The symbiotic relationship of corals and zooxanthellae is expected to exist since the early Triassic, 240 million years ago (Muscatine et al., 2005) and is essential for the existence of coral reefs (Barnes & Taylor, 1973; Birkeland, 1997; Osborne, 2000). Zooxanthellae belong to the phylum Dinophyta. While earlier studies assumed a single species *Symbiodinium microadriaticum* (Freudenthal, 1962; Lelektin, 2000; Osborne, 2000), the last two decades have unveiled a large diversity of zooxanthellae clades and types (Rowan et al., 1997; Baker et al., 2003; LaJeunesse et al., 2005). The tiny coccoid cells (only 5 – 15 µm in diameter; Freudenthal, 1962) live inside the endodermic cells of the coral, covered by one or more membranes of the host, which builds a vacuolar compartment (symbiosome). They reach average densities of 1.45 $\times 10^6$ cells per cm^2 of coral tissue (Drew, 1972) which can vary with light intensity, nutrient availability and temperature (Drew, 1972; Dubinsky & Jokiel, 1994; Fagoonee et al., 1999).

Photosynthesis is the basis of all life on earth. Photosynthetic pigments in plants and algae absorb light energy and convert it into chemical energy (ATP) and reducing power (NADPH) (Wright et al., 1997; Kirk, 2011). Several pigments, which capture light, and electron carrier, which use absorbed energy to create reducing power, are located in special types of membrane (thylakoid) within the chloroplast of the zooxanthellae (Kirk, 2011). Zooxanthellae exhibit six major photosynthetic pigments: chlorophyll *a* and c_2, peridinin, diadinoxanthin, diatoxanthin and β-carotene (Strain et al., 1944; Jeffrey & Haxo, 1968). The main function of chlorophylls is light absorption in so-called light-harvesting complexes (LHC) but they can also operate as electron donor and acceptor in reaction centres (RC) (Wright et al., 1997). Carotenoids are associated in photosynthetic pigments (Bresinsky et al., 2008) with main purpose on light harvesting (Wright et al., 1997). Peridinin is only found in dinoflagellates (Jeffrey & Haxo, 1968; Sitte et al., 2002) and is responsible for the golden-brown colour, which is typical for zooxanthellae (Hochberg et al., 2005). It is assumed, that there is an efficient energy transfer from the carotenoid peridinin to chlorophyll *a* (Haxo et al., 1976), which marks it as an important accessory pigment. Carotenes, such as peridinin, ß-carotene, diadinoxanthin and diatoxanthin also work photoprotective (Sieferman-Harms, 1987; Demers et al., 1991; Arsalane, et al. 1994Wright et al., 1997).

The symbiosis between corals and zooxanthellae also enables both partners to effectively take up nutrients and to re-use them as well as the photosynthates (Muscatine & Porter, 1977). Photosynthates are the products of zooxanthellar photosynthesis, such as oxygen, nitrogen, phosphate (Sargent & Austin, 1949; Osborne, 2000), amino acids or lipids (Muscatine, 1990) and carbon (Sargent & Austin, 1949; Muscatine & Carnichiari, 1969). Zooxanthellae fix carbon via the C3 carbon-fixation pathway (Calvin-Benson carbon reduction cycle) (Streamer et al., 1993). Main products are hexose phosphate, malate, aspartate and glucose (Streamer et al., 1993) and glycerol (Hofmann & Kremer, 1981). Fixed carbon is used for respiration and synthesis of new cell walls in the symbiont but can also be transferred to the host (Muscatine et al., 1984). Nitrogen taken up by zooxanthellae can be transformed into amino acids (Miller & Yellowlees, 1989) and thus, be transferred to the coral host where it is used for the buildup of proteins and biomass (Trench, 1993). The zooxanthellae translocate up to 99% of their photosynthetic products to the coral (Muscatine and Cernichiari, 1969). The polyps provide access to light, inorganic nutrients (CO_2, NH_3, PO_4^{3-}) and protection (Yellowlees et al., 2008).

1.3 Ocean acidification - a stressor to the symbiosis

This very delicate symbiosis between coral host and dinoflagellate algae is dependent on a very narrow range of stable environmental conditions and especially endangered by irreversible changes or degradations of the environment (Glynn, 1990). In this context the gradual changes in ocean chemistry and temperature due to the anthropogenically induced climate change is of major interest and importance.

Since pre-industrial times, temperature increased already by 0.85 °C (IPCC, 2014) and atmospheric CO_2 concentrations rose from 280 ppm to > 400 ppm (NOAA, 2013). Due to increased CO_2 uptake of the oceans, surface ocean pH already decreased by 0.1 pH units, which equals an increase of acidity of 26 % (IPCC, 2014). Today, surface ocean pH is leveled at 8.1 (NOAA, 2014) and is predicted to decrease by 0.3 – 0.5 pH units by 2100 (Caldeira and Wickett, 2005). The concentration of bicarbonate ions [HCO_3^-] is increasing while the one of carbonate ions [CO_3^{2-}] is declining as the following reaction shows (Orr et al., 2005):

$$CO_2 + CO_3^{2-} + H_2O \rightarrow 2HCO_3^- \quad (1)$$

In consequence the saturation states of calcite (Ω_c) and aragonite (Ω_a) are declining (Orr et al., 2005). This process is called ocean acidification (OA), a condition which is expected to make it

hard for some marine calcifying organisms, like corals, to build up biogenic calcium carbonate ($CaCO_3$) (Orr et al., 2005).

The fixation of carbon by the zooxanthellae supports the corals in building up their massive skeletons (sometimes 5-10 m across (Osborne, 2000)) by calcification (Birkeland, 1997; Osborne, 2000), i.e. the deposition of calcium carbonate ($CaCO_3$). This $CaCO_3$ is built within the calcifying fluid underneath the endodermic cells of scleractinian corals, the so-called *subcalicoblastic space* (Beaugrand, 2014) in the presence of calcium and bicarbonate (McConnaughey & Whelan, 1997):

$$Ca^{2+}\ HCO_3^- \rightarrow CaCO_3 + H^+ \qquad (2)$$

The fact, that calcification in light is much higher (~ threefold) than in darkness, suggests that calcification is linked to photosynthesis (cf. 'light-enhanced calcification'; Gattuso et al., 1999). How the mechanism works in detail is still not completely clear. Gattuso et al. (1999) assumed that a photosynthetic uptake of CO_2 would cause an increase of $CaCO_3$ saturation. A high ATP level which is needed for calcification could be maintained by high O_2 concentrations resulting from algal photosynthesis (Colombo-Pallotta et al., 2010). Due to photosynthetic CO_2 fixation by the algae, pH inside the coral rises and more carbonate ions for calcium carbonate precipitation are provided (Birkeland, 1997; Holcomb et al., 2014). On the other hand, Schneider & Erez (2006) assumed the opposite, i.e. calcification enhances photosynthesis through indirect carbon supply: When excess H^+ ions are transported out of the calcification site and arrive in the cavity of the polyp, they combine with HCO_3^- to form CO_2 which is used up by the zooxanthellae via photosynthesis. High concentration of CO_3^{2-} is expected to enhance the export of H^+ ions out of the subcalicoblastic space.

As OA causes an increase of H^+ ion concentration, the removal of H^+ ions out of the subcalicoblastic space of the coral, is prevented or hindered (Jury et al., 2010). This leads to a low pH inside the coral and as H^+ export and Ca^{2+} import are coupled (McConnaughey & Whelan, 1997), this will also result in a low concentration of Ca^{2+} (Zoccola et al., 2004). This in turn will impede calcification, as Ca^{2+} is needed (2). Whether and how much different species of corals will suffer from increased surface ocean pH depends on their capability to remove H^+ out of the calcifying fluid (Zoccola et al., 2004). Also, H^+ export and Ca^{2+} import are based on the usage of ATP mainly provided by respiration (found in Colombo-Pallotta et al. 2010; Wall & Edmunds, 2013). Thus, if a high concentration of H^+ is present in the ambient seawater, the affected export of H^+ will require more energy (Zoccola et al., 2004). If a coral suffers more or

less from low pH conditions, hence also depends on how much energy it can put up via respiration and also for how long (Wall & Edmunds, 2013).

1.4 Experiment

The symbiosis between corals and algae is not only complex and unique but also a very sensitive system. The coral not only receives huge support in form of nutrients and stability from the zooxanthellae but also relies on the dinoflagellates to cope better with changing climate conditions like OA (de Beer et al., 2000; Al-Horani et al., 2003; Vogel et al., 2015). Natural volcanic carbon dioxide seeps such as found in Papua New Guinea (PNG, Fabricius et al., 2011) simulate future climate change scenarios. Those vent sites perfectly enable *in situ* investigations on how OA effects the physiology of hermatypic corals and their symbiotic algae. In the present study the dependence of the coral host on the presence of the zooxanthellae was examined under the effect of OA. At both, a volcanic carbon dioxide seep and control reef site in PNG, two different coral taxa were investigated:: the massive *Porites lutea* (Milne Edwards and Haime, 1851) and the structurally complex *Seriatopora hystrix* (Dana, 1846), both hermatypic corals containing symbiotic dinoflagellates (zooxanthellae) (Veron, 2000). Indo-pacific *Porites* is a slow-growing, helm-shaped and long-living hermatypic coral with simple formation (Veron, 2000; Fabricius et al., 2011). *Porites lutea* colonies are known to grow at about 1 cm in height per year (Sheppard et al., 2009) and can be over 4 meters across (Veron, 2000). They are supposed to be the oldest living corals (Sheppard et al., 2009). Structurally complex and thin branching coral *Seriatopora hystrix* is widely spread in the indo-pacific ocean (Veron, 2000).

Recent studies at the PNG seep sites showed that massive *Porites spp.* seems to benefit from elevated pCO_2 as the cover of the coral had doubled (Fabricius et al., 2011) and the abundance increased significantly to 157.7 % (Strahl et al., 2015) at high pCO_2. The rate of net photosynthesis was almost double (43%) and at both control and vent site light calcification rates increased three fold (Strahl et al., 2015). But *Porites spp.* was not exclusively positively affected by high pCO_2: the taxonomic richness of hard corals such as *Porites spp.* was reduced by 39% at the seep sites compared to control sites and they were paler (Fabricius et al., 2011). Juveniles of *Porites spp.* also seemed to suffer as their density declined fourfold at high pCO_2 (Fabricius et al., 2011). The impact of high pCO_2 on the structurally complex *Seriatopora hystrix* however was found far stronger than in the massive *Porites*: the abundance was reduced three fold at high pCO_2 sites (Fabricius et al, 2011) and both, light and dark calcification rates were declined

(Strahl et al., 2015). A study on *Acropora millepora*, a coral similarly structured as *S. hystrix* (Veron, 2000), revealed a reduction of biomass (48%) due to simulated OA conditions and an additional decline in weight (96%), net photosynthesis (62%) when treated with reduced light conditions (Vogel et al., 2015).

1.5 Hypothesis

Regarding these prior results both *P. lutea* and *S. hystrix* are likely different in their reaction to high pCO_2. To investigate the immediate dependence of both coral species on their symbiotic algae under high pCO_2 a light exclusion experiment was performed to study the physiological reaction of the coral host after turning off the symbiotic energy supply. *Porites lutea* is expected to hardly suffer from high pCO_2 under normal light conditions (Fabricius et al., 2011; Comeau et al., 2013; Strahl et al., 2015) and is likely able to cope better with a lack of photosynthetic support in darkness due to its massive, slow growing and thick tissue (Veron, 2000). In contrast, branching and fast growing *Seriatopora hystrix* (Veron, 2000) is awaited to suffer more from elevated CO_2-levels as former studies have shown (e.g. Fabricius et al., 2011; Strahl et al., 2015) and might show a faster and more serious reaction to an inhibited photosynthetic input.

2. Material and Methods

2.1 Description of study sites

The present study was performed at a volcanic seep site of ~ 99 % CO_2 (latitude 9.82410 S, longitude 150.81759 E; in the following termed as vent site) and a nearby control reef site (9.82821 S, 150.82052 E) (cf. Stahl et al., 2015) with similar salinity, seawater temperature and geomorphology. The sites are located within the coral triangle at D´ Entrecasteaux Islands, Milne Bay Province, Papua New Guinea (Fig. 1). At the seep site the mean pH_{Total} was 7.8 and partial pressure (pCO_2) 862 ppm (Strahl et al., 2015) with a medium aragonite saturation state (Ω_a) of 2.9 (Fabricius et al., 2011). At the control reef, located 500 m south of the seep site, median pH_{Total} was 8.1, pCO_2 323 ppm (Strahl et al., 2015) and medium Ω_a 3.5 (Fabricius et al., 2011).

Figure 1 Study sites were at natural volcanic seep sites (red dot) within the coral triangle at D´ Entrecasteaux Islands, Milne Bay Province, Papua New Guinea. Modified after google maps: https://www.google.de/maps (last accessed: 07.07.15)

2.2 Coral sampling

For the experimental approach, 8 mother colonies of both, the branching *Seriatopora hystrix* and the massive *Porites lutea* were chosen and marked in a depth of 4 to 5.5 m at each study site (vent and control). A total of 96 fragments (8 mother colonies x 2 sample sites (vent and control)

x 2 treatments (light control and light exlusion) x 3 samplings) was sampled from each coral by chiselling (*P. lutea*) and clipping (*S. hystrix*) the fragments from the upper central part of the mother colonies.

2.3 Experimental setup

The experimental setup is documented in the pictures of Fig. 2: The fragments of both coral species were carefully attached to experimental plastic rails: A total of 16 rails per study site (vent and control) was prepared, each equipped with three fragments of every mother colony of the respective site and both coral species (Fig. 2c). The rails were then left at the site of their collection for 4 days for recovery. At each study site (vent and control) a total of 8 platforms was set up each equipped with a transparent and a dark flow pipe (open at both ends), fixed on a rotatable rack on top of an iron rod braced into the ground. The platform (1m above the ground) of each rack was provided with a current vane to move freely in the water and ensure free water flow through the perspex pipes (Fig. 2a,b). After the recovery period the experiment was started on May 21st 2013 by placing half of the rails (8) at each site in the transparent, the other half in the black flow pipes, making sure that no light reached any of the fragments in the dark pipes (Fig. 2b). One fragment of each flow pipe and mother colony (resulting in 8 replicate samples per treatment at each site and sampling) was then successively recollected on days 2, 10 and 17 in case of *S. hystrix* and on days 3, 11 and 16 in case of *P. lutea*. Immediately after collection 8 mm cores of *P. lutea* and small branches of *S. hystrix* of every sampled fragment were shock frozen in liquid nitrogen and kept at -80°C until further processing. Due to sampling complications, day -11-samples of *Porites lutea* could not be used for examination.

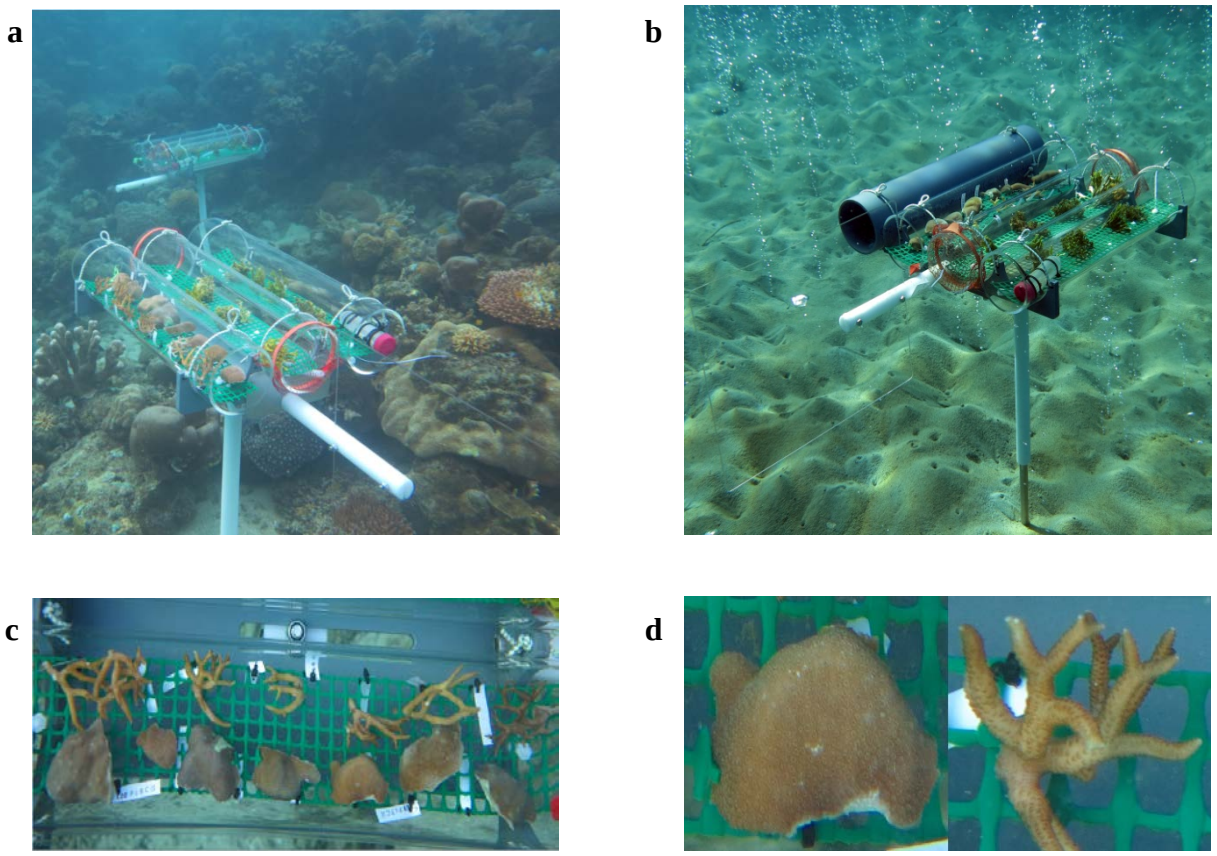

Figure 2 Picture documentation of experimental setup at control (a) and vent site (b) with transparent (control) and black (light exclusion) flow pipes. Additional pipes per platform were used for another experimental approach and were not part of the present study. Fragments of *P. lutea* and *S. hystrix* attached to plastic rails and fixed within flow pipe (c). Close up of *P. lutea* (left) and *S. hystrix* (right) on plastic rail. Photos by **© K. Fabricius**

2.4 Processing of coral tissue

Coral tissue was blown off the skeleton of each fragment with an airbrush and filtered seawater and collected as a thoroughly mixed, homogeneous stock solution (A) of known volume. The remaining chalk skeleton was dried overnight at 60 °C and later used for surface area analysis. 2 ml of the stock solution were filtrated onto GF/F glass microfiber filters (Ø 25 mm, Whatman®) wrapped in aluminium foil, labeled and stored at – 80 °C for later pigment alanysis. The rest of the stock solution (D) was centrifuged for 10 minutes at 0 °C (4400 rpm). The supernatant contained the coral´s tissue and the pellet the algal tissue. For coral biomass determination, 8 – 10 ml of the supernatant (F) were vortexed and filtrated on a pre-combusted and -weighed GF/C glass microfiber filter (Ø 25 mm, Whatman®) and dried overnight at 60 °C. 0.5 ml (H) of the remaining coral tissue solution was stored at -80°C in a separate cryovial for further protein examination. The pellet containing the symbiont´s tissue was 3 times resuspended in seawater, vortexed and centrifuged at 0 °C for 5 minutes (4400 rpm) to ensure a complete elution of any

remaining coral tissue before filling each sample to 3.5 ml with seawater (E). Two ml (G) of the symbiont solution (E) were filtrated on a pre-combusted and -weighed GF/C glass microfiber filter (Ø 25 mm, Whatman®) and dried over night at 60°C for later biomass determination. Half a ml (I) was stored at -80°C in a separate cryovial for further protein examination.

2.5 Surface area determination

The surface area (C) of the coral skeletons was determined geometrically by using a digital caliper (Insize®). Fragments of both coral species were measured to the nearest mm. Nubbins of *P. lutea* were mainly circular (Fig. 3a) or elliptic. Branches of *S. hystrix* had to be divided into several compartments for accurate calculation. Those were mainly cylindric (Fig. 3b) and/or cone shaped.

Common geometric forms were used for the surface calculation:

Circle: $C = r^2 \pi$

Cone: $C = \pi r s$

Cylinder: $C = 2 \pi r h$

Ellipse: $C = r_a r_b \pi$

Triangle: $C = ½ g h$

Trapezoid: $C = ½ h (c + f)$

C = surface area

a, b = opposite sides of the ellipse

c, f = opposite sides of the trapezoid

g = bottom side of the triangle

h = height

r = radius

$s = \sqrt{h^2 + r^2}$

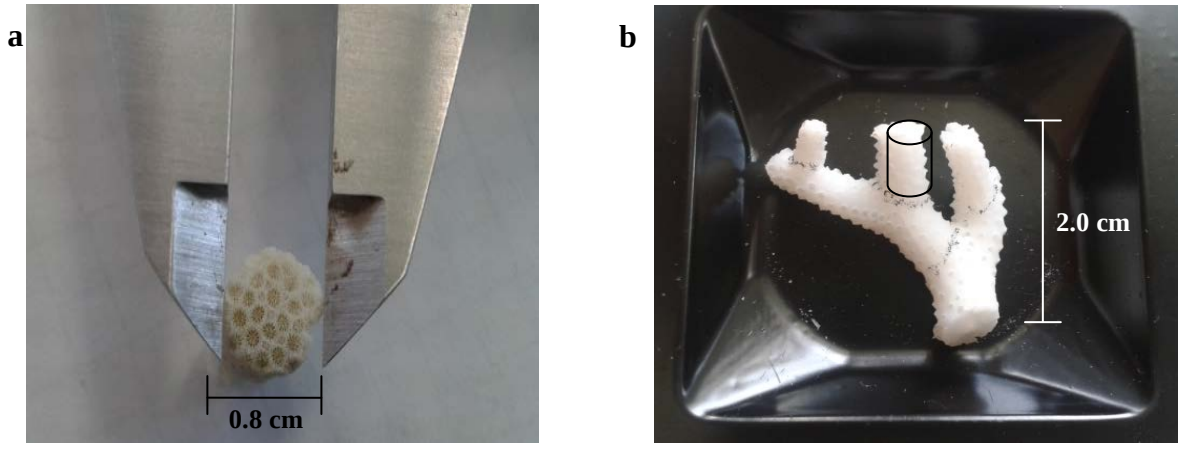

Figure 3 | Surface area analysis of *Porites lutea* which were mostly circular (a) while branches of S*eriatopora. hystrix* were composed and complex and had to be divided into smaller compartments such as single cylinders indicated by pencil lines (b).

2.6 Pigment analysis via HPLC

Filters for pigment analyses were transferred into prepaired apex vials (2ml) filled with zirconia / silica beads (Ø 0.5 mm) and 1.5 ml 100 % acetone and 50 µl of Canthaxanthin analytical standard for extraction. Before each extraction, the absorbance of Canthaxanthin had been measured at 750 nm and 474 nm (Spectronic Genesis 5 photometer) and noted for later correction and analysis. The Apex vials were then placed in a Precellys homogenizer for 20 seconds (5500 rpm) to homogenize the GF/F filters by the added zirconia / silica beads. After the following centrifugation (Heraeus Fresco 17 refrigerated centrifuge) for 5 minutes at 0 °C (1.500 x g) the supernatant was drawn up into a syringe needle (Omnifix®-F Solo 3), filtered through a syringe filter (Rotilabo®, pore size 0.20 µm) and stored at – 80 °C before the actual HPLC measurements started.

High performance liquid chromatography (HPLC) is a common method for separating and analyzing pigments. Each sample was dissolved in an eluent (liquid phase) and under high pressure, it was led through a column containing a stationary phase. Separation of the single molecules depends on size of the examined molecules, ionic charge and polarity. While the fluid phase is led through the stationary phase, the single analytes interact with the column and are separated at the same time (Madigan et al., 2013). Because of interaction with the stationary phase, they reach the detector at different times. If the pigments have a high affinity to the column, they need more time to migrate and vice versa (Wink, 2006). The result is a chromatogram with several peaks, which is displayed on a computer screen. The single pigments can be identified by their retention time (time that is needed between injection and detection) (Antranikian, 2006) and shape of the graph. Both are compared to a library containing graphs of standard pigments.

The pigment content of each sub-sample used for HPLC was calculated backward to the stock solution and then related to the surface area of each fragment as the following equation shows:

$$\frac{Pgs\left(\frac{ng}{l}\right)}{1000} = \mathrm{Pg_s}\left(\frac{ng}{ml}\right)$$

$$\rightarrow \mathrm{Pg_s}\left(\frac{ng}{ml}\right) \cdot \mathrm{A\ (ml)} = \mathrm{Pg_{st}\,(ng)}$$

$$\rightarrow \frac{\frac{Pgs\,(ng)}{1000}}{C\ (cm^2)} = \mathrm{Pg_{st}}\left(\frac{\mu g}{cm^2}\right)$$

A = stock solution

C = surface area

Pg_s = symbiont pigment content of sub-sample

Pg_{st} = total symbiont pigment content per area

2.7 Coral and symbiont biomass content

The biomass of both coral host and symbiont was determined gravimetrically by weighing the dried filters containing the filtrated coral or symbiont tissue, respectively. The biomass in mg cm^{-2} was then calculated backward to the stock solution and related to the surface area as the following equation shows:

Coral: $B_{ct}\left(\frac{mg}{cm^2}\right) = \left(\frac{Bc\ (mg)}{F\ (ml)} \cdot A\ (ml)\right) \cdot \frac{1}{C\ (cm^2)}$

Symbiont: $B_{st}\left(\frac{mg}{cm^2}\right) = \frac{\frac{Bs\ (mg) \cdot E\ (ml) \cdot A\ (ml)}{G\ (ml)}}{D\ (ml)} \cdot \frac{1}{C\ (cm^2)}$

A = stock solution

C = surface area

B_c = coral biomass of sub-sample

B_s = symbiont biomass of sub-sample

B_{ct} = total coral biomass per area

B_{st} = total symbiont biomass per area

D = remaining coral tissue solution

E = symbiont solution

F = sub-sample of coral biomass solution

G = sub-sample of symbiont biomass solution

2.8 Coral and symbiont protein content

The protein content of both coral host and symbiont was determined after Lowry et al. (1951) with a protein assay kit (DC Protein Assay Kit, Bio-Rad) and a bovine serum albumin standard. Concentrations were measured spectrophotometrically (Shimadzu UV 1800) at 750 nm and the protein content of each sub-sample calculated backward to the stock solution and related to the surface area as the following equation shows:

Coral: $P_{ct}\left(\frac{\mu g}{cm^2}\right) = \frac{Pc\ (\frac{\mu g}{ml}) \cdot A\ (ml)}{C\ (cm^2)}$

Symbiont: $P_{st}\left(\frac{\mu g}{cm^2}\right) = \frac{Ps\ (\frac{\mu g}{ml}) \cdot A\ (ml)}{C\ (cm^2)}$

A = stock solution

C = surface area

P_c = protein concentration per ml sub-sample of coral protein solution (H)

P_{ct} = total coral protein content

P_s = protein concentration per ml sub-sample of symbiont protein solution (I)

P_{st} = total symbiont protein content per area

2.9 Statistical analysis

For the statistical analyses that program Sigma Plot 11 was used. All data of pigment, biomass and protein concentrations were first tested for normality with the t-test (Shapiro-Wilk) as always 2 different treatments were compared (vent and control site versus light exclusion and light control). Shapiro-Wilk test is a high power test which was invented for data of low samples size (< 50) (Razali & Wah, 2011). If normality test failed, rank sum test (Mann-Whitney) was used. This test is also used for comparison of 2 data sets and is a very powerful test for non-parametric data (Herrmann, 1984).

3. Results

For the sake of clarity, only remarkable changes in pigment, biomass and protein concentrations are shown and described in this section. Complete figures of each parameter can be found in the appendix (from p. 49).

3.1 Zooxanthellate pigment concentration

Light control: vent versus control site:

Comparing vent and control nubbins in the light treatments of *Porites lutea*, concentrations of all pigments were clearly increased at the vent site from the start (Table 8; chlorophyll *a*: Fig. 4a,b; chlorophyll c_2: Fig. 5a,b; peridinin: Fig. 6a,b; diadinoxanthin: Fig. 7a,b; ß-carotene: Fig. 9a,b; diatoxanthin: Fig. 8a,b).

In light-treated vent fragments of *Seriatopora hystrix* concentrations of diadinoxanthin (Fig. 7 c,d), diatoxanthin (Fig. 8 c,d) and ß-carotene (Fig. 9 c, d) did not change during the time of the study compared to control fragments (Table 18), but chlorophyll *a* (Fig. 4 c, d), chlorophyll c_2 (Fig. 5 c, d) and peridinin (Fig. 6 c, d) in control nubbins were clearly increased at the beginning of the experiment (Table 18).

Light control versus light exclusion at vent site:

At the vent site, concentrations of chlorophyll *a*, chlorophyll c_2, peridinin and diadinoxanthin were clearly decreased in dark-treated compared to light-treated nubbins in both *Porites lutea* and *Seriatopora hystrix* at the end of the experiment (*P. lutea*: Table 3; chlorophyll *a* (Fig. 4a), chlorophyll c_2 (Fig. 5a), peridinin (Fig. 6a) and diadinoxanthin (Fig. 7a); *S. hystrix*: Table 13; chlorophyll *a* (Fig. 4c), chlorophyll c_2 (Fig. 5c), peridinin (Fig. 6c) and diadinoxanthin (Fig. 7c)).

Instead, concentrations of diatoxanthin in dark-treated *P. lutea* and *S. hystrix* showed no effect until day 16 or 17, respectively, and were then clearly elevated compared to light-treated corals (*P. lutea*: Table 3; Fig. 8a; *S. hystrix*: Table 13; Fig. 8c).

Light exclusion at vent site:

In the dark at the vent site, concentrations of chlorophyll *a*, chlorophyll c_2, perdinin, diadinoxanthin in both corals and ß-carotene in *S. hystrix* clearly decreased at the end of the experiment compared to the beginning (*P. lutea*: Table 1, chlorophyll *a*: Fig. 4a; chlorophyll c_2: Fig. 5a; peridinin: Fig. 6a; diadinoxanthin: Fig. 7a; *S. hystrix*: Table 11; chlorophyll *a*: Fig. 4b;

chlorophyll c_2: Fig. 5b, peridinin: Fig. 6b, diadinoxanthin: Fig. 7b and ß-carotene: Fig. 9b; *S. hystrix*: Table 25, chlorophyll *a*: Fig. 4c; chlorophyll c_2: Fig. 5c; peridinin: Fig. 6c; diadinoxanthin: Fig. 7c). In contrast to the other pigments however, concentrations of diatoxanthin (Fig. 8a) in *Porites lutea* were clearly elevated at the end of the experiment (Table 1).

Light exclusion: vent versus control site:

S. hystrix showed clearly increased pigment concentrations at the vent compared to the control site at the end of the experiment (Table 17; chlorophyll *a*: Fig. 4c,d: chlorophyll c_2: Fig. 5c,d; peridinin: Fig. 6c,d; diadinoxanthin: Fig. 7 c, d).

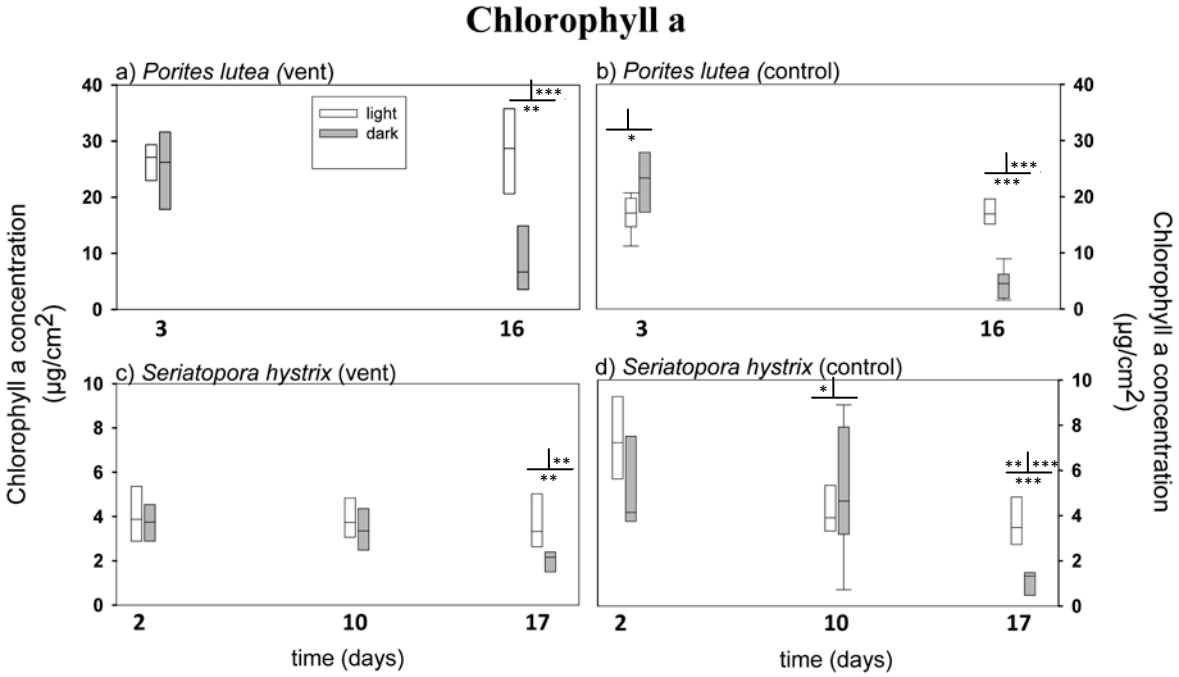

Figure 4 | Chlorophyll *a* concentration in zooxanthellae over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

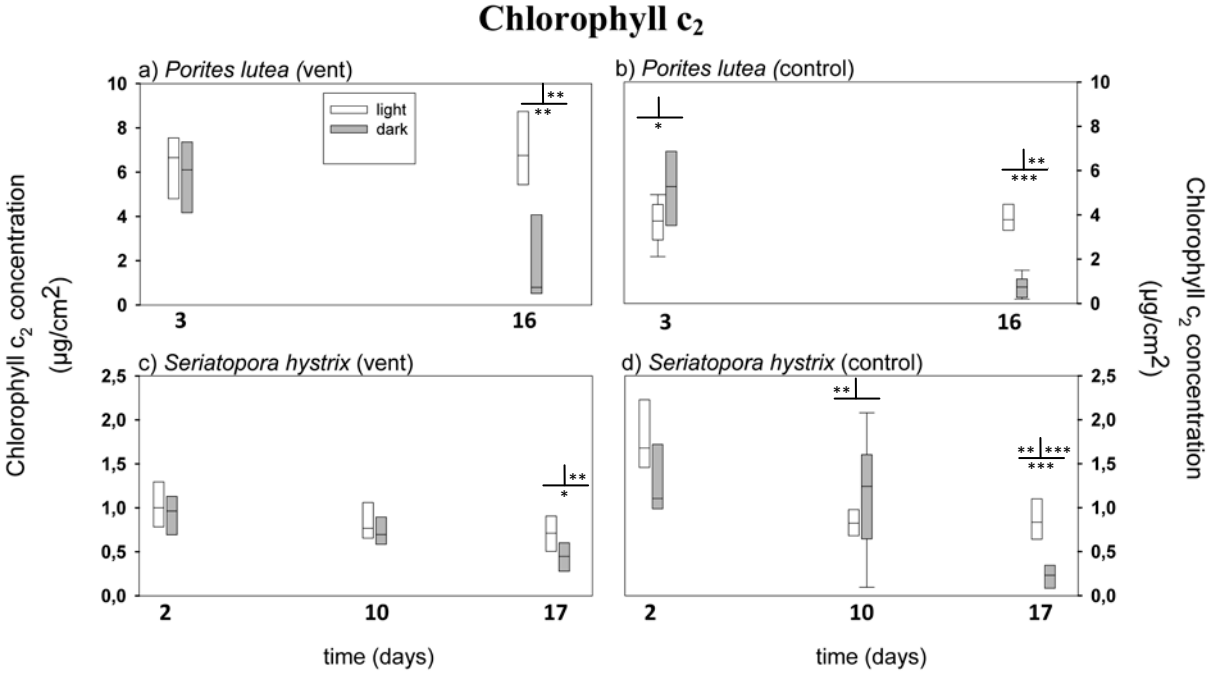

Figure 5 | **Chlorophyll c_2 concentration in zooxanthellae** over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

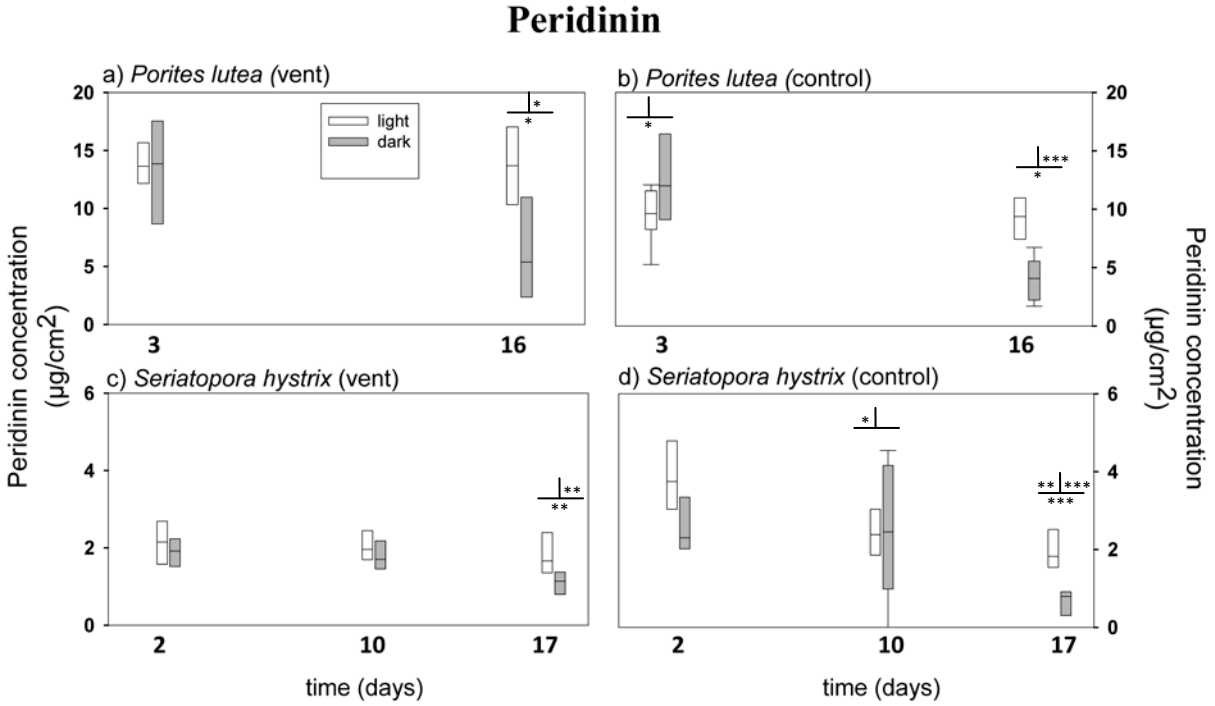

Figure 6 | **Peridinin concentration in zooxanthellae** over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

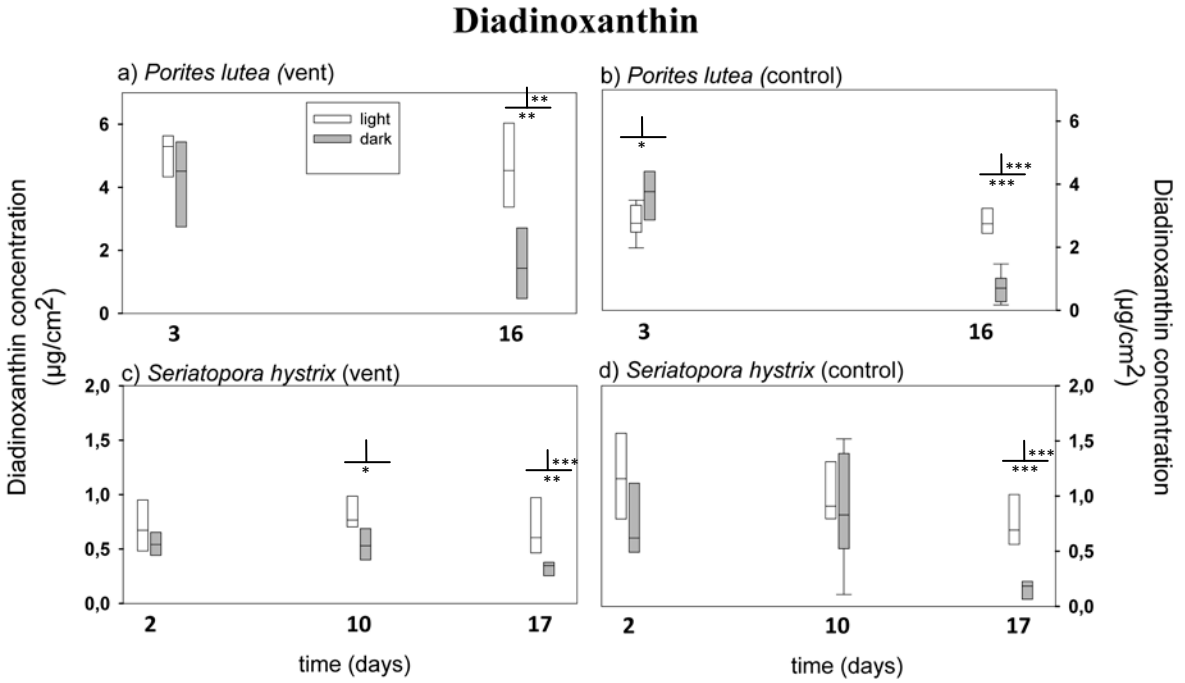

Figure 7 | Diadinoxanthin concentration in zooxanthellae over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

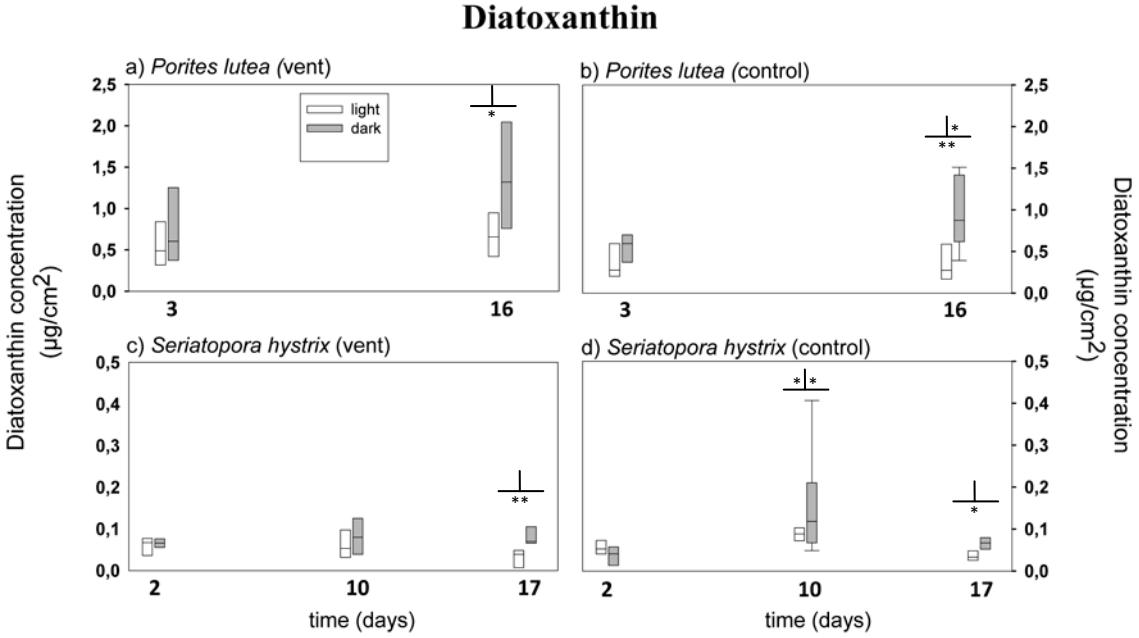

Figure 8 | Diatoxanthin concentration in zooxanthellae over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

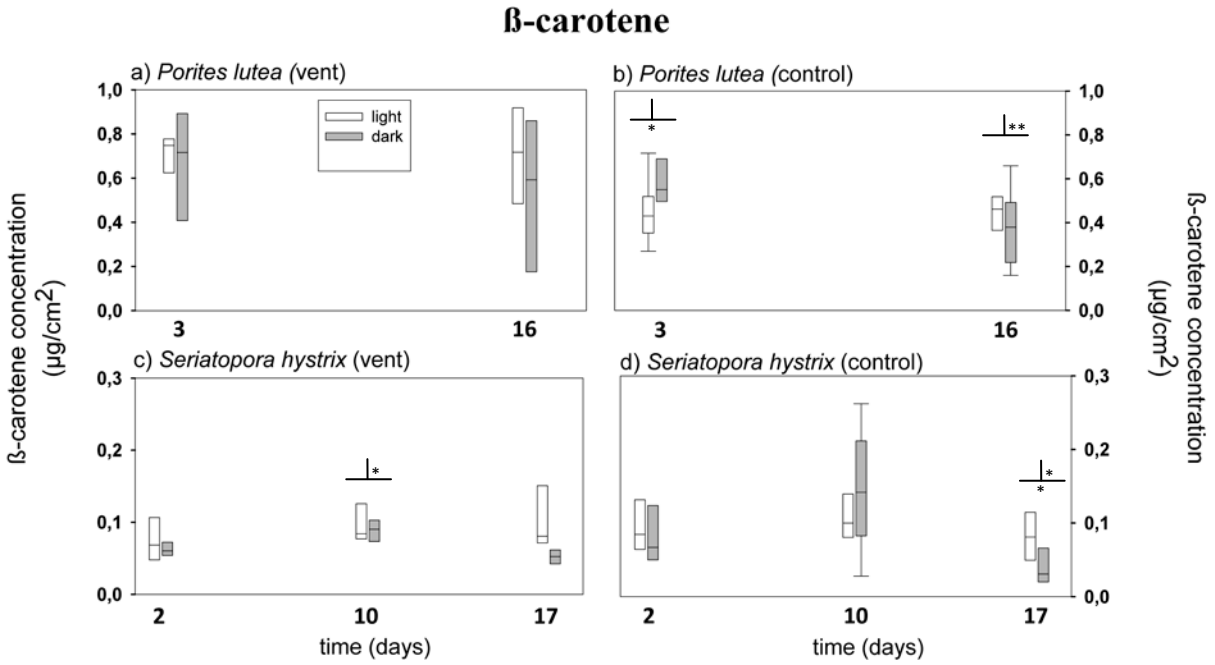

Figure 9 | **ß-carotene concentration in zooxanthellae** over a time period of 16 and 17 days, respectively, at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: *Porites lutea* (a,b) and *Seriatopora hystrix* (c,d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2/3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Tables 1-8 (*P. lutea*); 11-18 (*S. hystrix*) for detailed results.

3.2 Coral host and symbiont biomass concentration

No clear trend of change could be detected in host and symbiont biomass concentrations of *P. lutea* at both vent and control site and in host and symbiont biomass concentrations of *S. hystrix* at control site wherefore those figures are not presented here but can be looked up in the appendix (Fig.13-14, p. 49-50).

Light control at vent site:

Coral host and symbiont biomass of *S. hystrix* showed an increase in the light at the vent site at the end of the experiment (Table 19; Fig. 10).

Light control: vent versus control site:

S. hystrix showed elevated coral host biomass values at the end of the experiment while the symbiont biomass was lower at the beginning of the study (Table 19; Fig. 10).

Light control versus light exclusion at vent site:

Coral and symbiont biomass in dark-treated *S. hystrix* were clearly elevated at the beginning and in the middle of the experiment compared to light treated nubbins (Table 19; Fig. 10).

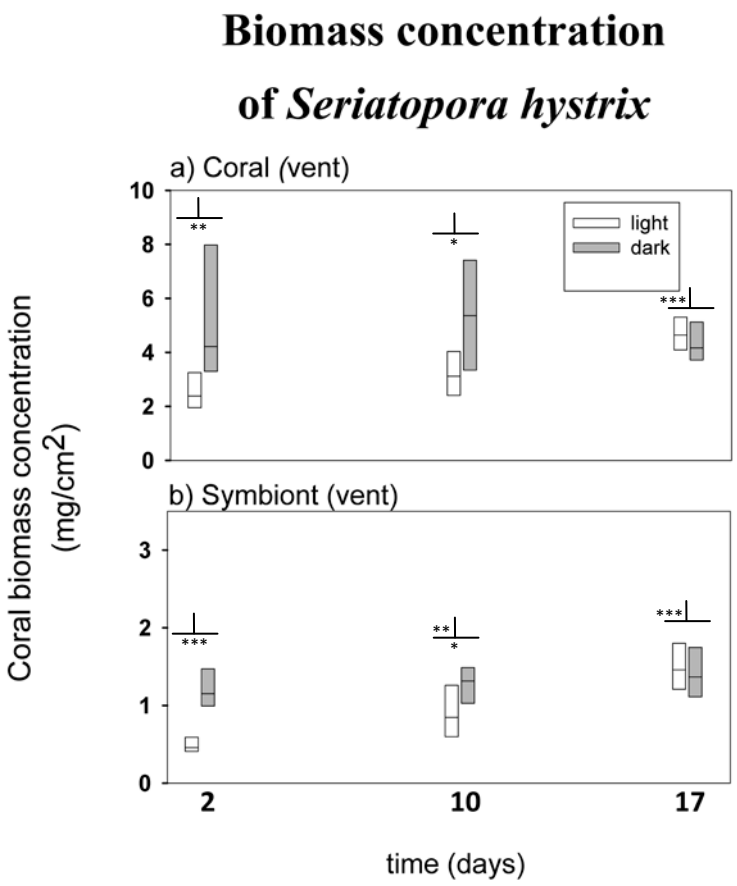

Figure 10 | Biomass concentration of *Seriatopora hystrix* over a time period of 17 days at vent (pH 7.8 and pCO_2 862 ppm) site: coral (a) and symbiont (b). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 19 for detailed results.

3.3 Coral host and symbiont protein concentration

Light control at vent site:

Symbiont and coral protein concentrations of *S. hystrix* showed clearly declined values at the end of the experiment (Table 20; Fig. 12) whereas symbiont protein concentrations of P. lutea were clearly elevated at the vent site at the end of the experiment (Table 10; Fig. 11b).

Light control versus light exclusion at vent site:

Both coral and symbiont protein concentrations of *S. hystrix* in the dark at the vent site were clearly lower than the light controls right from the beginning of the experiment (Table 20; Fig. 12).

Light exclusion at vent site:

Symbiont protein concentrations of *S. hystrix* were clearly lower at the end compared to the beginning of the experiment (Table 20; Fig. 12b).

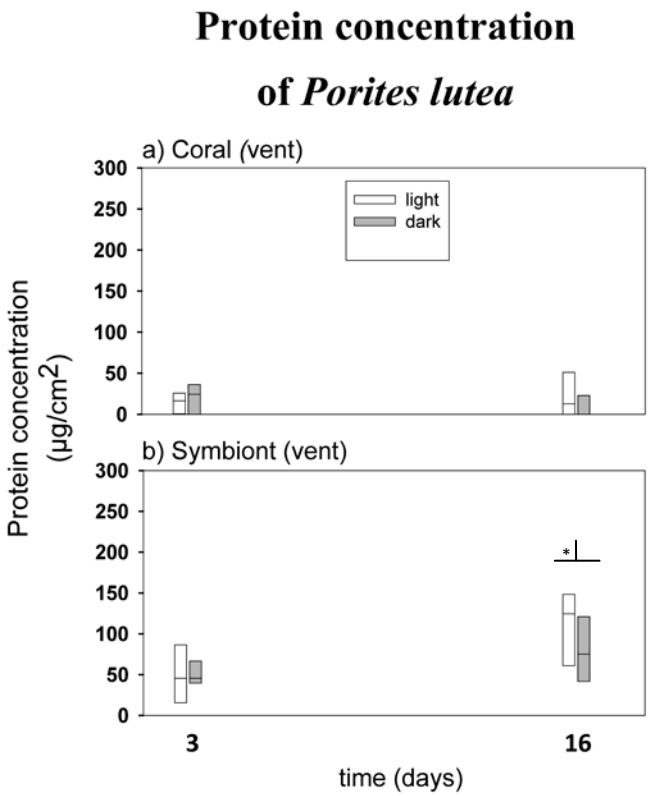

Figure 11 | Protein concentration of *Porites lutea* over a time period of 16 days, at vent (pH 7.8 and pCO_2 862 ppm) site: coral (a) and symbiont (b). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 10 for detailed results.

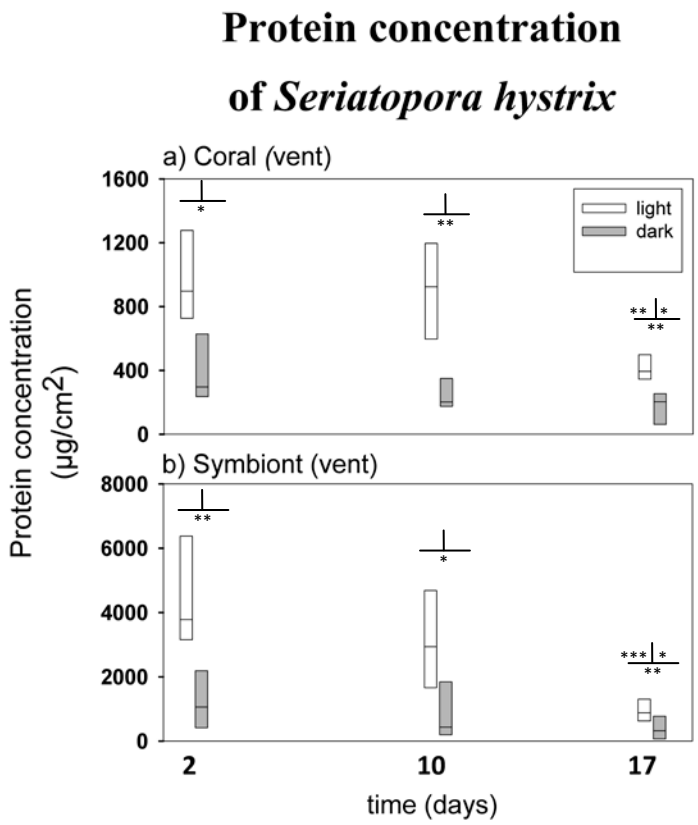

Figure 12 | Protein concentration of *Seriatopora hystrix* over a time period of 17 days at vent (pH 7.8 and pCO_2 862 ppm) site: coral (a) and symbiont (b). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 20 for detailed results.

4. Discussion

4.1 *Porites lutea*

4.1.1 Ocean acidification (OA) and light control conditions

Based on the results of the present study it can be assumed that *Porites lutea* did benefit from elevated pCO_2 under normal light conditions and was probably even able to stimulate its photosynthetic rates (cf. Dunne, 2010; Strahl et al., 2015). These findings are concurrent with former studies where cover of massive Porites spp. was at least doubled at the vent site (Fabricius et al., 2011; Strahl et al., 2015). Although pigment concentrations of *P. lutea* did not change throughout the experiment at the vent site per se, photosynthetic pigment concentrations were clearly increased compared to the control (Fig. 4-9a, b, Table 8). Elevated pCO_2 conditions under normal light were probably worthwhile for the algae to invest more energy into increasing pigment concentrations and thus, a more efficient photosynthetic rate (Dunne, 2010). It was found by Vogel et al. (2015) that corals suffer less from OA under normal light conditions than under reduced light intensity, explained by the fact that reduced photosynthetic active radiation (PAR) and high pCO_2 enhance each other (Dunne, 2010). Photosynthesis works against OA by enhancing pH inside the coral´s cells and on its surface (Vogel et al., 2015) and also increases Ω_a (de Beer et al., 2000) stimulating the deposition of $CaCO_3$ (Al-Horani et al., 2003).

The assumption of an increased photosynthetic rate is also supported by the fact that the zooxanthellate protein concentrations in vent nubbins were clearly increased at the end of the study compared to protein concentrations in control nubbins under same light conditions which stayed unchanged (Fig. 11, Table 10). It can be assumed that the zooxanthellae produced amino acids coming from fixed CO_2 (Hofmann & Kremer, 1981; Streamer et al., 1993). Neither coral protein (Fig. 11, Table 10) nor coral or symbiont biomass concentrations however changed during the study period in the light controls (Fig. 13, Table 9). This could be due to a lack or due to a not increased nutrient transfer from the algae to the coral for an additional buildup of proteins and biomass (Trench, 1993). It is also possible that the experimental duration was too short.

Algae from the genus *Symbiodinium* are haploid and the dinoflagellates living in symbiosis divide only mitotically. The cell cycle is both light and dark dependent. G1 to S phase and transition of S phase to G2/M phase happens during light and G2/M to G1 phase happens during

darkness (Smith and Muscatine, 1999; reviewed in Stambler, 2011, published in Dubinsky & Stambler, 2011) (G1 phase: algal cell growth; S phase: DNA doubling; G2 phase: cell preparation for mitosis (Alberts et al., 2004)). There is different information regarding doubling time of zooxanthellae in symbiosis. As reviewed in Stambler (2011), doubling time in zooxanthellae in the host is about 8 days without nutrient limitation. But under oligotroph conditions, doubling times can be extended to 70-100 days. Instead, Muscatine et al. (1984) observed division rates of symbiotic dinoflagellates in the coral *S. pistillata* under normal nutrient conditons under normal light and shade conditions. Algal cells doubled once within ~ 77 days during normal light and once within ~ 106 days during shade conditions and therefore 1-2 orders of magnitude lower than in free living dinoflagellates (Taylor, 1978). Thus, the division rate also depends on the type of zooxanthellae, light conditions, seasonal patterns and if they are living in symbiosis or in culture (reviewed in Stambler, 2011). But according to the different studies, it can be assumed that the doubling rate of symbiotic dinoflagellates is very slow in general. Thus, a change in symbiont biomass was unlikely to be detected within the short experimental duration of 16 days. It is also known, that the coral expels algae in case of a rapid increase or due to environmental changes (Stambler, 2011, reviewed in Dubinsky & Stambler, 2011), which is another factor which should be taken into account.

Zooxanthellae transfer up to 99% of their photosynthetic products to the coral (Muscatine and Cernichiari, 1969) but not only the amount but also the quality of the transferred organic material is important for coral tissue buildup. If transferred nutrients consist mainly of carbon and only little of nitrogen they will not preferentially used for coral tissue build up but rather be used as an energy resource (Muscatine et al., 1984). As nutrient content was not tested during this study it can only be assumed that an increase of coral protein and biomass content is very likely in a long-term perspective.

4.1.2 OA and light exclusion

In the dark treatment at the vent site, concentrations of all pigments (Fig. 4-7a, 9a; Table 1) (except for diatoxanthin (Fig. 8a, Table 1)) clearly decreased. Photosynthesis is light dependent and main functions of photosynthetic pigments are light absorption, light harvesting and photoprotection (Wright et al., 1997). Thus, if light is absent, photosynthesis is no longer performable as light energy is needed for activation of the reaction center (RC) and by this the

zooxanthellae do not need to invest any energy in keeping it up and do not gain any energy from it. This explains the decrease in pigment concentration.

Opposed to the general pigment decrease, concentrations of diatoxanthin (Fig. 8a, b, Table 1) were clearly increased during light exclusion at both vent and control site. Diatoxanthin and diadinoxanthin are part of the diadinoxanthin cycle, a photoprotective mechanism (Demers et al., 1991; Arsalane, et al. 1994; Wright et al., 1997; Kirk, 2011) which enables excess light energy to be dissipated as heat (Brown et al., 1999). Diatoxanthin epoxidase (DEP) transforms diatoxanthin into diadinoxanthin (Gross & Jakob, 2010). This enzyme is not only completely inhibited during high light conditions due to the light-driven proton gradient (Mewes and Richter 2002; Goss et al. 2006) but also during darkness due to a lack of NADPH (that can only be produced during light (Wright et al., 1997)), which is an essential cofactor to DEP. Thus, diatoxanthin concentrations increase during light exclusion as it cannot be converted backward into diadinoxanthin (Fig. 17; appendix p. 53) (Hager, 1975).

Despite of the clear changes in pigment concentrations however, protein contents of both, coral host and algal symbiont, did not change during the dark treatment (Fig. 11, Table 10). This could be explained by a lack of photosynthesis on the one hand, and by this, a lack of any further synthesis of photosynthates and on the other hand by a slowed down metabolism of the coral. This assumption can be supported by the fact that biomass concentrations did not change (Fig. 13 in appendix, Table 9) and *P. lutea* is a very slow growing coral (Veron, 2000). But as mentioned before, biomass buildup is a rather long-term process and changes are unlikely to be detected within a 16-day period. Stagnation in symbiont biomass concentrations could also be due to the fact, that parts of the algal cell cycle are light dependent (Smith & Muscatine, 1999; reviewed in Stambler, 2011, published in Dubinsky & Stambler, 2011). So, in constant darkness, it can be assumed that the cell cycle is ground to a halt and does not pass onto the G1 phase. However, Fitt (2000) still detected a low mitotic index of the observed zooxanthellae in the hydroid *Myrionema amboinense* which were kept in constant darkness. But anyhow, it can be assumed that cell division of symbiotic dinoflagellates is much slower during darkness than during normal light conditions. Light is not only necessary for the mitosis of the zooxanthellae. Photosynthesis provides carbon skeletons which are essential for assimilation of dissolved inorganic nitrogen (DIN) and for the completion of cytokinesis (Fitt, 2000).

Another explanation for the unchanged protein and biomass content of *P. lutea* could be nutrient gathering from heterotrophic feeding. During night, hermatypic corals are generally able

to catch food from surrounding waters (Dana, 1853; Birkeland, 1997; Osborne, 2000). Feeding on zooplankton enables the coral to gather nitrogen-rich nutrients which are mainly used for biomass build up (Fitt, 2000). This parameter was not tested during this study but as former investigations show (Edmunds & Davies, 1986; Grottoli et al., 2006), *Porites spp*. does mainly rely on autotrophic feeding. Grottoli et al. (2006) found evidence during an initiated 30 day bleaching event by the fact that *Porites spp.* meets its daily metabolic energy (DME) by using up existing energy reserves and mostly relies on organic carbon provided by zooxanthellar photosynthesis (only 21–35% of their DME demand was met heterotrophically).

Comparing dark treatments between vent and control site, no clear changes in pigment (Fig. 4a, b, Table 7) concentrations as well as biomass (Fig. 13 in appendix, Table 9) and protein (Fig. 11, Table 10) content of *P. lutea* and its symbiont could be detected. This leads to the assumption that there were no additive effects of OA and light exclusion in contrast to observations made by Vogel et al. (2015). Actually, OA should have a higher impact on corals under light exclusion as both symbiont and host respire and produce additional CO_2. At the same time, no CO_2 will be fixed as photosynthesis cannot work due to a lack of light. This in turn leads to enhanced reduction of pH and Ω_a and hence, $CaCO_3$ deposition is inhibited which eventually results in dissolution of the coral´s skeleton. Furthermore, without photosynthesis the coral receives a lack of energy and thus, it will no longer be able to grow (Vogel et al., 2015). But as *P. lutea* does not seem to be affected from both OA and light exclusion, it might be able to keep its energy demands on a minimum level and / or to still actively export excess H^+ coming from elevated pCO_2 despite of darkness. It might also have a high density of active ion exchanger Ca^{2+} -ATPase in their calicoblastic cells which would facilitate a control of its inner pH (also suggested in Strahl et al., 2015). Essential energy might come from a higher respiration rate and as examined in Strahl et al. (2015), dark respiration rate of *Porites spp*. was particularly increased during a similar length of light exclusion (14 days). But again, changes in biomass concentration are not informative regarding the experimental duration and thus, changes or non-changes should not be used for any conclusions regarding the effect of OA and light exclusion on the coral *P. lutea*.

4.2 *Seriatopora hystrix*

4.2.1 OA and light control conditions

Protein concentrations of both coral host and algal symbiont decreased in the light control at the vent site (Fig. 12, Table 20). It can be assumed that the zooxanthellae were likely not able to transfer additional photosynthates (amino acids) to the coral host possibly because of their own demand for it. It is also possible that *S. hystrix* needed to invest any available energy reserves in actively exporting excess H^+, caused from the high pCO_2 and low pH conditions, out of the subcalicoblastic space (Zoccola et al., 2004) and thus, suffered from OA. Similar observations were made during former studies (cf. Fabricius et al., 2011; Strahl et al., 2015) where the abundance of branching corals such as *S. hystrix* was clearly reduced at the vent site and the corals were obviously suffering from OA.

Pigment concentrations stayed unchanged in the light control treatments and comparing the vent and control site, no clear trend was detectable regarding higher or normal pCO_2 conditions. Concentrations of chlorophyll a (Fig. 4c, d), c_2 (Fig. 5c, d) and peridinin (Fig. 6c, d) increased initially at the control site, but were similar to the ones at the vent site until the end of the experimental period (Table 18). Obviously, the coral was not able to benefit from additional CO_2 concentrations and this in turn means that *S. hystrix* suffers from OA over time which was firstly recognizable in the decreased protein content at the end of the study. Strahl et al. (2015) also found that oxygen production rates were slightly lower at the vent site. The increased biomass contents of both coral and symbiont (Fig. 10; 14 in appendix, Table 19) are not very meaningful as already mentioned because biomass buildup in corals and its symbiont can be a very slow process (Taylor, 1978; reviewed in Stambler, 2011, published in Dubinsky & Stambler, 2011). So it is possible, that the higher biomass concentrations of the coral and its algae in dark treated nubbins compared to those of light control did still remain from former environmental conditions.

4.2.2 OA and light exclusion

Under high pCO_2 and light-exclusion, symbiont protein concentrations were clearly decreased within the 17-day-period (Fig. 12b, Table 20). Similarly pigment concentrations declined strongly until the end of the study (Fig. 4-7c, d; 9c, d; Table 11) except for diatoxanthin

(8c, d, Table 11), which showed an increase in the dark. The latter is equally to *P. lutea* and might also be due to the lack of NAD(P)H and thus, inhibited DEP. Due to the absence of light, there was no need for the algae to invest energy in keeping pigment concentrations up and as photosynthesis was prevented, no photosynthates were produced and used to restock algal and/or coral energy reserves (protein content). Therefore a decrease in biomass would have been expected but the opposite was detected until the mid of the 17 days of experimental period (Fig.10; 14 in appendix, Table 19). This could be again due to short length of the experiment as biomass buildup is time-consuming and it is unlikely, that changes can be detected after 10 or 17 days. It is also possible that the coral switched to heterotrophic feeding while zooxanthellae were put out of action.

As dark-treated protein (Fig. 12; Table 20) concentrations of both coral and algae were clearly decreased at the vent compared to the control site, it can be assumed, that light exclusion and OA did additively affect *S. hystrix*. Similar findings were published by Vogel et al. (2015) concerning *Acropora millepora*, similary shaped as *S. hystrix* (Veron, 2000).

5. Conclusions and Perspectives

Overall the present study showed that *P. lutea* did not seem to suffer from OA under normal light conditions but rather benefited from enhanced CO_2 concentrations. *P. lutea* is apparently very effective in exporting excess H^+ out of the calcifying fluid and by this not limited in using additional CO_2 for a productive photosynthesis. But as there is only a slight trend visible at the end of the present experiment, which lasted only for a limited number of days, conclusions should be made cautiously. A long-term study would be more useful to strengthen this conclusion.

S. hystrix on the other side did suffer from OA even under light conditions during the time of the experiment documented in the decrease of energy reserves in both, coral host and symbiont. Thus, the coral has probably more problems with actively exporting excess H^+ ions and is not able to compensate this energy expenditure by the use of the additional CO_2. Due to a lack of clear difference between vent and control treatments however, final conclusions should be drawn cautiously, especially because the *S. hystrix* might have suffered from the experimental setup itself indicated by a decrease in pigment concentrations in the light control treatments (Fig. 4-8d, Table 29).

Regarding OA and light exclusion, both coral taxa were expected to suffer most as there was a lack of photosynthetic support from the zooxanthellae. Surprisingly, *P. lutea* was completely unaffected under these conditions and is probably quite independent from its zooxanthellae over a limited period of time. In comparison, *S. hystrix* was more fragile than *P. lutea* as symbiotic protein concentrations started to decrease while they stayed unaffected in *P. lutea*. Thus, *S. hystrix* relies more on its symbiotic algae than *P. lutea*.

Regarding future climate change scenarios, *P. lutea* is expected to have rather no problems dealing with OA and is expected to be a dominant coral species in future coral reefs. This can already be seen at natural volcanic carbon dioxide seeps in Papua New Guinea (Fabricius et al, 2011). In contrast, *S. hystrix* seems to be more fragile and is awaited to be less abundant in coral reefs of the future. This can also be observed at carbon dioxide seeps in PNG as cover of branching corals was reduced three fold compared to control sites (Fabricius, 2011). Anyhow, the symbiosis is essential for the welfare of the corals. Thus, not only OA can be a serious threat to coral reefs but also a decreasing water quality and linked turbidity. The latter can either be caused from coastal runoff, which results from forest clearing, or from dredging (reviewed in

Rogers, 1990) which is practiced by the fishing industry. Thus, additive effects of OA and increasing turbidity could be a serious problem to sensitive corals such as *S. hystrix*.

6. References

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7. Appendix

7.1 Raw data of *Porites lutea* and *Seriatopora hystrix*

The raw data of both corals can be found on the enclosed CD.

7.2 Statistical analysis

Parametric test: t-test (Shapiro-Wilk)

Non-parametric test: Rank sum test (Mann-Whitney)

7.2.1 Pigment concentration of *Porites lutea*

Table 1: Pigment concentration of *Porites lutea*: light exclusion at vent site

Pigment		**Comparison day 3 + 16**
Chlorophyll *a*	p	<0.001
	Test type	parametric
Chlorophyll c_2	p	0.004
	Test type	non-parametric
Peridinin	p	0.038
	Test type	non-parametric
Diadinoxanthin	p	0.001
	Test type	parametric
Diatoxanthin	p	0.055
	Test type	parametric
ß-carotene	p	0.585
	Test type	parametric

Table 2: Pigment concentration of *Porites lutea*: light control at vent site

Pigment		**Comparison day 3+16**
Chlorophyll *a*	p	0.867
	Test type	non-parametric
Chlorophyll c_2	p	0.694
	Test type	non-parametric
Peridinin	p	0.955
	Test type	non-parametric
Diadinoxanthin	p	0.463
	Test type	non-parametric

Diatoxanthin	p	0.656
	Test type	parametric
ß-carotene	p	1.000
	Test type	non-parametric

Table 3: Pigment concentration of *Porites lutea*: light control versus light exclusion at vent site

Pigment		Day 3	Day 16
Chlorophyll *a*	p	0.477	0.001
	Test type	parametric	non-parametric
Chlorophyll c_2	p	0.554	0.001
	Test type	parametric	non-parametric
Peridinin	p	0.542	0.023
	Test type	parametric	parametric
Diadinoxanthin	p	0.145	0.001
	Test type	parametric	non-parametric
Diatoxanthin	p	0.383	0.040
	Test type	non-parametric	non-parametric
ß-carotene	p	0.578	0.295
	Test type	parametric	parametric

Table 4: Pigment concentration of *Porites lutea*: light exclusion at control site

Pigment		Comparison day 3 + 16
Chlorophyll *a*	p	<0.001
	Test type	parametric
Chlorophyll c_2	p	0.001
	Test type	non-parametric
Peridinin	p	<0.001
	Test type	parametric
Diadinoxanthin	p	<0.001
	Test type	parametric
Diatoxanthin	p	0.034
	Test type	non-parametric
ß-carotene	p	0.007
	Test type	parametric

Table 5: Pigment concentration of *Porites lutea*: light control at control site

Pigment		Comparison day 3 + 16
Chlorophyll *a*	p	0.934
	Test type	parametric
Chlorophyll c_2	p	0.641
	Test type	parametric
Peridinin	p	0.818
	Test type	parametric

Diadinoxanthin	p	0.841
	Test type	parametric
Diatoxanthin	p	0.959
	Test type	non-parametric
ß-carotene	p	0.919
	Test type	parametric

Table 6: Pigment concentration of *Porites lutea*: light control versus light exclusion at control site

Pigment		Day 3	Day 16
Chlorophyll *a*	p	0.012	<0.001
	Test type	parametric	parametric
Chlorophyll c_2	p	0.016	<0.001
	Test type	parametric	parametric
Peridinin	p	0.039	<0.001
	Test type	parametric	parametric
Diadinoxanthin	p	0.023	<0.001
	Test type	parametric	parametric
Diatoxanthin	p	0.348	0.007
	Test type	parametric	parametric
ß-carotene	p	0.034	0.248
	Test type	parametric	parametric

Table 7: Pigment concentration of *Porites lutea*: light exclusion: vent versus control site

Pigment		Day 3	Day 16
Chlorophyll *a*	p	0.652	0.074
	Test type	parametric	parametric
Chlorophyll c_2	p	0.541	0.290
	Test type	parametric	non-parametric
Peridinin	p	0.833	0.244
	Test type	parametric	non-parametric
Diadinoxanthin	p	0.508	0.057
	Test type	parametric	non-parametric
Diatoxanthin	p	0.303	0.169
	Test type	parametric	parametric
ß-carotene	p	0.639	0.290
	Test type	parametric	non-parametric

Table 8: Pigment concentration of *Porites lutea*: light control: vent versus control site

Pigment		Day 3	Day 16
Chlorophyll *a*	p	<0.001	0.028
	Test type	parametric	non-parametric
Chlorophyll c_2	p	<0.001	0.010
	Test type	parametric	non-parametric
Peridinin	p	0.002	0.028

	Test type	parametric	non-parametric
Diadinoxanthin	p	<0.001	0.021
	Test type	parametric	non-parametric
Diatoxanthin	p	0.189	0.124
	Test type	non-parametric	parametric
ß-carotene	p	0.001	0.050
	Test type	parametric	non-parametric

7.2.2 Biomass concentration of *Porites lutea*

Table 9: Biomass concentration of *Porites lutea*

site	treatment	day	organism	test-type	p-value
control	light control	3 + 16	coral	parametric	0.445
			symbiont	non-parametric	0.361
	light exclusion	3 + 16	coral	non-parametric	0.397
			symbiont	parametric	0.204
	light control versus exclusion	3	coral	parametric	0.030
			symbiont	non-parametric	0.916
		16	coral	parametric	0.468
			symbiont	parametric	0.022
vent	light control	3 + 16	coral	non-parametric	0.694
			symbiont	parametric	0.935
	light exclusion	3 + 16	coral	non-parametric	0.902
			symbiont	parametric	0.237
	light control versus exclusion	3	coral	non-parametric	0.017
			symbiont	non-parametric	0.261
		16	coral	parametric	0.902
			symbiont	parametric	0.599
vent versus control	light exclusion	3	coral	parametric	0.341
			symbiont	non-parametric	1.000
		16	coral	non-parametric	0.574
			symbiont	parametric	0.212
	light control	3	coral	non-parametric	0.535
			symbiont	parametric	0.801
		16	coral	parametric	0.457
			symbiont	parametric	0.015

7.2.3 Protein concentration of *Porites lutea*

Table 10: Protein concentration of *Porites lutea*

site	treatment	day	organism	test-type	p-value
control	light control	3 + 16	coral	non-parametric	0.388
			symbiont	parametric	0.124
	light exclusion	3 + 16	coral	non-parametric	0.480
			symbiont	non-parametric	0,916
	light control versus exclusion	3	coral	non-parametric	0,825
			symbiont	non-parametric	0,011
		16	coral	non-parametric	0,178
			symbiont	non-parametric	0,413
vent	light control	3 + 16	coral	parametric	0,573
			symbiont	parametric	0,035
	light exclusion	3 + 16	coral	parametric	0,131
			symbiont	parametric	0,296
	light control versus exclusion	3	coral	parametric	0,372
			symbiont	parametric	0,907
		16	coral	non-parametric	0,336
			symbiont	parametric	0,197
vent versus control	light control	3	coral	non-parametric	0,830
			symbiont	parametric	0,324
		16	coral	parametric	0,295
			symbiont	parametric	0,065
	light exclusion	3	coral	non-parametric	0,902
			symbiont	non-parametric	0,097
		16	coral	non-parametric	0,122
			symbiont	non-parametric	0,832

7.2.4 Pigment concentration of *Seriatopora hystrix*

Table 11: Pigment concentration of *Seriatopora hystrix*: light exclusion at vent site

Pigment		Comparison day 2 + 10	Comparison day 2 + 17	Comparison day 10 + 17
Chlorophyll *a*	p	0.721	0.003	0.006
	Test type	non-parametric	parametric	non-parametric
Chlorophyll c_2	p	0.500	0.751	0.006
	Test type	parametric	parametric	non-parametric
Peridinin	p	0.798	0.001	<0.001
	Test type	non-parametric	parametric	non-parametric
Diadinoxanthin	p	0.798	<0.001	0.004
	Test type	non-parametric	parametric	non-parametric
Diatoxanthin	p	0.383	0.336	0.963
	Test type	parametric	non-	parametric
ß-carotene	p	0.021	0.337	0.006
	Test type	non-parametric	parametric	non-parametric

Table 12: Pigment concentration of *Seriatopora hystrix*: light control at vent site

Pigment		Comparison day 2 + 10	Comparison day 2 + 17	Comparison day 10 + 17
Chlorophyll *a*	p	0.878	0.574	0.382
	Test type	non-parametric	non-parametric	non-parametric
Chlorophyll c_2	p	0.382	0.105	0.505
	Test type	non-parametric	non-parametric	non-parametric
Peridinin	p	0.959	0.279	0.328
	Test type	non-parametric	non-parametric	non-parametric
Diadinoxanthin	p	0.234	0.798	0.105
	Test type	non-parametric	non-parametric	non-parametric
Diatoxanthin	p	0.785	0.173	0.174
	Test type	parametric	parametric	parametric
ß-carotene	p	0.130	0.282	0.574
	Test type	non-parametric	parametric	non-parametric

Table 13: Pigment concentration of *Seriatopora hystrix*: light control versus light exclusion at vent site

Pigment		Day 2	Day 10	Day 17
Chlorophyll *a*	p	0.456	0.279	0.004
	Test type	parametric	non-parametric	non-parametric
Chlorophyll c_2	p	0.477	0.645	0.014
	Test type	parametric	non-parametric	non-parametric
Peridinin	p	0.319	0.234	0.006
	Test type	parametric	non-parametric	non-parametric

Diadinoxanthin	p	0.128	0.028	0.004
	Test type	parametric	non-parametric	non-parametric
Diatoxanthin	p	0.681	0.444	0.009
	Test type	parametric	parametric	non-parametric
ß-carotene	p	0.227	0.798	0.053
	Test type	parametric	non-parametric	parametric

Table 14: Pigment concentration of *Seriatopora hystrix*: light exclusion at control site

Pigment		**Day 2**	**Day 10**	**Day 17**
Chlorophyll *a*	p	0.196)	0.520	<0.001
	Test type	parametric	parametric	parametric
Chlorophyll c_2	p	0.121	0.216	<0.001
	Test type	parametric	parametric	parametric
Peridinin	p	0.166	0.953	<0.001
	Test type	parametric	non-parametric	parametric
Diadinoxanthin	p	0.220	0.439	<0.001
	Test type	parametric	parametric	parametric
Diatoxanthin	p	0.502	0.444	0.029
	Test type	parametric	non-parametric	non-parametric
ß-carotene	p	0.749	0.345	0.025
	Test type	parametric	parametric	parametric

Table 15: Chlorophyll *a* concentration in *Seriatopora hystrix*: light control at control site

Pigment		**Comparison day 2 + 10**	**Comparison day 2 + 17**	**Comparison day 10 + 17**
Chlorophyll *a*	p	0.028	0.004	0.462
	Test type	parametric	parametric	parametric
Chlorophyll c_2	p	0.002	0.002	0.674
	Test type	parametric	parametric	parametric
Peridinin	p	0.035	0.003	0.243
	Test type	parametric	parametric	parametric
Diadinoxanthin	p	0.732	0.067	0.109
	Test type	parametric	parametric	parametric
Diatoxanthin	p	0.018	0.065	0.002
	Test type	parametric	non-parametric	parametric
ß-carotene	p	0.282	0.613	0.216
	Test type	non-parametric	parametric	parametric

Appendix

Table 16: Pigment concentration of *Seriatopora hystrix*: light control versus light exclusion at control site

Pigment		Comparison day 2 + 10	Comparison day 2 + 17	Comparison day 10 + 17
Chlorophyll *a*	p	0.818	<0.001	0.004
	Test type	parametric	non-parametric	non-parametric
Chlorophyll c_2	p	0.598	<0.001	0.004
	Test type	parametric	parametric	non-parametric
Peridinin	p	0.609	<0.001	0.034
	Test type	parametric	non-parametric	non-parametric
Diadinoxanthin	p	0.841	<0.001	0.004
	Test type	parametric	non-parametric	non-parametric
Diatoxanthin	p	0.015	0.209	0.112
	Test type	non-parametric	non-parametric	non-parametric
ß-carotene	p	0.087	0.046	0.006
	Test type	parametric	parametric	non-parametric

Table 17: Pigment concentration of *Seriatopora hystrix*: light exclusion: vent versus control site

Pigment		Day 2	Day 10	Day 17
Chlorophyll *a*	p	0.189	0.291	0.009
	Test type	non-parametric	parametric	parametric
Chlorophyll c_2	p	0.091	0.203	0.023
	Test type	parametric	parametric	parametric
Peridinin	p	0.072	0.361	0.025
	Test type	non-parametric	non-parametric	parametric
Diadinoxanthin	p	0.336	0.218	0.003
	Test type	non-parametric	parametric	parametric
Diatoxanthin	p	0.225	0.160	0.535
	Test type	parametric	parametric	non-parametric
ß-carotene	p	0.172	0.163	0.239
	Test type	parametric	non-parametric	parametric

Table 18: Pigment concentration of *Seriatopora hystrix*: light control: vent versus control site

Pigment		Day 2	Day 10	Day 17
Chlorophyll *a*	p	0.022	0.950	0.878
	Test type	parametric	non-parametric	non-parametric
Chlorophyll c_2	p	0.017	0.852	0.442
	Test type	parametric	non-parametric	non-parametric
Peridinin	p	0.011	0.345	0.721
	Test type	parametric	non-parametric	non-parametric
Diadinoxanthin	p	0.075	0.228	0.505
	Test type	parametric	non-parametric	non-parametric
Diatoxanthin	p	0.744	0.215	0.798
	Test type	parametric	parametric	non-parametric
ß-carotene	p	0.368	0.662	0.439
	Test type	parametric	non-parametric	parametric

7.2.5 Biomass concentration of *Seriatopora hystrix*

Table 19: Biomass concentration of *Seriatopora hystrix*

site	treatment	day	organism	test-type	p-value
control	light control	2 + 10	coral	parametric	0.096
			symbiont	parametric	0.457
		2 + 17	coral	non-parametric	0.234
			symbiont	non-parametric	0.505
		10 + 17	coral	non-parametric	0.491
			symbiont	parametric	0.904
	light exclusion	2 + 10	coral	parametric	0.215
			symbiont	non-parametric	0.290
		2 + 17	coral	non-parametric	0.620
			symbiont	non-parametric	0.805
		10 + 17	coral	parametric	0.302
			symbiont	parametric	0.359
	light control versus exclusion	2	coral	non-parametric	0.397
			symbiont	non-parametric	0.955
		10	coral	parametric	0.032
			symbiont	parametric	0.384
		17	coral	non-parametric	0.867
			symbiont	non-parametric	0.536
vent	light control	2 + 10	coral	parametric	0.083
			symbiont	non-parametric	0.005
		2 + 17	coral	parametric	<0.001
			symbiont	non-parametric	<0.001
		10 + 17	coral	parametric	0.009
			symbiont	parametric	0.006
	light exclusion	2 + 10	coral	non-parametric	0.721
			symbiont	parametric	0.351
		2 + 17	coral	non-parametric	0.878
			symbiont	non-parametric	0.279
		10 + 17	coral	parametric	0.378
			symbiont	non-parametric	0.382
	light control versus exclusion	2	coral	non-parametric	0.007
			symbiont	parametric	<0.001
		10	coral	non-parametric	0.038
			symbiont	parametric	0.047
		17	coral	non-parametric	0.505
			symbiont	non-parametric	0.442
vent versus control	light control	2	coral	parametric	0.196
			symbiont	parametric	<0.001
		10	coral	parametric	0.051
			symbiont	non-parametric	0.158
		17	coral	non-parametric	0.007

			symbiont	non-parametric	0.195
	light exclusion	2	coral	non-parametric	0.029
			symbiont	non-parametric	0.779
		10	coral	parametric	0.236
			symbiont	parametric	0.394
		17	coral	non-parametric	0.072
			symbiont	non-parametric	0.189

7.2.6 Protein concentration of *Seriatopora hystrix*

Table 20: Protein concentration of *Seriatopora hystrix*

site	treatment	day	organism	test-type	p-value
control	light control	2 + 10	coral	non-parametric	0.573
			symbiont	parametric	0.159
		2 + 17	coral	non-parametric	0.442
			symbiont	parametric	0.043
		10 + 17	coral	non-parametric	0.852
			symbiont	parametric	0.722
	light exclusion	2 + 10	coral	parametric	0.137
			symbiont	non-parametric	0.266
		2 + 17	coral	parametric	0.498
			symbiont	non-parametric	0.165
		10 + 17	coral	non-parametric	1.000
			symbiont	parametric	0.695
	light control versus exclusion	2	coral	parametric	0.266
			symbiont	non-parametric	0.463
		10	coral	non-parametric	0.263
			symbiont	non-parametric	0.953
		17	coral	non-parametric	0.463
			symbiont	parametric	0.767
vent	light control	2 + 10	coral	non-parametric	1.000
			symbiont	parametric	0.248
		2 + 17	coral	non-parametric	0.005
			symbiont	non-parametric	<0.001
		10 + 17	coral	non-parametric	0.003
			symbiont	non-parametric	0.001
	light exclusion	2 + 10	coral	non-parametric	0.195
			symbiont	non-parametric	0.574
		2 + 17	coral	non-parametric	0.038
			symbiont	non-parametric	0.038
		10 + 17	coral	non-parametric	0.574
			symbiont	parametric	0.150
	light control	2	coral	non-parametric	0.015

	versus exclusion				
			symbiont	non-parametric	0.005
		10	coral	non-parametric	0.001
			symbiont	parametric	0.013
		17	coral	parametric	0.002
			symbiont	parametric	0.008
vent versus control	light control	2	coral	parametric	0.068
			symbiont	parametric	0.025
		10	coral	non-parametric	0.228
			symbiont	parametric	0.058
		17	coral	parametric	0023
			symbiont	parametric	0.188
	light exclusion	2	coral	parametric	0.145
			symbiont	non-parametric	0.232
		10	coral	non-parametric	0.061
			symbiont	non-parametric	0.413
		17	coral	non-parametric	0.014
			symbiont	parametric	0.006

7.3 Additional figures

7.3.1 Biomass concentration of *Porites lutea*

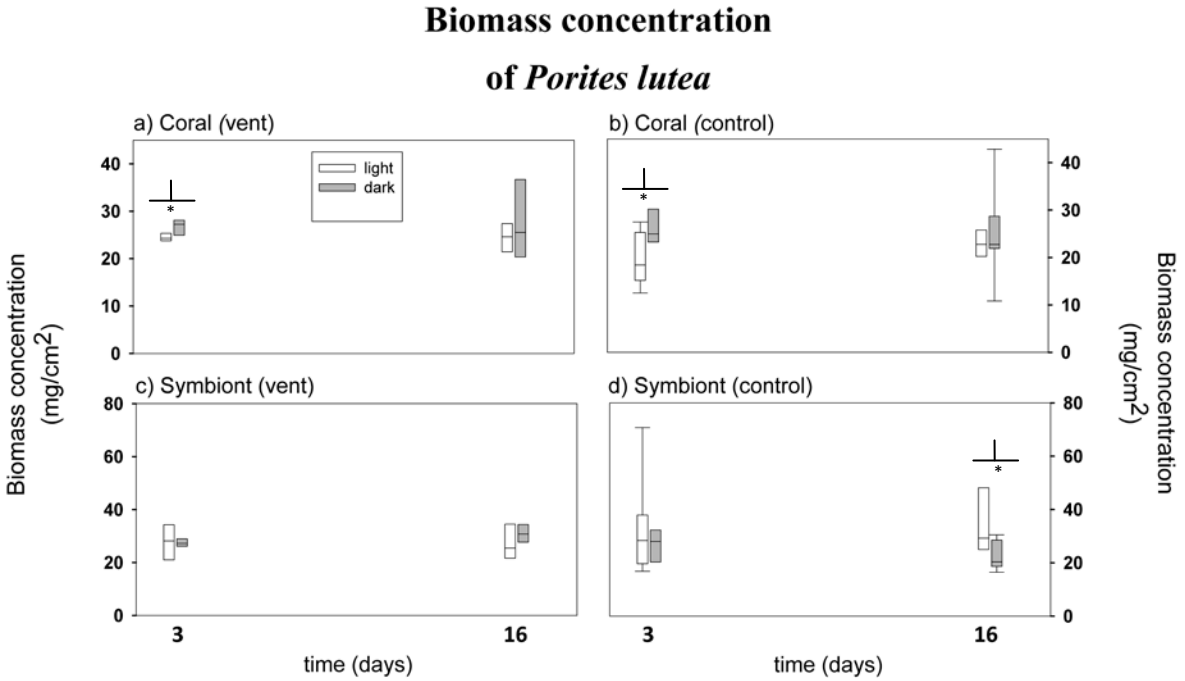

Figure 13 | **Biomass concentration of *Porites lutea*** over a time period of 16 days at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: coral (a, b) and symbiont (c, d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 3: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 9 for detailed results.

7.3.2 Biomass concentration of *Seriatopora hystrix*

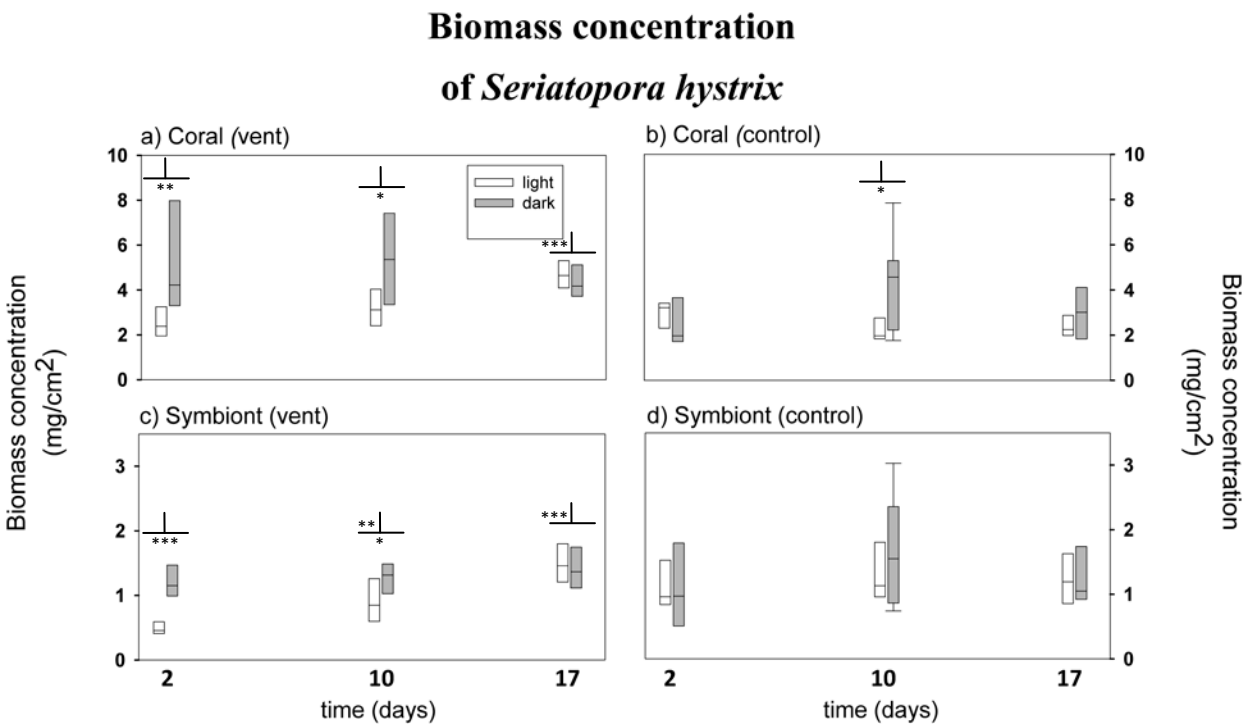

Figure 14 | Biomass concentration of *Seriatopora hystrix* over a time period of 17 days at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: coral (a, b) and symbiont (c, d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 19 for detailed results.

7.3.3 Protein concentration of *Porites lutea*

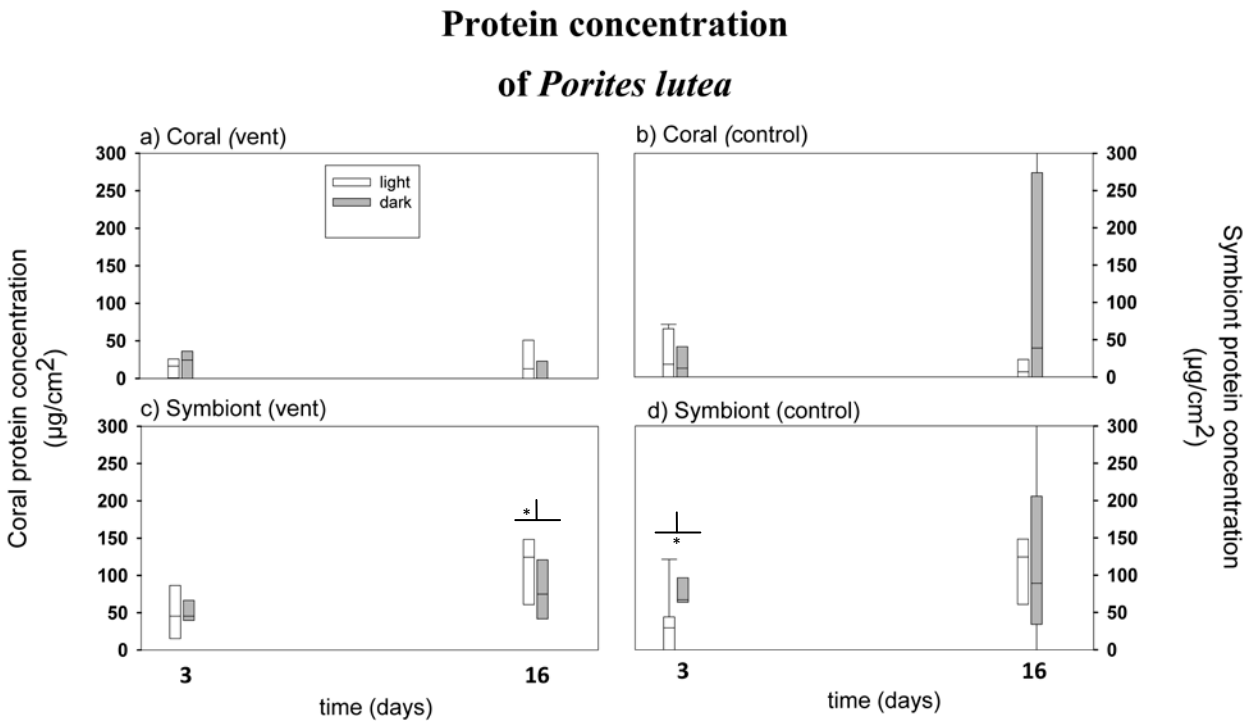

Figure 15 | **Protein concentration of *Porites lutea*** over a time period of 16 days at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: coral (a, b) and symbiont (c, d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 3: left side: light treatment. right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 10 for detailed results.

7.3.4 Protein concentration of *Seriatopora hystrix*

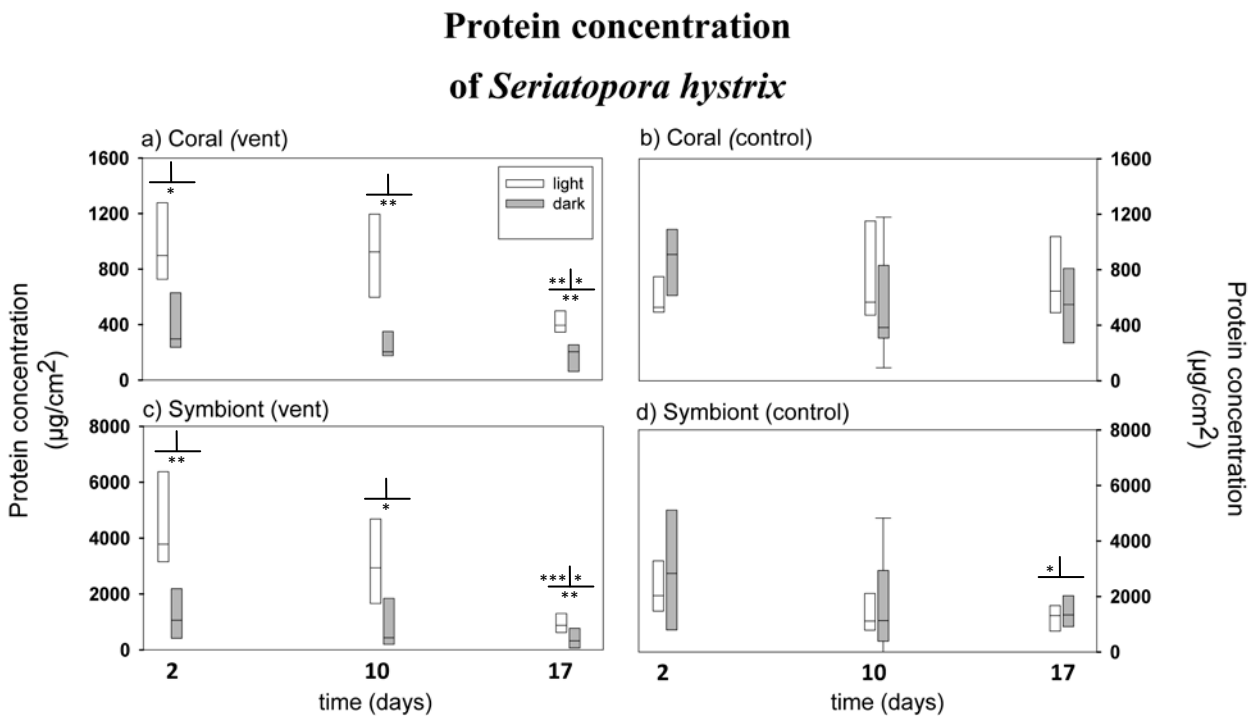

Figure 16 Protein concentration of *Seriatopora hystrix* over a time period of 17 days at vent (pH 7.8 and pCO_2 862 ppm) and control (pH 8.1 and pCO_2 323 ppm) site: coral (a, b) and symbiont (c, d). Central tendency box plots (median as solid line with 25th and 75th percentile and non-outlier range). Asterisks indicate results of parametric t-test / non-parametric rank sum test between treatments (light (white) and dark (grey)) and sampling date: Below lines light versus dark treatment at each point in time, above lines comparison to condition at day 2: left side: light treatment, right side: dark treatment. Significance levels: *p<0.05, **p<0.01, ***p<0.001. See Table 20 for detailed results.

7.3.5 Scheme of NADPH dependent diatoxanthin epoxidase (DEP)

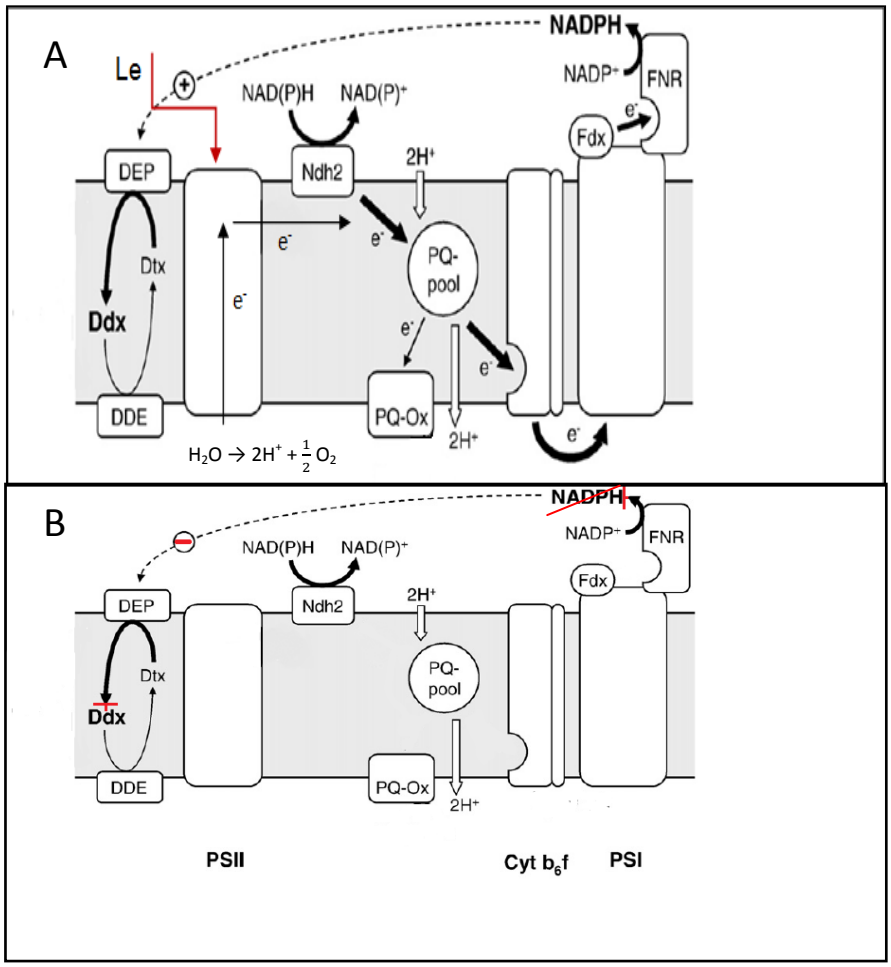

Figure 17 NADPH dependent diatoxanthin epoxidase (DEP). Activation during light (A) and inactivation during darkness (B). For photoprotection, monoepoxide diadinoxanthin (Ddx) is converted into non-epoxide diatoxanthin (Dtx) via diadinoxanthin-ep-oxidase (DDE). Thus, Dtx dissipates excess light as heat. DEP catalyzes the reversible reaction. During darkness, DEP is inhibitied due to a lack of NADPH which can only be produced via light driven electron (e^-) transport. Other abbreviations: Ndh: NADPH-dehydrogenase; Le: light energy; NADPH dehydrogenase; PQ: plastoquinone; PQ-Ox: plastoquinone oxidase; Fdx: ferredoxin; FNR: ferredoxin-$NADP^+$ oxidoreductase; PS I: photosystem I; PS II: photosystem II; Cyt b_6 f: cytochrome b_6/f complex (Figure modified after Grouneva et al., 2009).

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9. Declaration of originality

I hereby declare that the work submitted is my own and that information which has been directly or indirectly taken from other sources has been noted as such.

Bremen, 10.08.2015

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Signature