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Photobiology of the zoanthid Zoanthus sociatusin intertidal and subtidal habitats

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Abstract. Intertidal environments are boundaries between marine and terrestrial ecosystems that are subject to rapid fluctuations across tidal cycles. This study investigates, for the first time, the photobiology of symbiotic zoanthids inhabiting different tidal environments: subtidal, intertidal pools and intertidal areas exposed to air during low tide. More specifically, we assessed the photochemical efficiency, *Symbiodinium* density and photosynthetic pigments profile of *Zoanthus sociatus* during low tide. Photochemical efficiency was lower and cell density higher in air exposed zoanthids. The profile of photosynthetic pigments also varied significantly among tidal habitats, particularly photoprotective pigments such as dinoxanthin and diadinoxanthin. Differences were also observed for the pigment content per cell, but the proportion of particular pigments (peridinin/chlorophyll-*a* and diatoxanthinþdiadinoxanthin/chlorophyll-*a*) remained stable. Results suggest that aerial exposure conditions induce reversible downregulation of photochemical processes but no photophysiological impairment or bleaching. These findings provide a baseline for future studies addressing the prevalence of these overlooked cnidarians in environmentally dynamic reef flats.

Additional keywords: aerial exposure, chlorophyll fluorescence, photosynthetic pigments, *Symbiodinium*,

tidal pool. Received 14 January 2015, accepted 15 September 2015, published online 10 December 2015

Introduction

Intertidal environments are among the most physiologically challenging marine habitats as organisms are exposed to dra matic fluctuations in environmental conditions across tidal cycles (Kaiser *et al.* 2011). The main factors displaying the highest variability in these stressful environments are temper ature, dissolved oxygen, salinity, solar radiation, desiccation and wave exposure, as well as air exposure duration (Teixeira *et al.* 2013). The exposure of organisms directly to high irradiance, UV-radiation and strong winds is particularly rele vant for intertidal animals that developed photosynthetic sym bioses such as benthic cnidarians living in association with photosynthetic dinoflagellates (Shick and Dykens 1984; Romaine *et al.* 1997; Leggat *et al.* 2006). Although photosyn thesis provides nutritional benefits to the animal host, it also poses major risks. Photosynthetic symbionts are very sensitive to environmental variations that can disturb the complex inter action between the animal host and the endosymbiotic dinoflagellates and may lead to bleaching and, ultimately, death (*a*) of the host (Venn *et al.* 2008). This is critical for symbiotic cnidarians such as scleractinian corals, anemones and zoan tharians Hexacorallia) are inhabiting intertidal reef flats that are periodically exposed during low tide (Brown *et al.* 1994; Fadlallah *et al.* 1995; Eakin and Glynn 1996). Zoantharians (order Zoantharia, Anthozoa:

Sample location

Brazil
38 10 60.00 W

Tidal pool
Air exposed
Subtidal

12 46 50.00 S

(*b*)

Atlantic Ocean 200 m

38 10 60.00 W

colonial cnidarians widely distributed in tropical and subtropical environments. Similar to corals and anemones, some zoanthar ians also harbour symbiotic dinoflagellates of the genus *Sym biodinium* (Reimer *et al.* 2006; Rabelo *et al.* 2014). Moreover, zoantharians have been responsible for phase shifts in coral reefs (Yang *et al.* 2013; Cruz *et al.* 2015), which are one of the most conspicuous effects of the 'coral reef crisis' (Done 1999; Hughes *et al.* 2010). However, research on zoantharian ecology has been scarce as compared with other cnidarian groups (Hill 2013). The prevalence of zoantharian species hosting photosyn thetic symbionts in reef flats and in wide range of tidal heights (Sebens 1982; Rabelo *et al.* 2015) suggests the presence of photobiological processes well prepared to deal with environ mental variability associated with tidal cycles. However, photo biological adaptations of symbiotic zoantharians differently exposed to tides remain poorly explored. Few studies addressing the photobiological processes of zoantharians are available (Karlson 1988; Kemp *et al.* 2006; Hibino *et al.* 2013; Leal *et al.* 2015*a*), and, to our best knowledge, the photosynthetic pigments of zoantharians have never been investigated.

This study aims to characterise the still poorly explored photobiology of symbiotic zoanthids and provide a baseline for future studies addressing photobiological processes of sym biotic zoantharians. Specimens were collected in three different tidal environments: subtidal (ST), intertidal pools (TP) and emerged intertidal areas during low tide (IT). Particularly, we assessed the photochemical efficiency, photosynthetic pigments profile and *Symbiodinium* cell density of the zoanthid *Zoanthus sociatus* on each of the environments referred above. Note that IT zoanthids are exposed to air during low tide whereas TP organisms are subject to larger environmental variability as compared

with ST individuals due to a reduced (or absent) water exchange during low tide (Morris and Taylor 1983).

Materials and methods

Portions of colonies of *Z. sociatus*(,25 cm^2 each) were sampled during low tide with a rock pick and hammer and the polyps in the marginal areas were excluded from the analysis to minimise potential bias associated from damaging. Sampling was per formed near Arembepe Beach, Bahia, Brazil ($12846^{0}26^{00}$S, $38810^{0}30^{00}$W; Fig. 1) in December 2013 (maximal tidal range is 2.3 m). Specimens were collected from three habitats differently exposed to tidal cycles: ST, TP and IT. These habitats follow a tidal gradient, as ST and IT are respectively at the lowest and highest tidal levels with TP in between. ST specimens were always underwater (,1-m mean depth at low tide) and were collected from a lagoon between tidal flats and a beachrock wall that is exposed during low tide (Fig. 1). TP and IT specimens were collected from the beachrock. TP zoanthids were also submerged throughout the tidal cycle but became isolated dur ing low tide due to non-existent water exchange with the

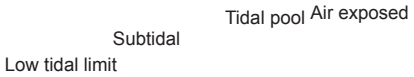


Fig. 1. Map of Arembepe Beach, Bahia, Brazil, showing the collection site and the lagoon and intertidal beachrock barrier where *Zoanthus sociatus* were collected. Dark grey shades indicate intertidal rocky structures where *Z. sociatus* occur.

surrounding areas. TP area ranged from 1 to 3 m^2 with maximum pool depth varying from 0.1 to 0.5 m and zoanthids always being collected at 0.3-m mean depth to minimise the effect of light variations between ST and TP organisms. IT specimens were located in high intertidal areas that are exposed for ,4 h during low tide and submerged during high tide. IT specimens were directly exposed to sun and not in cracks or shaded areas. Five replicate samples were taken from ST and IT areas separated by at least 100 m and different TP. Samples from ST and TP were individually placed in labelled plastic boxes with ,500 mL of seawater and transported (during ,30 min) to the laboratory in an isothermal box. Samples collected in IT habitats were transported in labelled plastic boxes without water.

Laboratory procedures

In vivo chlorophyll fluorescence

In vivo chlorophyll (Chl) fluorescence was measured non intrusively through Pulse Amplitude Modulation (PAM) fluo rometry, using a Diving PAM fluorometer (Walz; Effeltrich, Germany). The fluorometer's fibre optic was positioned per pendicularly and ,2 mm above the polyp surface. Measure ments were carried out in three separate polyps in each zoanthid colony. Measurements were taken with the zoanthids inside the transport plastic boxes to minimise manipulation and guarantee that polyps were open. IT zoanthids had their polyps closed as they were measured without water. This method was first validated in a preliminary study where no significant differences were observed (*t* test, t ¼ 1.1549, P ¼ 0.3) between Chl fluores cence of IT zoanthids measured with and without water, i.e. with open and closed polyps respectively. Zoanthids were dark adapted for 30 min (during transport) before any measurement. One saturation pulse (0.8 s) was applied to determine the minimum- or dark-level fluorescence (F_o), and maximum fluorescence (F_m), following the procedures described by

Rocha *et al.* (2013*a*). F_o and F_m were used to determine the maximum quantum yield of PSII (Schreiber *et al.* 1986):

$$F_v = F_m ¼ F_m \quad F_o F$$

Tukey's HSD test was used when ANOVA revealed significant

ð1Þ

compared among zoanthids from different habitats using a one way ANOVA. Assumptions of normality and homoscedasticity were confirmed with Shapiro test and Bartlett test respectively.

differences (P , 0.05). All results are presented as mean and one standard deviation. Analyses were performed using R

Symbiodinium cell density and photosynthetic pigments A composed sample with five polyps was collected from each *Z. sociatus* colony fragment for quantification of *Symbiodinium* cell density. Samples were stored in 50-mL conical centrifuge tubes with filtered seawater, stained with Lugol's iodine (Sigma–Aldrich, Sintra, Portugal) and stored at 48C until cell counting. Cells were counted in an improved Neubauer haemocytometer (Boerco, Hamburg, Germany), and the number of *Symbiodinium* in the aliquot was calculated from the average of five replicate counts for each colony. Another composed sample with five polyps from each *Z. sociatus* colony was prepared for quantification of photosynthetic pigments. Samples were stored in 15 mL conical centrifuge tubes with filtered seawater and homogenised. Samples were centrifuged (15 000*g*, 10 min, 48C), the pellet immediately frozen in liquid nitrogen and freeze-dried for later analysis of photosynthetic and accessory pigments.

Freeze-dried samples of 0.046–0.083 g were placed in a screw-cap centrifuge tube with 3 mL of 95% cold-buffered methanol (2% ammonium acetate) containing 0.05 mg L^{1} trans-b-apo-8^{0}-carotenal (Fluka, Buchs, Switzerland) as internal standard. The samples were sonicated for 5 min in an ice-water bath, placed at 208C for 1 h and centrifuged at 1100*g* for 5 min at 38C. Supernatants were filtered through Fluoropore PTFE membrane filters (0.2-mm pore size; Merck Millipore Ltd, Bill erica, MA, USA) to remove cell debris. Immediately before injection, 1 mL of sample was mixed with 400 mL of Milli-Q water in 2-mL amber glass sample vials, and vials were placed in the HPLC cooling rack (48C). Method procedures for HPLC analyses (using a monomeric C8 column with a pyridine containing mobile phase) are fully described in Zapata *et al.* (2000). The detection limit and quantification procedure of this method were done according to Mendes *et al.* (2007). Pigments were identified from both absorbance spectra and retention times from the signals in the photodiode array detector (SPD M20A) or fluorescence detector

(RF-10AXL; excitation 430 nm, emission 670 nm) (Shimadzu Corporation, Kyoto, Japan). Peaks were integrated using LC-Solution software, but all peak integrations were checked manually and corrected where necessary. The HPLC system was previously calibrated with pigment standards from DHI (Institute for Water and Environment, Denmark). For correction losses and volume changes, the concentrations of the pigments were normalised to the internal standard.

Symbiont cell density and pigment data were normalised to zoanthid dry weight. Data are shown as *Symbiodinium* cell density per dry weight (cell g ^{1}), pigment content per dry weight (mg g ^{1}) and pigment content per cell (pg cell ^{1}).

Statistical analysis

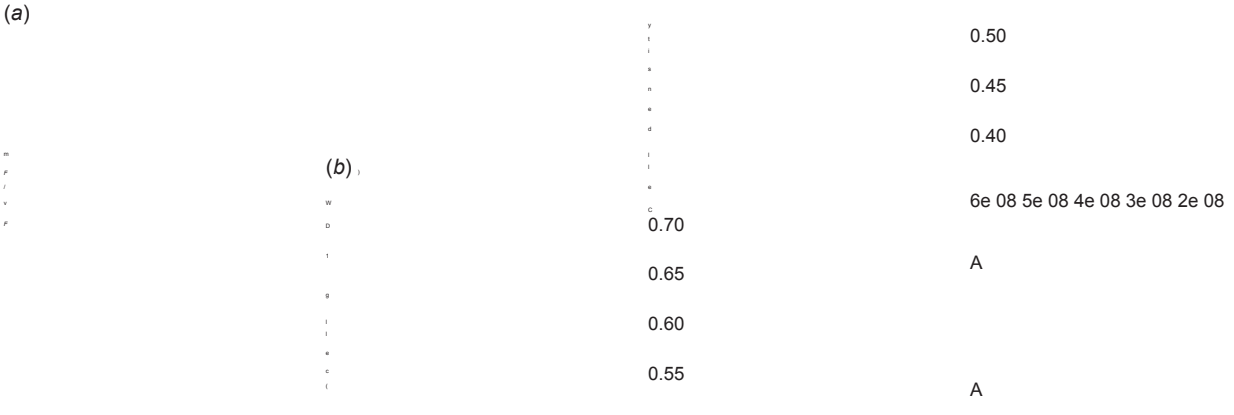
Maximum quantum yield of PSII (F_v/F_m), *Symbiodinium* cell density and concentration of photosynthetic pigments were

(R Development Core Team 2015) and detailed statistical data is provided in Table S1 in the Supplementary material. Multivar iate statistical analyses were performed to assess differences among habitats as regards the profile of photosynthetic pig ments. Prior to statistical analysis, pigment data was log(x þ 1) transformed to emphasise compositional differences among samples rather than quantitative differences (Clarke and Gorley 2006). Similarity percentages (SIMPER) were determined to analyse the percentage contribution of each pigment to dis similarities among habitats. A similarity–difference matrix was constructed using the Euclidean distance and used for permu tational multivariate analysis of variance (PERMANOVA) to assess significant differences in the photosynthetic pigment profile of *Z. sociatus* among habitats. All multivariate statistical tests (SIMPER and PERMANOVA) were performed using PRIMER v6 with the add-on PERMANOVAþ.

Results and discussion

Maximum quantum yield of PSII (F_v/F_m) is among the most used photophysiological parameters to estimate photochemical efficiency and as a proxy of photophysiological health (Maxwell and Johnson 2000). Results suggest a decrease in F_v/F_m with increasing level of tidal habitat, i.e. F_v/F_m was higher in ST specimens and lower in IT zoanthids (Fig. 2*a*), but still within the levels observed for photophysiologically healthy symbiotic zoantharians and corals Warner *et al.* 1999; Fitt *et al.* 2001; Wijgerde *et al.* 2014; Leal *et al.* 2015*a*). Reduced photochem ical efficiency in IT zoanthids is likely associated with stress from exposure conditions. However, this does not necessarily indicate that photophysiological mechanisms are damaged in these specimens. Diurnal patterns of reversible downregulation have already been identified in symbiotic corals associated with irradiance variability (Brown *et al.* 1999). Moreover, exposure of the scleractinian coral *Stylophora pistillata* to air also resulted in temporary photoinhibition but no apparent photo physiological damage (Romaine *et al.* 1997). The results here observed thus suggest that IT zoanthids show temporary downregulation of photochemical processes during low tide but no photodamage or bleaching, as evidenced by the similar, or even higher, symbiont cell density they displayed when com pared with specimens from other habitats (Tukey HSD, *P* , 0.05; Fig. 2*b*). This may be a consequence of tissue retraction, as *Z. sociatus* in IT habitats close their polyps during low tide as a photoprotection mechanism (Brown *et al.* 2002).

The photosynthetic pigments profile of *Z. sociatus* varied among habitats (PERMANOVA, *P* , 0.05). However, the con tent of individual pigments was similar across zoanthids, apart from chlorophyll-*a* (Chl-*a*), chlorophyll-c_2 (Chl-c_2), b-carotene (b-Car) and pheophytin-*a* (Phe-*a*) (Table 1), and also within the range observed for other symbiotic cnidarians (Rocha *et al.* 2013*a*, 2013*b*). Chl-*a* content was higher in *Z. sociatus* from TP and lower in ST zoanthids. In contrast, the contents of b-Car and Phe-*a* were higher in ST specimens. Quantitative differences in photosynthetic pigments are usually associated with irradiance or the balance between the rates of light collection and light use (Venn *et al.* 2006; Kuguru *et al.* 2010; Leal *et al.* 2015*a*). However, it is unlikely that large irradiance differences are observed among the surveyed habitats and that irradiance is a limiting factor in any of the habitats here analysed. Higher Chl-*a* content is usually associated with increased depths or lower irradiance (Titlyanov and Titlyanova 2002), and ST zoanthids that were collected at the highest depth (,1 m deep at low tide) displayed the lowest concentration of most photosynthetic pigments (Table 1). Although changes in Chl-*a* were likely associated with factors other than light, such as changes in temperature (Bingham *et al.* 2011), results suggest that irradi ance may be affecting the photosynthetic pigments profile, particularly photoprotective pigments that explain a large por tion of variability recorded among habitats (SIMPER, Table 2). Dinoxanthin (Dinox) and diadinoxanthin (DD) accounted alto gether for ,32.6% of data variability between ST and TP, 31.3% between ST and IT, and 17.3% between TP and IT



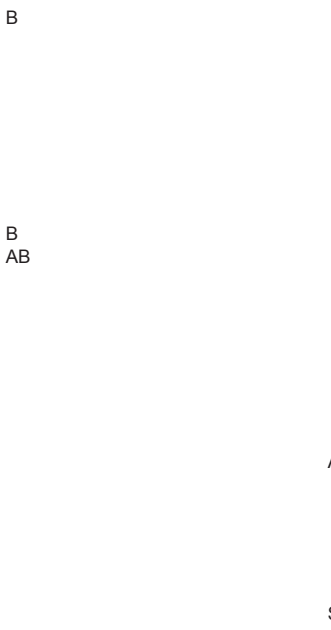


Fig. 2. Average (standard deviation) maximum quantum yield of PSII (*a*) and *Symbiodinium* cell density (*b*) measured in *Zoanthus sociatus* from subtidal (ST), tidal pools (TP) and intertidal habitats (IT). Significant differences among habitats (P , 0.05) are noted with different letters.

Table 1. Photosynthetic pigment composition of tissue and Symbiodinium cells of Zoanthus sociatusfrom different habitats: subtidal (ST), tidal pools (TP) and intertidal (IT)

Different superscript letters denote significant differences (P , 0.05) among habitats for each pigment. b-Car, b-carotene; Chl-*a*, chlorophyll-*a*; Chl-c_2, chlorophyll-c_2; Chl-c_1, chlorophyll-c_1; Diadino, DD; Diato, DT; Dinox, dinoxanthin; Perid., peridinin; Phe-*a*, pheophytin-*a*

Pigment Tissue pigment content (mg g ^{1}) Cellular pigment content (pg cell ^{1}) ST TP IT ST TP IT

Chl-*a* 789.32 18.31^{A} 969.62 111.47^{B} 881.31 63.56^{AB} 1.75 0.38^{A} 3.14 0.41^{B} 2.42 1.07^{AB} Chl-c_2 251.88 30.70^{A} 294.76 38.77^{AB} 319.49 31.63^{B} 0.54 0.14^{A} 0.95 0.12^{B} 0.84 0.42^{AB} Chl-c_1 6.36 1.50 5.33 1.11 5.22 1.35 0.01 0.00 0.02 0.01 0.01 0.01 Perid 464.17 21.43 497.94 69.24 510.80 87.30 0.98 0.16^{A} 1.62 0.27^{B} 1.43 0.73^{AB} DD 146.43 33.07 156.06 23.34 166.07 33.37 0.32 0.11^{A} 0.50 0.06^{B} 0.46 0.22^{AB} DT 39.47 19.79 34.89 1.13 38.38 15.22 0.08 0.04 0.12 0.03 0.10 0.03 Dinox 30.42 10.64 40.09 9.59 43.16 9.10 0.07 0.03^{A} 0.13 0.02^{B} 0.12 0.06^{AB} b-Car 13.22 2.18^{A} 12.50 2.65^{A} 6.74 1.67^{B} 0.03 0.01^{A} 0.04 0.01^{B} 0.02 0.01^{A} Phe-*a* 108.38 1.93^{A} 80.57 9.77^{B} 60.50 13.35^{C} 0.19 0.06 0.26 0.05 0.19 0.11 Ratios

Perid./Chl-*a* 0.57 0.03 0.52 0.06 0.58 0.07 0.57 0.03 0.52 0.06 0.58 0.07 DD þ DT/Chl-*a* 0.22 0.04 0.20 0.02 0.23 0.02 0.22 0.04 0.20 0.02 0.23 0.02

flagellates and helps to protect against reactive oxygen species that are released with light induced stress (Venn *et al.* 2006; Rodrı´guez *et al.* 2009), whereas DD is associated with non photochemical quenching and is a main photoprotective pig ment (Hoegh-Guldberg and Jones 1999; Levy *et al.* 2006). The difference between the photosynthetic pigment profile of TP and IT zoanthids was mostly explained by b-Car (Table 2), which was notably lower in IT specimens (Table 1). Although this contrasts with the role of b-Car as a photoprotective pigment (Stambler and Dubinsky 2004), it is possible that its reduced content in IT zoanthids is associated with its role as a pool for the synthesis of DD (Goericke and Welschmeyer 1992; Levy *et al.* 2006).

Table 2. Similarity percentage analysis (SIMPER) identifying the top five photosynthetic pigments that contribute to differences recorded among Zoanthus sociatus from different habitats

Habitats: ST, subtidal; TP, tidal pools; IT, intertidal. Pigments: b-Car, b-carotene; Chl-*a*, chlorophyll-*a*; Chl-c_2, chlorophyll-c_2; Diadino, DD; Diato, DT; Dinox, dinoxanthin; Perid., peridinin; Phe-*a*, pheophytin-*a*

Pigment ST *v*. TP Pigment ST *v*. IT Pigment TP *v*. IT

Dinox 19.0% Dinox 19.5% b-Car 32.4% DD 13.6% b-Car 11.9% Phe-*a* 14.1%

Chl-c_2 13.1% DT 11.8% DD 9.6% Chl-*a* 12.8% DD 10.7% Perid 8.6% Perid. 11.5% Chl-c_2 10.4% Chl-*a* 8.4%

Statistically significant differences among habitats were also observed for the photosynthetic pigment profile per cell (PERMANOVA, P , 0.05), and also for most photosynthetic pigments alone (Table 1). Overall, and in contrast to ST speci mens, *Z. sociatus* from TP displayed a higher pigment cell content, which is in agreement with the pattern recorded for pigment content per dry weight (Table 1) and *Symbiodinium* density (Fig. 2*b*). This result is particularly notable for the main photosynthetic pigments, such as Chl-*a* and peridinin (Perid), but also for photoprotective pigments such as DD and Perid that accounted for most of the variability among habitats for cell pigment content. Particularly, Chl-*a* accounted for 41–45% of data variability among habitats, whereas Perid accounted for 27–29%. Despite the variability observed for pigment content per cell or tissue, no differences were recorded for pigment ratios among zoanthids from different environments (Table 1). This suggests that although pigment cell content changes, the proportion among photosynthetic pigments remains stable.

Photophysiological variability among habitats here observed may be explained by symbiont identity harboured by *Z. sociatus* (Kemp *et al.* 2006; Reimer *et al.* 2006, 2013). Although we were unable to assess the identity of *Symbiodinium* in the present study, it is known that *Z. sociatus* from north-eastern Brazil are associated with either clades A or C, specifically subclades A3 and C1 (Rabelo *et al.* 2014), but may also harbour clades A4 and B1 as observed in Mexico (LaJeunesse 2002). Moreover, recent findings suggest that *Symbiodinium* subcladal diversity shows functional differences (Leal *et al.* 2015*b*; Suggett *et al.* 2015), thus highlighting the cryptic diversity within this genus. It is, therefore, possible that *Symbiodinium* functional diversity pres ent in *Z. sociatus* may support the resilience of zoanthids to environmental fluctuations throughout seasons (Reimer *et al.* 2007) and intertidal habitats (Kamezaki *et al.* 2013).

Conclusions

Results here observed suggest that photophysiological condition of *Z. sociatus* varies with tidal habitat.

Particularly, zoanthids from IT habitats showed low photochemical efficiency and high *Symbiodinium* density. Although this provides evidence that air exposure conditions are not notably affecting the photo physiology of these photosynthetic symbionts, it is important to note that this study only provides a snapshot of temporal and spatial variability. Further studies are needed to assess if photoprotective pigments are also responsible for the large fraction of the differences here recorded between IT and the other two habitats (ST and TP) across seasons. Photo physiological differences between habitats suggest different mechanisms in response to environmental variability, which may be associated with *Symbiodinium* diversity (Kamezaki *et al.* 2013; Rabelo *et al.* 2014).

This is the first study assessing the photosynthetic pigment profile of zoantharians, and is among the few investigations on the effects of aerial exposure on the photobiology of symbiotic cnidarians. Although the photobiology of other symbiotic cni darians has been thoroughly investigated, particularly scleracti nian corals and anemones, the still poor understanding of

inherent optical properties of zoantharian tissue such as symbi ont density tissue thickness and multiple scattering, impair accurate comparisons with previous results. Nevertheless, the differences here recorded provide a promising basis for future work to improve our knowledge on the ecology of symbiotic zoantharians.

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References

Bingham, B. L., Freytes, I., Emery, M., Dimond, J., and Muller-Parker, G. (2011). Aerial exposure and body temperature of the intertidal sea anemone *Anthopleura elegantissima. Invertebrate Biology* **130**, 291–301. doi:10.1111/J.1744-7410.2011.00241.X

Brown, B. E., Dunne, R. P., Scoffin, T. P., and Le Tissier, M. D. A. (1994). Solar damage in intertidal corals. *Marine Ecology Progress Series* **105**, 219–230. doi:10.3354/MEPS105219

Brown, B., Ambarsari, I., Warner, M., Fitt, W., Dunne, R., Gibb, S., and Cummings, D. (1999). Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. *Coral Reefs* **18**, 99–105. doi:10.1007/S003380050163

Brown, B. E., Downs, C. A., Dunne, R. P., and Gibb, S. W. (2002). Preliminary evidence for tissue retraction as a factor in photoprotection of corals incapable of xanthophyll cycling. *Journal of Experimental Marine Biology and Ecology* **277**, 129–144. doi:10.1016/S0022-0981 (02)00305-2

Clarke, K., and Gorley, R. (2006). 'PRIMER v6: User Manual/Tutorial.' (PRIMER-E: Plymouth.)

Cruz, I. C. S., Kikuchi, R. K. P., Longo, L. L., and Creed, J. C. (2015). Evidence of a phase shift to *Epizoanthus gabrieli*Carlgreen, 1951 (Order Zoanthidea) and loss of coral cover on reefs in the Southwest Atlantic. *Marine Ecology* **36**, 318–325. doi:10.1111/MAEC.12141

Done, T. J. (1999). Coral community adaptability to environmental change at the scales of regions, reefs and reef zones. *American Zoologist* **39**, 66–79. doi:10.1093/ICB/39.1.66

Eakin, C. M., and Glynn, P. W. (1996). Low tidal exposures and reef mortalities in the eastern Pacific. *Coral Reefs* **15**, 120. doi:10.1007/ BF01771901

Fadlallah, Y. H., Allen, K. W., and Estudillo, R. A. (1995). Mortality of shallow reef corals in the western Arabian Gulf following aerial expo sure in winter. *Coral Reefs* **14**, 99–107. doi:10.1007/BF00303430

Fitt, W., Brown, B., Warner, M., and Dunne, R. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* **20**, 51–65. doi:10.1007/S003380100146

Goericke, R., and Welschmeyer, N. (1992). Pigment turnover in the marine diatom *Thalassiosira weissflogii*: 1. The 14CO2-labelling kinetics of chlorophyll-*a*. *Journal of Phycology* **28**, 498–507. doi:10.1111/J.0022- 3646.1992.00498.X

Hibino, Y., Todd, P., Ashworth, C. D., Obuchi, M., and Reimer, J. D. (2013). Monitoring colony colour and zooxanthellae (*Symbiodinium* spp.) con dition in the reef zoanthid *Palythoa tuberculosain* Okinawa, Japan. *Marine Biology Research* **9**, 794–801. doi:10.1080/17451000.2013. 766344

Hill, R. (2013). Evidence of light-induced phenotypic plasticity in zoanthids: editorial comment on the feature article by Wei *et al. Marine Biology* **160**, 1051. doi:10.1007/S00227-013-2225-5

Hoegh-Guldberg, O., and Jones, R. (1999). Photoinhibition and photopro tection in symbiotic dinoflagellates from reef-building corals. *Marine Ecology Progress Series* **183**, 73–86. doi:10.3354/MEPS183073

Hughes, T. P., Graham, N. A. J., Jackson, J. B. C., Mumby, P. J., and Steneck, R. S. (2010). Rising to the challenge of sustaining coral reef resilience. *Trends in Ecology & Evolution* **25**, 633–642. doi:10.1016/J.TREE.2010. 07.011

Kaiser, M. J., Attrill, M. J., Jennings, S., Thomas, D. N., Barnes, D. K. A., Brierley, A. S., Hiddink, J. G., Kaartokallio, H., Polunin, N. V. C., and Raffaelli, D. G. (2011). 'Marine Ecology: Processes, Systems, and Impacts.' (Oxford University Press: Oxford, UK.)

Kamezaki, M., Higa, M., Hirose, M., Suda, S., and Reimer, J. D. (2013). Different zooxanthellae types in populations of the zoanthid *Zoanthus sansibaricus* along depth gradients in Okinawa, Japan. *Marine Biodi versity* **43**, 61–70. doi:10.1007/S12526-012-0119-2

Karlson, R. H. (1988). Size-dependent growth in two zoanthid species: a contrast in clonal strategies. *Ecology* **69**, 1219–1232. doi:10.2307/ 1941277

Kemp, D. W., Cook, C. B., Lajeunesse, T. C., and Brooks, W. R. (2006). A comparison of the thermal bleaching responses of the zoanthid *Palythoa caribaeorum* from three geographically different regions in south Florida. *Journal of Experimental Marine Biology and Ecology* **335**, 266–276. doi:10.1016/J.JEMBE.2006.03.017

Kuguru, B., Achituv, Y., Gruber, D. F., and Tchernov, D. (2010). Photo acclimation mechanisms of corallimorpharians on coral reefs: photo synthetic parameters of zooxanthellae and host cellular responses to variation in irradiance. *Journal of Experimental Marine Biology and Ecology* **394**, 53–62. doi:10.1016/J.JEMBE.2010.07.007

LaJeunesse, T. (2002). Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141**, 387–400. doi:10.1007/S00227-002-0829-2

Leal, M. C., Jesus, B., Ezequiel, J., Calado, R., Rocha, R. J. M., Cartaxana, P., and Sero^dio, J. (2015*a*). Concurrent imaging of chlorophyll fluores cence, chlorophyll-*a* content and green fluorescent proteins-like proteins of symbiotic cnidarians. *Marine Ecology* **36**, 572–584. doi:10.1111/ MAEC.12164

Leal, M. C., Hoadley, K., Pettay, D. T., Grajales, A., Calado, R., and Warner, M. E. (2015*b*). Symbiont type influences trophic plasticity of a model cnidarian-dinoflagellate symbiosis. *The Journal of Experimental Biology* **218**(6), 858–863. doi:10.1242/JEB.115519

Leggat, W., Ainsworth, T. D., Dove, S., and Hoegh-Guldberg, O. (2006). Aerial exposure influences bleaching patterns. *Coral*

Reefs **25**, 452–452. doi:10.1007/S00338-006-0128-3

Levy, O., Achituv, Y., Yacobi, Y., Stambler, N., and Dubinsky, Z. (2006). The impact of spectral composition and light periodicity on the activity of two antioxidant enzymes (SOD and CAT) in the coral Favia favus. *Journal of Experimental Marine Biology and Ecology* **328**, 35–46. doi:10.1016/J.JEMBE.2005.06.018

Maxwell, K., and Johnson, G. (2000). Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* **51**, 659–668. doi:10.1093/ JEXBOT/51.345.659

Mendes, C. R., Cartaxana, P., and Brotas, V. (2007). Determination of phytoplankton and microphytobenthos pigments: comparing resolution and sensitivity of a C18 and C8 method. *Limnology and Oceanography, Methods* **5**, 363–370. doi:10.4319/LOM.2007.5.363

Morris, S., and Taylor, A. C. (1983). Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science* **17**, 339–355. doi:10.1016/0272-7714(83) 90026-4

R Development Core Team (2015). R: a language and environment for statistical computing. (R Foundation for Statistical Computing: Vienna, Austria.) Available at http://www.R-project.org [Verified 7 October 2015].

Rabelo, E. F., Rocha, L. L., Colares, G. B., Bomfim, T. A., Nogueira, V. L. R., Katzenberger, M., Matthews-Cascon, H., and Melo, V. M. M. (2014). *Symbiodinium* diversity associated with zoanthids (Cnidaria: Hexacor allia) in Northeastern Brazil. *Symbiosis* **64**, 105–113. doi:10.1007/ S13199-014-0308-9

Rabelo, E. F., Soares, M. O., Bezerra, L. E. A., and Matthews-Cascon, H. (2015). Distribution pattern of zoanthids (Cnidaria: Zoantharia) on a tropical reef. *Marine Biology Research* **11**, 584–592. doi:10.1080/ 17451000.2014.962542

Reimer, J., Takishita, K., and Maruyama, T. (2006). Molecular identification of symbiotic dinoflagellates (*Symbiodinium* spp.) from *Palythoa* spp. (Anthozoa: Hexacorallia) in Japan. *Coral Reefs* **25**, 521–527. doi:10.1007/S00338-006-0151-4

Reimer, J. D., Ono, S., Tsukahara, J., Takishita, K., and Maruyama, T. (2007). Non-seasonal clade-specificity and subclade microvariation in symbiotic dinoflagellates (*Symbiodinium* spp.) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia) at Kagoshima Bay, Japan. *Phycological Research* **55**, 58–65. doi:10.1111/J.1440-1835.2006.00446.X

Reimer, J. D., Irei, Y., Fujii, T., and Yang, S.-Y. (2013). Molecular analyses of shallow-water zooxanthellate zoanthids (Cnidaria: Hexacorallia) from Taiwan and their *Symbiodinium* spp. *Zoological Studies* **52**, 38. doi:10.1186/1810-522X-52-38

Rocha, R. J. M., Calado, R., Cartaxana, P., Furtado, J., and Seroˆdio, J. (2013*a*). Photobiology and growth of leather coral *Sarcophyton* cf. *glaucum* fragments stocked under low light in a recirculated system. *Aquaculture* **414–415**, 235–242. doi:10.1016/J.AQUACULTURE. 2013.08.018

Rocha, R. J. M., Seroˆdio, J., Leal, M. C., Cartaxana, P., and Calado, R. (2013*b*). Effect of light intensity on post-fragmentation photobiological performance of the soft coral *Sinularia flexibilis*. *Aquaculture* **388–391**, 24–29. doi:10.1016/J.AQUACULTURE.2013.01.013

Rodrı´guez, J. J. G., Miro´n, A. S., Camacho, F. G., Garcı´a, M. C. C., Belarbi, E. H., Chisti, Y., and Grima, E. M. (2009). Causes of shear sensitivity of the toxic dinoflagellate *Protoceratium reticulatum*. *Biotechnology Progress* **25**, 792–800. doi:10.1002/BTPR.161

Romaine, S., Tambutte´, E., Allemand, D., and Gattuso, J.-P. (1997). Photosynthesis, respiration and calcification of a zooxanthellate scler actinian coral under submerged and exposed conditions. *Marine Biology* **129**, 175–182. doi:10.1007/S002270050158

Schreiber, U., Schliwa, U., and Bilger, W. (1986). Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quench ing with a new type of modulation fluorometer. *Photosynthesis Research* **10**, 51–62. doi:10.1007/BF00024185

Sebens, K. P. (1982). Intertidal distribution of zoanthids on the Caribbean coast of Panama: effects of predation and desiccation. *Bulletin of Marine Science* **32**, 316–335.

Shick, J. M., and Dykens, J. A. (1984). Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: photosynthesis, respiration, and behaviour under intertidal conditions. *The Biological Bulletin* **166**, 608–619. doi:10.2307/1541166

Stambler, N., and Dubinsky, Z. (2004). Stress effects on metabolism of hermatypic coral. In 'Coral Health and Disease'. (Eds E. Rosernberg and Y. Loya.) pp. 195–215. (Springer-Verlag: Berlin.)

Suggett, D. J., Goyen, S., Evenhuis, C., Szabo, M., Pettay, D. T., Warner, M. E., and Ralph, P. J. (2015). Functional diversity of photobiological

Photobiology of the zoanthid Zoanthus sociatus *Marine and Freshwater Research* G

traits within the genus *Symbiodinium* appears to be governed by the interaction of cell size with cladal designation. *New Phytologist* **208**, 370–381. doi:10.1111/NPH.13483

Teixeira, T., Diniz, M. S., Calado, R., and Rosa, R. (2013). Coral physio logical adaptations to air exposure: heat shock and oxidative stress responses in *Veretillum cynomorium*. *Journal of Experimental Marine Biology and Ecology* **439**, 35–41. doi:10.1016/J.JEMBE.2012.10.010

Titlyanov, E., and Titlyanova, T. (2002). Reef-building corals – symbiotic autotrophic organisms: 2. Pathways and mechanisms of adaptation to light. *Russian Journal of Marine Biology* **28**, S16–S31. doi:10.1023/ A:1021833821493

Venn, A. A., Wilson, M. A., Trapido-Rosenthal, H. G., Keely, B. J., and Douglas, A. E. (2006). The impact of coral bleaching on the pigment profile of the symbiotic alga, *Symbiodinium*. *Plant, Cell & Environment* **29**, 2133–2142. doi:10.1111/J.1365-3040.2006.001587.X

Venn, A., Loram, J., and Douglas, A. (2008). Photosynthetic symbioses in animals. *Journal of Experimental Botany* **59**, 1069–1080. doi:10.1093/ JXB/ERM328

Warner, M., Fitt, W., and Schmidt, G. (1999). Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 8007. doi:10.1073/PNAS.96.14.8007

Wijgerde, T., Melis, A. V., Silva, C. I. F., Leal, M. C., Vogels, L., Mutter, C., and Osinga, R. (2014). Red light represses the photophysiology of the scleractinian coral *Stylophora pistillata*. *PLoS One* **9**, e92781. doi:10.1371/JOURNAL.PONE.0092781

Yang, S. Y., Bourgeois, C., Ashworth, C. D., and Reimer, J. D. (2013). *Palythoa* zoanthid 'barrens' in Okinawa: examination of possible environmental cues. *Zoological Studies* **52**, 39. doi:10.1186/1810- 522X-52-39

Zapata, M., Rodrı´guez, F., and Garrido, J. L. (2000). Separation of chlor ophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. *Marine Ecology Progress Series* **195**, 29–45. doi:10.3354/MEPS195029