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RESEARCH ARTICLE

Thicker host tissues moderate light stress in a cnidarian endosymbiont

James L. Dimond[1,*], Benjamin J. Holzman[2] and Brian L. Bingham[3]

[1]Shannon Point Marine Center, Western Washington University, Anacortes, WA, USA, [2]Cascadia Community College, Bothell, WA, USA and [3]Department of Environmental Sciences, Western Washington University, Bellingham, WA, USA

*Author for correspondence (jdimond@gmail.com)

SUMMARY

The susceptibility of algal–cnidarian holobionts to environmental stress is dependent on attributes of both host and symbiont, but the role of the host is often unclear. We examined the influence of the host on symbiont light stress, comparing the photophysiology of the chlorophyte symbiont *Elliptochloris marina* in two species of sea anemones in the genus *Anthopleura*. After 3 months of acclimation in outdoor tanks, polyp photoprotective contraction behavior was similar between the two host species, but photochemical efficiency was 1.5 times higher in *A. xanthogrammica* than in *A. elegantissima*. Maximum relative electron transport rates, derived from rapid light curves, were 1.5 times higher in *A. xanthogrammica* than in *A. elegantissima* when symbionts were inside intact tissues, but were not significantly different between host species upon removal of outer (epidermis and mesoglea) tissue layers from symbiont-containing gastrodermal cells. Tissues of *A. xanthogrammica* were 1.8 times thicker than those of *A. elegantissima*, with outer tissue layers attenuating 1.6 times more light. We found no significant differences in light absorption properties per unit volume of tissue, confirming the direct effect of tissue thickness on light attenuation. The thicker tissues of *A. xanthogrammica* thus provide a favorable environment for *E. marina* – a relatively stress-susceptible symbiont – and may explain its higher prevalence and expanded range in *A. xanthogrammica* along the Pacific coast of North America. Our findings also support a photoprotective role for thicker host tissues in reef corals that has long been thought to influence variability in bleaching susceptibility among coral taxa.

Key words: cnidarian, symbiosis, light stress, photosynthesis, photoprotection, bleaching.

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INTRODUCTION

Gathering sufficient light for photosynthesis is essential for cnidarians such as corals and sea anemones that host algal endosymbionts. At the same time, however, light and photosynthesis can be sources of stress. Excess light, often in conjunction with elevated temperature, can overburden symbiont photosynthetic processes and lead to the disruptive loss of algal symbionts or their pigments known as bleaching (Weis, 2008). Tolerance of light and heat varies broadly among symbiont taxa, causing variation in bleaching susceptibility and resulting in ecological zonation of symbionts according to prevailing habitat conditions (Baker, 2003; van Oppen et al., 2009). Symbiont identity has thus emerged as a key determinant of both the distribution and stress susceptibility of algal–cnidarian holobionts.

Meanwhile, several studies have concluded that the host plays an equally important role in holobiont stress susceptibility (Bhagooli and Hidaka, 2003; Goulet et al., 2005; Abrego et al., 2008; Fitt et al., 2009). Hosts may differ in their ability to tolerate or limit symbiont stress because of variation in, for example, antioxidant enzymes, heat shock proteins, UV-absorbing compounds, photoprotective pigments and behaviors, and heterotrophic capacity (Salih et al., 2000; Brown et al., 2002; Grottoli et al., 2006; Fitt et al., 2009; Baird et al., 2009). The resistance of certain scleractinian coral taxa to bleaching is well known [the so-called 'winners' (Loya et al., 2001; van Woesik et al., 2011)], yet the mechanisms behind their resistance are not clearly understood, although hypotheses include differences in acclimatization capacity, morphology-mediated mass transfer efficiency and tissue thickness (Gates and Edmunds, 1999; Loya et al., 2001). Many 'winners' have relatively thick tissue, which has been speculated to provide bleaching resistance through increased photoprotective capacity (Hoegh-Guldberg, 1999; Loya et al., 2001). Photoprotection of symbionts by host tissues is illustrated by studies showing increased light sensitivity of isolated *versus in hospite* symbionts (Muller-Parker, 1984; Bhagooli and Hidaka, 2003; Goulet et al., 2005), as well as by studies documenting considerable attenuation of light through host tissues (Kühl et al., 1995; Magnusson et al., 2007; Kaniewska et al., 2011). Although there is evidence of the importance of tissue quality in photoprotection, including the presence of fluorescent and non-fluorescent pigments (Salih et al., 2000; Dove et al., 2008) and UV-absorbing mycosporine-like amino acids (MAAs) (Shick et al., 1995), tissue thickness can differ substantially among host taxa (Loya et al., 2001) and may be even more important to symbiont photophysiology and ecology. To date, however, the potential for thicker host tissues to mitigate symbiont stress has not been evaluated.

Here, we were able to test the hypothesis that thicker host tissues provide greater photoprotection and moderate symbiont stress in a simple, tractable experimental system. We measured symbiont photophysiology and host tissue properties in two closely related (Geller and Walton, 2001) sympatric intertidal sea anemones hosting a relatively stress-susceptible symbiont. Along the Pacific coast of North America, the large solitary anemone *Anthopleura xanthogrammica* and the smaller clonal anemone *A. elegantissima*

host both dinoflagellate (*Symbiodinium* spp.) and chlorophyte (*Elliptochloris marina*) symbionts. Relative to *Symbiodinium muscatinei*, *E. marina* exhibits low tolerance of high light and temperature, and is typically found in cooler, low-light habitats (Bates, 2000; Verde and McCloskey, 2001; Verde and McCloskey, 2002; Secord and Muller-Parker, 2005; Dimond et al., 2011). Interestingly, however, *E. marina* occurs approximately 6 degrees of latitude farther south and much higher in the intertidal zone in *A. xanthogrammica* than in *A. elegantissima* (Secord and Augustine, 2000), indicating that host factors have an important effect on symbiont distribution patterns. In this study, we show that *E. marina* exhibits significantly less light stress in *A. xanthogrammica* than in *A. elegantissima*, resulting from increased light attenuation through *A. xanthogrammica*'s thicker tissues. Our results suggest that host tissue thickness is both physiologically and ecologically relevant to cnidarian symbioses, and support a long-standing yet previously untested hypothesis about the role of thicker tissues in buffering symbiont light stress.

MATERIALS AND METHODS

Experimental conditions

Sea anemones were collected at mean lower low water (±0.5 m) at two locations. Medium-sized *Anthopleura xanthogrammica* (Brandt 1835) (mean ± s.d. basal diameter=7.7±0.8 cm) were collected at Slip Point, WA (48°15′51″N, 124°14′55″W), on 20 April 2011, and large *A. elegantissima* (Brandt 1835) (mean basal diameter=3.4±0.5 cm) were collected at Cone Island, WA (48°35′34″N, 122°40′27″W), on 21 April 2011. At Shannon Point Marine Center (48°30′32″N, 122°41′02″W), individuals of each species were paired together in 10 independent outdoor tanks (69 l volume, 48 cm water depth) each receiving flow-through seawater at a rate of approximately 2 l min^{-1}. Mean temperature of the tanks, as measured by Hobo WaterTemp Pro dataloggers (Onset Computer, Bourne, MA, USA) in two of them, was 11.3±0.5°C over the experimental period. The anemones were fed a weekly ration of one live mussel (*Mytilus trossulus*) proportional to anemone body size. We verified the symbiont identity within anemones by viewing symbionts extracted from an excised tentacle under light microscopy; all anemones had >99% *Elliptochloris marina* Letsch 2009 both at the beginning and the end of the experiment. Hemacytometer counts at the end of the experiment revealed *Symbiodinium muscatinei* (LaJeunesse and Trench, 2000) at 0.1% relative abundance in one *A. xanthogrammica*; *S. muscatinei* were not detected in the other 19 anemones. *Elliptochloris marina* is easily distinguished from *Symbiodinium* spp. due to its smaller size (8–10 μm) and green color. Recent phylogenetic analysis of *E. marina* confirmed its monophyly regardless of host species or geographic location, and that analysis included specimens from locales close to those sampled in our study (Letsch et al., 2009). More recent and thorough population genetics analysis of *rbc*L and ITS2 sequences has again found no significant geographic, seasonal or host-species-specific population structure in *E. marina* (M. Letsch, personal communication).

Sea anemone behavior and symbiont chlorophyll fluorescence

Anemones were acclimated together in the outdoor tank system for 3 months (April–July 2011) before collecting data on their photobiology. This acclimation period is assumed to be sufficient based on the results of Buchsbaum (Buchsbaum, 1968), who monitored *A. elegantissima* hosting *Symbiodinium* after transplantation to full sunlight and found stabilization of most animal and algal pigments after only 7 weeks. We first assessed anemone photoprotective expansion/contraction behavior together with symbiont chlorophyll fluorescence hourly between approximately dawn and dusk over 2 days. Photosynthetically active radiation (PAR; 400–700 nm) over the tanks was measured with a LI-COR LI-190 2π quantum sensor (LI-COR Biosciences, Lincoln, NE, USA). The ranking scheme of Shick and Dykens (Shick and Dykens, 1984) was used to quantify anemone expansion/contraction behavior (Fig. 1A; 4=fully expanded, 3=75% expanded, 2=50% expanded, 1=25% expanded, 0=fully contracted). Given that *Anthopleura* spp. are known for their photoprotective contraction behavior under high

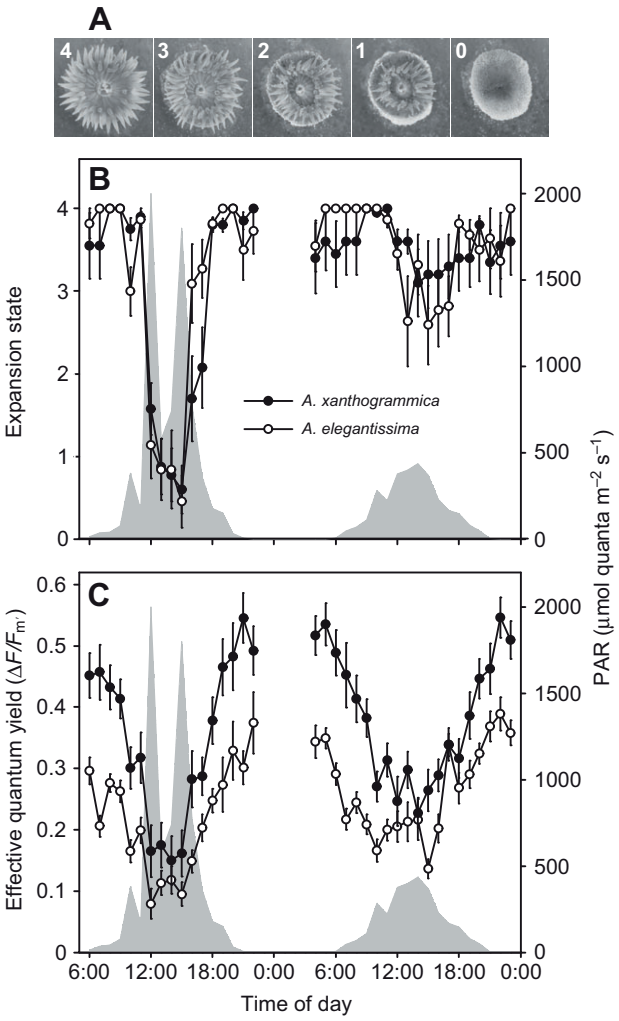

Fig. 1. Diel cycles of sea anemone expansion/contraction behavior and symbiont photosystem II (PS II) quantum yield in *Anthopleura xanthogrammica* and *A. elegantissima*. (A) Numerical ranking scheme of anemone (*A. elegantissima* shown) photoprotective expansion/contraction behavior based on Shick and Dykens (Shick and Dykens, 1984): 4=fully expanded, 3=75% expanded, 2=50% expanded, 1=25% expanded, 0=fully contracted. (B) Time series of anemone expansion/contraction behavior (means ± s.e.m.) in outdoor flow-through seawater tanks over a 2-day period in July 2011. There was no significant difference between species (P=0.69). (C) Time series of PS II quantum yield (ΔF/F_m'; means ± s.e.m.) of *Elliptochloris marina* in its two host anemones over the same period. Pre-dawn values on the second day represent the dark-adapted maximum quantum yield (F_v/F_m), which was significantly different between species (P<0.001). Both time series are superimposed over ambient irradiance levels (gray), shown on the right axes.

irradiance (Shick and Dykens, 1984), we deemed it necessary to evaluate potential differences in behavior between species that could influence the light dose experienced by different tissues, particularly the oral disk and tentacles where most fluorescence data were taken.

The quantum yield of photosystem II (PS II) was measured using a pulse-amplitude modulated (PAM) fluorometer (DIVING-PAM, Heinz Walz, Effeltrich, Germany). The DIVING-PAM measures PS II fluorescence by using a weak measuring light (~0.15 μmol quanta m^{-2} s^{-1}) to assess the minimum fluorescence (F_o in a dark-adapted state or F in a light-adapted state), followed by a 0.8 s saturation pulse (>10,000 μmol quanta m^{-2} s^{-1}) that closes all PS II reaction centers to assess maximum fluorescence (F_m in a dark-adapted state or F_m' in a light-adapted state). Pre-dawn values were used for assessment of the dark-adapted maximum quantum yield ($F_v/F_m=[F_m–F_o]/F_m$), whereas all other values represented the effective quantum yield ($\Delta F/F_m'=[F_m'–F]/F_m'$) under varying degrees of illumination. The effective quantum yield reflects the proportion of absorbed light energy that is used in photochemistry, whereas the maximum quantum yield is the potential capacity of PS II to use absorbed light energy for photochemistry when chlorophyll reaction centers are in a relaxed, dark-adapted state. Maximum quantum yield is a sensitive indicator of PS II light history, stress and photoinhibition (Maxwell and Johnson, 2000). During fluorescence measurements, the fluorometer probe was held 5 mm from the anemone tissue surface, and care was taken to avoid shading the tissue with the probe or eliciting anemone contraction behavior by touching the tissue surface. When anemones were in expanded posture, fluorescence readings were taken on the oral disk adjacent to the first row of tentacles. Fluorescence readings of contracted anemones were taken on the exposed column surface.

Rapid light curves

To evaluate the influence of the intact host tissue environment on PS II function in *E. marina*, we performed rapid light curves (RLCs) (Ralph and Gademann, 2005) with the DIVING-PAM on freshly excised anemone tentacles as well as on symbionts extracted from freshly excised tentacles the week following behavior and fluorescence measurements in the experimental tanks. RLCs measure $\Delta F/F_m'$ at a range of light intensities emanating from the fluorometer probe within a time span of 90 s, and provide a test of the ability of PS II reaction centers to tolerate short-term increases in light intensity. RLCs were performed in a darkened room with the fluorometer probe mounted 5 mm over a large Petri dish filled with seawater and maintained at 12°C. For each anemone, one to four freshly excised intact tentacles were tested, and one to four additional tentacles had their symbiont-containing gastrodermal cells squeezed out of the severed end of the tentacles using a dental pick, leaving behind the epidermal and mesogleal tissue. This extraction method could be performed on a single tentacle within ~15 s and there was no evidence that cells were physiologically affected by this procedure (see Results). The number of tentacles used was dependent on their size and symbiont density, as it was necessary to obtain sufficient symbionts for a strong fluorescence signal. Samples were mounted on polycarbonate membrane filters supported on glass slides and tested within 1–2 min of being transferred to the dark room. The RLC protocol involved eight successive increases in PAR between 0 and 2700 μmol quanta m^{-2} s^{-1} applied for 10 s between each measurement of $\Delta F/F_m'$. This allowed quantification of the relative electron transport rate (rETR) as $rETR=\Delta F/F_m'\times PAR$. The maximum rETR ($rETR_{max}$) was determined by fitting data with the hyperbolic tangent function (Jassby and Platt, 1976; Ralph and Gademann, 2005).

Host tissue properties

To obtain relaxed tissue samples, three to four tentacles from each anemone were excised at their base after reversible narcotization with menthol crystals. Relaxed tentacles were fixed in 4% paraformaldehyde in phosphate buffered saline for 24 h. Light attenuation by epidermal and mesogleal tissue layers was determined by cutting a tentacle open lengthwise and removing gastrodermal cells by gently scraping with a scalpel under a dissecting microscope, then placing the strip of tentacle tissue over the PAR sensor (2.5 mm diameter) of the DIVING-PAM. The probe of the DIVING-PAM was positioned 2 cm directly over the PAR sensor and a constant beam of actinic light (450 μmol quanta m^{-2} s^{-1}) was applied, with PAR readings taken both with and without the tissue strip covering the PAR sensor. The area of each tissue strip was determined by photography under the dissecting microscope and subsequent image analysis (ImageJ, National Institutes of Health, Bethesda, MD, USA) after calibration with a stage micrometer. The tissue strip was then frozen at –70°C for later determination of tissue light absorbance. Through image analysis, we were able to confirm that very few symbionts (gastrodermis) remained on the tissue strips (only 0.97±0.68% of tissue area still held gastrodermal cells). We were also able to verify that mesoglea was not removed from these strips during the dissection process; epidermis was instantly stripped from mesoglea during the homogenization process, whereas the tougher mesoglea initially remained intact until it eventually broke up and became homogenized. We confirmed this by viewing partially homogenized tissue strips under epifluorescence microscopy, noting that the non-fluorescent mesoglea with no adherent epidermis remained largely intact until further homogenization.

A second set of tentacles was embedded in optimum cutting temperature compound (Tissue-Tek O.C.T. Compound, Sakura Finetek, Flemingweg, The Netherlands) and frozen at –70°C for later sectioning and measurement of tissue thickness. Frozen tentacle blocks were sectioned at 20 μm using a rotary microtome (A0820, AO Scientific Instruments, Buffalo, NY, USA) and tissue sections were photographed under epifluorescence microscopy (Leitz DMR, Leica Microsystems, Wetzlar, Germany) with a 450–490 nm excitation filter. The thickness of epidermal, mesogleal and gastrodermal tissue layers was measured at the thickest part of each section by image analysis after calibration with a stage micrometer.

The *in vitro* absorption spectrum of tentacle tissue was determined by homogenizing the frozen epidermal and mesogleal strips described above in 0.1 mol l^{-1} phosphate buffer with a motorized Teflon tissue grinder. Following 24 h under refrigeration, tissue extracts were centrifuged at 10,000 ***g*** for 5 min and read on an Agilent 8453A UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Absorbance was normalized to epidermis volume based on measurements of epidermis area and thickness as described above.

Chlorophyll analysis

Chlorophyll content of *E. marina* was assessed by both spectrophotometry and flow cytometry. For both analyses, symbiont cell suspensions were obtained by homogenizing frozen (–70°C) excised tentacles in filtered seawater with a motorized Teflon tissue grinder. Spectrophotometry was performed according to methods described by Dimond et al. (Dimond et al., 2011). Flow cytometry was performed using a BD FACSCalibur instrument (BD Biosciences, Franklin Lakes, NJ, USA) with a 488 nm excitation laser. Relative cell size was determined *via* the forward scatter (FSC) detector, whereas chlorophyll fluorescence (FL3) was measured *via* the 650 nm long-pass detector. Acquisition was set to 10,000 cells

accumulating in a pre-set gate on the FSC–FL3 plot, with logarithmic amplification on both detectors. Histograms of FL3 and FSC were modeled manually in Cyflogic 1.2 (CyFlo Ltd, Turku, Finland) to obtain population means.

Photosynthetic efficiency after acclimation to low light

To evaluate photosynthetic efficiency in *E. marina* in the absence of full sunlight and demonstrate that there were no inherent differences in symbiont photophysiology between host species, we measured F_v/F_m in anemones several months after our experiments when sea anemones had become acclimated to an indoor, low-light environment. In August 2011, the anemones were transferred to an indoor flow-through seawater tank where they received natural light through north-facing windows. Five months later, we measured F_v/F_m in the anemones in the evening following 30 min in complete darkness. Irradiance levels at mid-day during this period were approximately 30–50 μmol quanta m^{-2} s^{-1}.

Statistical analyses

Expansion/contraction behavior and PS II quantum yield of the two anemone species over two diel cycles were evaluated with repeated-measures ANOVA followed by tests of simple main effects. Paired Student's *t*-tests were used for all other comparisons except for F_v/F_m of symbionts under low-light conditions, for which samples were not paired in individual tanks and a standard Student's *t*-test was used. All data sets were homoscedastic except for epidermis thickness, which was log-transformed prior to analysis to stabilize variances. Statistical significance was determined at α=0.05 and all analyses were performed using SPSS Statistics 18 (IBM, Armonk, NY, USA).

RESULTS

Host behavior and symbiont chlorophyll fluorescence

Over two diel cycles, we found no significant difference ($F_{1,18}$=0.16, P=0.69) in expansion/contraction behavior of the two anemone species (Fig. 1B), indicating that anemone tissue surfaces received similar light doses throughout the day. There were, however, significant differences in PS II quantum yield (Fig. 1C) between the two anemone species for most but not all time periods, as indicated by a significant time × species interaction effect ($F_{36,648}$=2.93, P<0.001). Simple main effects showed that although quantum yields were often not significantly different between species during the middle of the day, quantum yields in *A. xanthogrammica* were significantly higher the majority (76%) of the time. Most importantly, the pre-dawn maximum quantum yield (F_v/F_m) at 04:00 h on day 2 was 1.5 times higher (P<0.001) in *A. xanthogrammica* than in *A. elegantissima* (Fig. 1C).

Rapid light curves

The $rETR_{max}$ of symbionts in intact whole tentacles (Fig. 2) was significantly (1.5 times) higher in *A. xanthogrammica* (t=2.5, d.f.=9, P=0.03). In contrast, when symbionts were extruded from tentacles, $rETR_{max}$ values of symbionts from the two anemone species were not significantly different (t=1.3, d.f.=9, P=0.21). Thus, the presence of intact epidermal and mesogleal tissue layers was largely responsible for differences in $rETR_{max}$ between anemone species, suggesting that there were differences in the internal light environment because of host-tissue properties. We were able to rule out potential negative effects of the gastrodermal extrusion process as initial measurements of $\Delta F/F_m'$, within minutes of tissue extrusion and seconds before commencement of the RLC, showed no significant difference (t=1.4, d.f.=19, P=0.19) between symbionts remaining in intact whole tentacles (0.198±0.077) and symbionts extruded from epidermis and mesoglea (0.185±0.077).

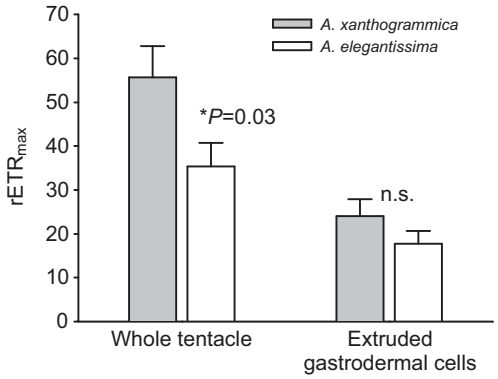

Fig. 2. Maximum relative electron transport rates ($rETR_{max}$; means ± s.e.m.) of *E. marina* in freshly excised whole tentacles of *A. xanthogrammica* and *A. elegantissima* in comparison to symbionts remaining inside gastrodermal cells but removed from outer (epidermis + mesoglea) tissue layers of a second set of freshly excised tentacles.

Host tissue properties

Examination of host tissue properties revealed similar overall tissue morphology of the two host species, including the presence of green fluorescent protein in the epidermis (Fig. 3A). However, all tissue layers were considerably thicker in *A. xanthogrammica* than in *A. elegantissima* (Fig. 3A,B). Overall, tissues were 1.8 times thicker in *A. xanthogrammica* with significant differences in epidermis (t=2.5, d.f.=9, P=0.03), mesoglea (t=4.6, d.f.=9, P=0.001) and gastrodermis (t=5.6, d.f.=9, P<0.001). Attenuation of PAR by strips of tentacle epidermis and mesoglea was significantly (1.6 times) greater in *A. xanthogrammica* than in *A. elegantissima* (t=4.3, d.f.=9, P=0.002; Fig. 3C). The absorption spectra of tissue extracts (Fig. 4) showed slightly higher epidermis volume-specific absorption of *A. elegantissima* tissue, but differences were not significant based on overlap of 95% confidence intervals.

Chlorophyll analysis

Spectrophotometric analysis of cellular chlorophyll content in *E. marina* indicated that symbionts from *A. elegantissima* had significantly more chlorophyll *a* and *b* than those from *A. xanthogrammica*, but similar ratios of chlorophyll *a* to chlorophyll *b* (Table 1). Flow cytometric analysis also verified that symbionts from *A. elegantissima* had significantly higher chlorophyll content, as indicated by higher FL3 red fluorescence. However, FL3 fluorescence normalized to relative cell size (FSC) showed no significant difference between symbionts from the two host species. There was a trend towards higher mean cell size (FSC) of symbionts from *A. elegantissima*, but this was not significant (Table 1).

Photosynthetic efficiency after acclimation to low-light

After 5 months in the indoor tank under low-light conditions, F_v/F_m of the symbionts in the two hosts was indistinguishable (*A. xanthogrammica*=0.708±0.017, *A. elegantissima*=0.696±0.019; t=1.5, d.f.=18, P=0.16), indicating no inherent host-specific differences in the photophysiology of the symbionts in the absence of strong light. These F_v/F_m values were similar to those obtained during the summer in other non-experimental anemones of both species hosting *E. marina* in indoor seawater tables exposed to natural sunlight.

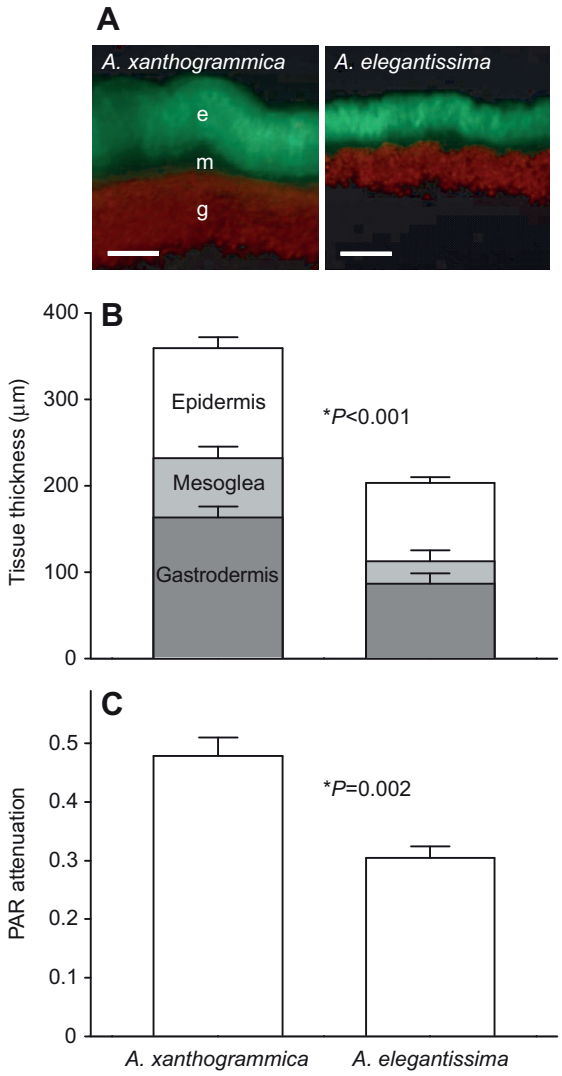

Fig. 3. Tissue properties of *A. xanthogrammica* and *A. elegantissima*. (A) Epifluorescence micrographs of representative tentacle sections of the two host species, showing epidermal green fluorescence from host fluorescent proteins and gastrodermal red fluorescence from symbiont chlorophyll. Tissue layers are labeled as follows: e, epidermis; m, mesoglea; g, gastrodermis. Scale bars, 100 μm. (B) Thicknesses (means ± s.e.m.) of epidermis, mesoglea and gastrodermis. (C) Attenuation of photosynthetically active radiation (PAR; means ± s.e.m.) by strips of tentacle tissue with gastrodermis removed (epidermis and mesoglea only). Attenuation is expressed as the proportion of total downwelling PAR attenuated by host tissues.

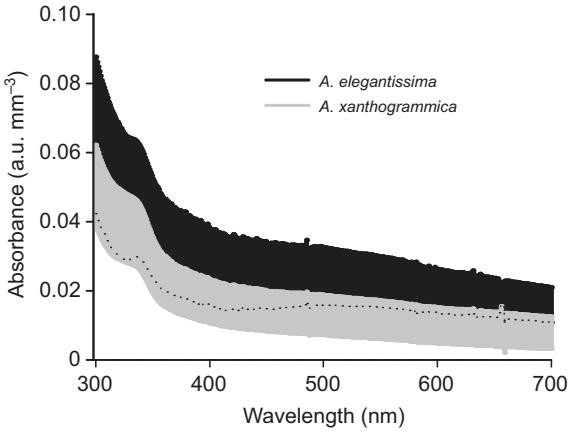

Fig. 4. *In vitro* absorption spectra of host tissue extracts in phosphate buffer normalized to epidermal tissue volume. Spectra represent upper and lower bounds of 95% confidence intervals, with confidence intervals of *A. elegantissima* superimposed as a dotted line over the *A. xanthogrammica* spectrum where they would be covered by spectra overlap. *Anthopleura xanthogrammica* tissue extracts absorbed less light per mm^3 tissue volume than *A. elegantissima* extracts, but overlap of 95% confidence intervals indicates no significant difference between species (P>0.05).

DISCUSSION

Measurements of *E. marina* chlorophyll fluorescence and host tissue properties indicate that symbionts in *A. xanthogrammica* received a considerably lower light dose than those in *A. elegantissima*, largely because of the attenuation of light by outer tissue layers. Light attenuation differences are clearly better explained by tissue thickness than by light-absorbing compounds or pigments. We suspect that the similar magnitude of differences between the species in F_v/F_m (35% difference between the means), $rETR_{max}$ (35% difference), tissue light attenuation (36% difference) and tissue thickness (43% difference) is not a coincidence. Collectively, the data suggest that *A. xanthogrammica* symbionts received at least one-third less light than symbionts in *A. elegantissima*. A higher degree of light stress related to these differences in light dose is indicated by the lower F_v/F_m in *A. elegantissima*, and is analogous to the effects of light dose on F_v/F_m commonly observed in reef corals based on depth and degree of shading (Gorbunov et al., 2001; Warner et al., 2002; Brown and Dunne, 2008). Reduced F_v/F_m is symptomatic of the general phenomenon of photoinhibition, a decline in photochemical efficiency that can be caused by both an increase in photoprotective processes and damage to PS II (Fitt et al., 2001; Gorbunov et al., 2001).

In addition to reduced photochemical efficiency, increased light exposure is typically associated with reduced cellular chlorophyll content. Higher chlorophyll content in *E. marina* from *A. elegantissima* was unexpected given that they received more light and had lower photochemical efficiency than symbionts in *A. xanthogrammica*. However, slightly larger cell sizes among *E. marina* in *A. elegantissima* may be responsible for their higher chlorophyll content; chlorophyll fluorescence normalized to relative cell size showed no host species effect. Cell size in *Symbiodinium* and a majority of marine and freshwater phytoplankton has been found to increase with irradiance (Lesser and Shick, 1989; Thompson et al., 1991). Another consideration is that *E. marina* chlorophyll content does not always change predictably in response to changes in irradiance. *Elliptochloris marina* living within *A. elegantissima* show little seasonal variation in cell-specific chlorophyll content despite large fluctuations in irradiance (Verde and McCloskey, 2007; Dimond et al., 2011). We have also found that *E. marina* chlorophyll content within *A. elegantissima* does not consistently increase during long-term experimental shading (J.L.D., B.L.B. and G. Muller-Parker, unpublished).

A photoprotective function of host tissues has been suggested by several studies comparing the physiology of algal symbionts *in hospite* with that of symbionts removed from the host. Photosynthesis–irradiance experiments have shown that *Symbiodinium* spp. freshly isolated from anemone hosts (*Aiptasia* spp.) are more likely to exhibit photoinhibition than those in intact hosts (Muller-Parker, 1984; Goulet et al., 2005), and that isolated symbionts are also much more susceptible to heat stress (Goulet et

Table 1. Spectrophotometric and flow cytometric analyses of *Elliptochloris marina* chlorophyll content in the two host species, *Anthopleura elegantissima* and *A. xanthogrammica*

	Host	Mean ± s.d.	*t*	d.f.	*P*
Spectrophotometric analysis					
Chl *a* (pg cell^{-1})	*A. elegantissima*	1.83±0.21	7.48	9	**<0.001**
	A. xanthogrammica	1.13±0.15			
Chl *b* (pg cell^{-1})	*A. elegantissima*	0.93±0.13	6.22	9	**<0.001**
	A. xanthogrammica	0.55±0.10			
Chl *a*:chl *b*	*A. elegantissima*	1.98±0.21	–1.04	9	0.33
	A. xanthogrammica	2.08±0.21			
Flow cytometric analysis					
FL3 (red fluorescence)	*A. elegantissima*	192±28.4	2.89	9	**0.02**
	A. xanthogrammica	166±16.6			
FSC (forward scatter)	*A. elegantissima*	1165±92.21	1.93	9	0.09
	A. xanthogrammica	1080±127.1			
FL3/FSC	*A. elegantissima*	0.166±0.024	0.90	9	0.39
	A. xanthogrammica	0.156±0.025			

Significant *P*-values are shown in bold.

al., 2005). Bhagooli and Hidaka (Bhagooli and Hidaka, 2003) showed that F_v/F_m of isolated symbionts from several scleractinian coral species was considerably more sensitive to heat and light stress than in intact hosts. In those experiments, the key role of light in symbiont stress physiology was highlighted by the fact that exposure to elevated temperature in darkness did not affect F_v/F_m, and that isolated symbionts showed reduced F_v/F_m at lower light levels than symbionts in intact hosts (Bhagooli and Hidaka, 2003). Thus, intact host tissues provide critical photoprotection that can moderate the effects of thermal stress. The photoprotective capacity of host tissues has been mostly ascribed to fluorescent and non-fluorescent pigments (Salih et al., 2000; Dove et al., 2008) and UV-absorbing MAAs (Shick et al., 1995), but here we have demonstrated the importance of tissue thickness alone.

The attenuation of light by epidermal and mesogleal tissues accounts for most of the difference in the effective quantum yield of PS II between the two host species, as shown by measurements of $rETR_{max}$ in whole tentacles compared with extruded gastrodermal cells. The epidermis of both *Anthopleura* species absorbs and scatters light *via* fluorescent and non-fluorescent pigments (Buchsbaum, 1968) and MAAs (Shick et al., 2002). Although the epidermis may account for much of the photoprotective capacity of outer tissue layers, we hypothesize that the mesoglea also plays a role through light scattering. The mesoglea is a matrix of collagen and mucopolysaccharides, and collagen has a relatively high refractive index (Johnsen and Widder, 1999). Among several pelagic cnidarians, Johnsen and Widder (Johnsen and Widder, 1998) found that mesoglea transparency ranged from 6 to 89% at 400 nm. Notably, of all tissue layers in our study, the mesoglea showed the greatest difference in thickness (62%) between host species whereas the epidermis showed the least (29%). In addition to the outer tissue layers, the gastrodermis of *A. xanthogrammica* was nearly twice as thick as that of *A. elegantissima*, which may also contribute to photoprotection *via* the self-shading of successive layers of algal symbionts in gastrodermal cells.

Host-specific differences in the distribution patterns of *Anthopleura* spp. symbionts suggest that the ecological implications of host tissue thickness are significant. Whereas *E. marina* is largely absent from *A. elegantissima* south of central Oregon (~44°N), in *A. xanthogrammica* it occurs approximately 6° farther south into northern California (~38°N) (Secord and Augustine, 2000). Similarly, where *E. marina* co-occurs in the two host species, it is typically only hosted by *A. elegantissima* in the low intertidal zone, whereas in *A. xanthogrammica* it is hosted much further up the shore (Secord and Augustine, 2000). Furthermore, *E. marina* is more prevalent in *A. xanthogrammica* even among similar-sized individuals of the two host species living adjacent to each other in the same habitat (Bates et al., 2010). The symbiotic dinoflagellate *S. muscatinei*, meanwhile, is considerably more prevalent in *A. elegantissima* than in *A. xanthogrammica.* This likely relates to the higher photophysiological performance of *S. muscatinei* at high light and temperature compared with *E. marina* (Verde and McCloskey, 2001; Verde and McCloskey, 2002). The results of our study therefore suggest a mechanism for the higher prevalence and expanded range of *E. marina* in *A. xanthogrammica*, particularly in high-irradiance habitats. Perhaps the distribution and host-specificity patterns of symbionts on tropical reefs are similarly influenced by differences in host tissue thickness, as suggested by LaJeunesse (LaJeunesse, 2002) in reference to differences in host tissue opacity.

The differences in tissue thickness of the two sea anemone species undoubtedly relates to their greatly different adult sizes. *Anthopleura elegantissima* rarely exceeds a basal diameter of 4 cm, whereas *A. xanthogrammica* can sometimes exceed 16 cm basal diameter (Sebens, 1981a). This fourfold difference in maximum diameter equates to a 32-fold difference in biomass based on the equations of Sebens (Sebens, 1981a) relating diameter to ash-free dry weight. Using these equations, we estimate that our medium-sized *A. xanthogrammica* had a biomass approximately eight times that of our large-sized *A. elegantissima*. Back-calculation of tentacle size scaling from equations of total tentacle surface area and tentacle number *versus* ash-free dry weight (Sebens, 1981a) suggest that scaling in the two species is similar, and that juvenile *A. xanthogrammica* the size of adult *A. elegantissima* have similar-sized tentacles. Juvenile *A. xanthogrammica* therefore may not have thicker tissues than adult *A. elegantissima*, in which case symbiosis with *E. marina* may not be advantageous for juvenile *A. xanthogrammica* (or *E. marina*) living in high-irradiance habitats. Interestingly, however, juvenile *A. xanthogrammica* occur almost exclusively within beds of their principal adult prey, the mussel *Mytilus californianus*, remaining in the shaded interstices of these beds until they reach a basal diameter of approximately 3.5 cm (Sebens, 1981b). This may allow them to initiate and maintain successful symbioses with *E. marina* at an early age and small size.

Our study supports the hypothesis that variability in bleaching susceptibility among reef corals may be related to the

photoprotective function of thicker host tissues (Hoegh-Guldberg, 1999; Loya et al., 2001). Bleaching typically involves the synergistic effects of light and temperature on symbiont photosynthetic processes (Hoegh-Guldberg, 1999; Weis, 2008), and if one of these stressors is lessened, the total stress will be reduced. We have shown that light stress in an algal symbiont can differ by approximately one-third in relation to host tissue thickness differences of a similar magnitude. Patterns of bleaching and post-bleaching survival among different reef coral taxa suggest that variation in tissue thickness may have profound ecological consequences (Loya et al., 2001; van Woesik et al., 2011). In Japan, Loya et al. (Loya et al., 2001) observed that bleaching susceptibility and subsequent mortality after the 1998 mass coral bleaching event was much lower among massive, thick-tissued species than in species with thinner tissues and branching morphologies. Hypotheses for these trends included enhanced mass transfer efficiency in encrusting or mounding morphologies *versus* branching morphologies, and the photoprotective capacity of thicker tissues (Loya et al., 2001). On average, tissue thickness of thick- and thin-tissued taxa in their study differed by 68% (Loya et al., 2001), considerably more than the differences we measured between *Anthopleura* species. Although thin-tissued branching corals provide inter-branch shading and vertically oriented surfaces that may result in more effective light attenuation than previously thought (Kaniewska et al., 2011), our findings show that thicker host tissues alone can provide considerable symbiont photoprotection. It is likely that variation in this relatively simple trait has ecological implications ranging from the biogeography, zonation and host specificity of cnidarian symbionts to the health and persistence of reef corals in increasingly warmer seas.

LIST OF ABBREVIATIONS

F_v/F_m	maximum quantum yield of photosystem II
PAM	pulse amplitude modulated
PAR	photosynthetically active radiation
PS II	photosystem II
$rETR_{max}$	maximum relative electron transport rate
RLC	rapid light curve
$\Delta F/F_m'$	effective quantum yield of photosystem II

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