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Unravelling coral photoacclimation: *Symbiodinium* strategy and host modification

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Abstract. Light is often the most abundant resource within the nutrient poor waters surrounding coral reefs. Consequently, zooxanthellae (*Symbiodinium spp.*) must continually photoacclimate to optimise productivity and ensure coral success. To accurately assess *Symbiodinium* photoacclimation *in situ*, differences in acclimation strategies and bio-optical signatures need to be characterised between genetic types of *Symbiodinium.* Using a systematic series of laboratory experiments, eight types of *Symbiodinium* were cultured and examined using techniques such as active (FIRe) fluorescence, Photosystem I (PSI) and II counts and spectrophotometry. Two key 'strategies' of photoacclimation are known to exist amongst microalgae: a preferential modification of the light harvesting antennae (σ-based) or of the reaction centre bed (n-based) for PSII and/or PSI. Our measurements demonstrated that acclimation strategies employed by *Symbiodinium* were highly varied between algal type but despite this variability, many optical signatures were conserved. Acclimation strategies of intact *Acropora formosa* and *Seriatopora caliendrum* at two light levels were further examined using fluorescence and optical signatures to determine host contribution to acclimation. Overall, our results demonstrated that (1) biophysical (active fluorescence, photosystem-specific) but not bio-optical signatures were highly variable between algal types; consequently, bio-physical signatures that are altered by an adaptation of the algal community structure may be misinterpreted as photoacclimation and (2) host acclimation and modification of the light environment plays a key role in *Symbiodinium* photoacclimation.

Key words: *Symbiodinium,* chlorophyll *a* fluorescence, photoacclimation, absorption

Introduction

In the oligotrophic waters surrounding coral reefs, optimisation of photosynthesis is crucial for the success of the symbiotic partnership between the microalgae (*Symbiodinium spp*) and the host coral. This is achieved through the process of photoacclimation (phenotypic modification of the photosynthetic apparatus in response to changes in light availability). However, this phenomenon is not ubiquitous for all coral-*Symbiodinium* assemblages.

Multiple genetic types of *Symbiodinium* exist, and have been categorised into 8 major clades, A-H (Coffroth and Santos 2005). These clades can be further split into sub-clades according to the ribosomal Internal Transcribed Region 2 (ITS2) (LaJeunesse et al. 2001, Robison and Warner 2006). It was initially assumed that different clades conformed as 'eco-types' such as thermally tolerant or sensitive, but recent evidence has shown that variability exists within each clade (Iglesias-Prieto et al. 1994, 1997, Tchernov et al. 2004, Robison and Warner 2006, Hennige et al. 2009).

A coral species may comprise of a mixed community of *Symbiodinium* types, including different clades as well as subclades (LaJeunesse et al. 2004, Goulet 2006). The importance of this for coping with environmental change (including light) is debated (Baker 2001, Hoegh-Guldberg et al. 2002) but centres around whether corals can adaptively bleach (expel sub-optimal zooxanthellae and 'uptake' suitable types), or whether the *Symbiodinium* community already *within* the coral will 'shuffle' in dominance as conditions favour growth of one type over others. Regardless of potential benefits related to 'hosting' multiple *Symbiodinium* types, the presence of multiple types complicates interpretation of any *in hospite* photoacclimation study where the genetic identity of *Symbiodinium* is unknown, as genetic variability may 'mask' potential photoacclimation responses (Hennige et al. 2008, 2009).

Several photoacclimation strategies are known to exist for microalgae (Falkowski and Owens 1981, Suggett et al. 2007): cells can preferentially undergo changes to the light harvesting antennae

(photosynthetic unit, PSU, size) or of the reaction centre pool size (PSU number). This 'choice' varies, but recent work on phytoplankton has identified two strategies which conform to environmental conditions such as nutrient and light availability (Moore et al. 2006, Warner et al. 2006, Six et al. 2008). The two strategies involve either changes to PSU size or number and were termed σ versus n–type acclimation strategies respectively (Six et al. 2008). N-type acclimation tends to be found in generalist microalgae species which live in variable light environments and are not nutrient limited. Conversely, σ-type acclimation is better suited to uniform or low light environments (Six et al. 2008).

Methods for assessing photoacclimation are often destructive, so recent *in situ* coral photoacclimation studies have turned towards non-invasive techniques, such as chlorophyll *a* (chl *a*) fluorescence and optical reflectance. Chl *a* fluorescence can be used to infer photosynthetic efficiency and effective cross section-absorption (σ) of *in hospite Symbiodinium* (Gorbunov et al 2001). Consequently, studies have used chl *a* fluorescence to assess coral photoacclimation across environmental gradients (Ralph et al. 1999, Hennige et al. 2008). However, many studies, past and present, do not account for the community composition of *in hospite Symbiodinium*; consequently, studies often assess photoacclimation on the basis that the genotype of *Symbiodinium* does not change.

These coral-*Symbiodinium* assemblages are often considered as a single entity – the holobiont. Since some hosts only associate with certain *Symbiodinium* types, are some holobionts more suited to certain environments than others? To answer this, a better understanding is needed of the photobiological variability between different *Symbiodinium* types, and also how the host can play a role in modifying the internal light environment to optimise *Symbiodinium* productivity.

This study addressed 2 primary objectives: (1) to categorise variability between different *Symbiodinium* types using bio-optical and bio-physical techniques; and (2) to assess whether host acclimation and modification plays a role in *Symbiodinium* photoacclimation.

Material and Methods

Isolated Symbiodinium - Eight *Symbiodinium* types identified using the ribosomal ITS2 region; A1, A1.1, A2, A3, B1, B1*, B1** and F2 (Table 1) were cultured in 2L flasks at 26°C at two photon flux densities (PFD, 100 (LL) and 650 (HL) μmol photons m^{-2} s^{-1}) on a 14:10 light: dark cycle (see Hennige et al. 2009). Cultures were semi-continuous in artificial seawater media ASP-8A, (Provasoli et al. 1957) and were bubbled gently with air passed via a carbon column to keep pH constant (ca. 8.3). In exponential phase, aliquots of 500 ml were gravity filtered to concentrate material for subsequent measurements (Suggett et al. 2007). Triplicates were taken from sequential generations.

Table 1: *Symbiodinium* type according to ITS2 region, the host and region they were isolated from (adapted from Hennige et al. 2009)

Algal type	Host	Host origin
A1	*Cassiopeia xamachana*	Florida
A1.1	*Condylactis gigantea*	Jamaica
A2	*Montastrea spp.*	Florida
A3	*Tridacna maxima*	Palau
B1	*Aiptasia pallida*	Bermuda
B1*	*Aiptasia puchella*	Hawaii
B1**	*Acropora spp.*	Aquarium (UK)
F2	*Meandrina meandrites*	Jamaica

Corals – small fragments (ca. 6 cm) of *Seriatopora caliendrum* and *Acropora formosa* (from London Aquarium) were maintained in separate 250 ml water jacketed vessels under a light: dark cycle of 12: 12 at 26°C for 12 weeks. Media was circulated through the vessels at 40 ml $hour^{-1}$. Each vessel was integrated with an inflow, an outflow and an aerator to provide water exchange and circulation. A planktonic food supplement was added to the media reservoir to provide a constant external nitrogen source to all vessels. There were two light treatments; low light, ca. 40, and high light, ca. 170 μmols photons m^{-2} s^{-1}. These light levels represent reef depths in Indonesia of ca. 20 and 10 m respectively (June – September).

N versus σ strategies– Photosynthetic unit sizes, which are used to assess σ–type acclimation, were defined as the ratio of chl *a* to reaction centres in PSI (RCI) or RCII (Suggett et al. 2007, Hennige et al. 2009), and are considered to be indicative of the concentration of functional reaction centres (RCs). Cell counts were determined using a haemocytometer, and subsequently used to calculate n–type acclimation as mol RCII(I) $cell^{-1}$.

To assess chl *a* fluorescence of *Symbiodinium*, a Fluorescence Induction and Relaxation (FIRe) fluorometer (Satlantic), which generates single turnover (ST) and multiple turnover (MT) fluorescence transients was used (Fig. 1). A ST protocol is where the primary electron acceptor Q_A, is fully reduced through a simultaneous single turnover event of all PSII reaction centres (Suggett et al. 2008).

A U-3000 spectrophotometer with ϕ–60 integrating sphere (Hitachi) was used to determine sample optical density as outlined in Suggett et al. (2007) and Hennige et al. (2009).

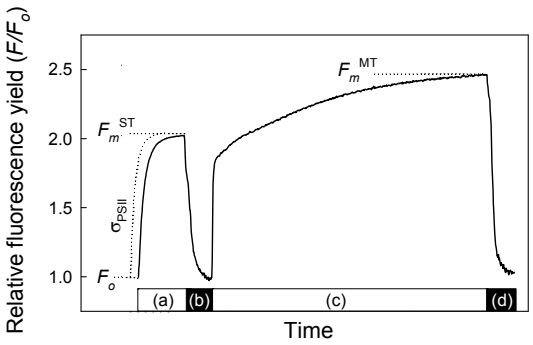

Figure 1: Example fluorescence induction trace using a FIRe fluorometer. Minimum fluorescence, F_o, maximum fluorescence (single turnover) F_m^{ST}, multiple turnover F_m^{MT}, and effective cross-section absorption of PSII (σ_{PSII}) are illustrated. Letters a - d refer to fluorescence induction stages along a time scale; a) describes the rise in fluorescence following a 100 µs single turnover (ST) event, b) is the 500 ms relaxation stage following the ST event, c) is the fluorescence rise from a multiple turnover (MT) flash over 600 ms and d) is the relaxation stage following the MT event over 1 s (adapted from Hennige et al. 2009)

To assess host and freshly isolated zooxanthellae absorption, coral tissue was removed from the coral skeletons using a waterpik. The slurry was separated into host and zooxanthellae portions by centrifuging twice at 1500***g*** for 15 min (Levy et al. 2003). The zooxanthellae pellet was re-suspended in a known quantity of seawater and the supernatant was regarded as the host fraction for subsequent spectrophotometry.

Surface area of the coral skeletons was quantified using tin foil and Image Tool analysis (UTHSCSA). Chlorophyll content of each sample and per colony was calculated using methanol and a spectrophotometer in accordance with Porra et al. (1989).

Results

Photophysiology - under steady state growth, significant variability was observed between algal types in F_v/F_m^{ST} ($F_{7,16} = 59.0$ and 35.8, $p < 0.001$ for LL and HL respectively) and σ_{PSII} ($F_{7,16} = 15.62$ and 3.81, $p < 0.05$ for LL and HL respectively), (see also Fig. 2). Mean values for F_v/F_m^{ST} and σ_{PSII} were ca. 25% and 5% lower under HL than LL respectively, but the magnitude of change was type-dependent.

Photosynthetic unit size and concentration- values of photosynthetic unit (PSU) 'size', chl *a*: RCII (I) used to assess σ–type acclimation, were highly variable with algal type (Data not shown). Most algal types exhibited a decrease in chl *a*: RCII between LL and HL but this was highly variable; algal type A1.1 exhibited an increase whilst type A2 exhibited no change in chl *a*: RCII (Table 3).

Changes to chl *a*: RCI between LL and HL were also highly variable between type, either increasing (A1.1), decreasing (B1, F2) or exhibiting little or no change (A1, A2, A3, B1*, B1**). Similarly, the cellular quotas (n-type acclimation) of both RCI and RCIIs were highly variable between algal types and upon an increase in growth PFD, most types decreased cellular RC content (Table 3). F2 was the only type to significantly increase cellular RC content from low to high growth PFD. Consequently, RC stoichiometry of PSII to PSI (RCII: RCI) did not vary in a consistent pattern for algal type or growth PFD.

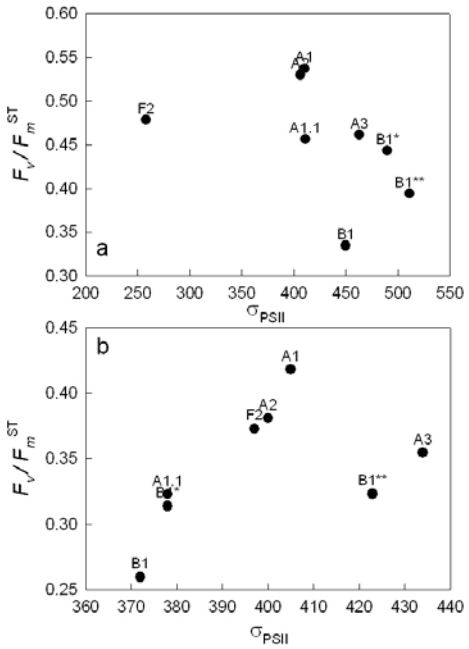

Figure 2: Maximum photochemical efficiency (F_v/F_m^{ST}) and effective cross section absorption of PSII (σ_{PSII}) of all *Symbiodinium* types under LL (a) and HL (b). For absolute values refer to Hennige et al. 2009

Table 3: Percentage difference in RC cell^{-1} (n) and chl *a* RC^{-1} (σ) for PSII and PSI between low and high light replicates. For absolute values refer to Hennige et al. 2009. Significant differences between LL and HL cultures are denoted with * (t-test, $p < 0.05$)

Algal Type	PSII		PSI	
	Number (RCII cell^{-1})	Size (chl *a* RCII^{-1})	Number (RCI cell^{-1})	Size (chl *a* RCI^{-1})
A1	-41.79	-17.46	-56.43*	10.27
A1.1	-72.62*	57.96*	-61.22*	11.55
A2	-57.08*	0.22	-46.69*	-36.24*
A3	14.93	-14.51	11.08	-3.68
B1	-73.90*	-44.53*	-78.96*	-31.18
B1*	-61.98*	-9.36	-46.41*	-35.70*
B1**	-6.9	-36.76	-51.9*	11.29
F2	34.95*	-57.07*	60.68*	-63.95*

Optical absorption, a^* (m^2 mg chl a^{-1}), was variable between algal type at LL and HL (Fig. 3), but was similar in shape for all algal types. Consequently, a^* was significantly correlated with pigment concentrations (Hennige et al. 2009). a^* at HL was

higher than at LL (Fig. 3) but percentage increase in absorption was type dependent.

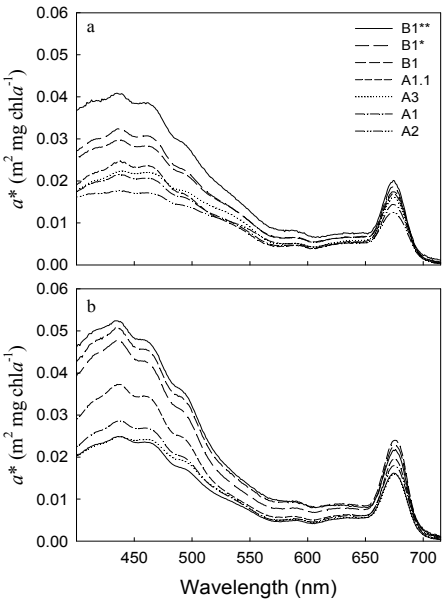

Figure 3: Optical absorption normalised to chlorophyll *a*, *a** (m^2 mg chl a^{-1}), from 400 to 715 nm for all *Symbiodinium* types except F2 under (a) LL and (b) HL.

Partitioning bio-optical signatures –freshly isolated zooxanthellae bulk absorption (Fig. 4a,c) was comparable to cultured *Symbiodinium* optical absorption (Fig. 3). Host tissue absorbed primarily at ca. 400 nm and decreased linearly to ca. 700 nm (Fig. 3a,b). Host absorption presented here was comparable to previous studies (Enriquez et al. 2005) but was higher in magnitude. Slurry absorption in Fig. 4a, was presented as the sum of both host and zooxanthellae fractions, which could differ by between ca. 2 – 10 % in magnitude of the measured slurry optical absorption.

When host fractions from *S. caliendrum* and *A. formosa* were compared between low light (LL) and high light (HL), HL corals had higher absorption. Additionally, *A. formosa* had an absorption peak (at both light levels) at ca. 500 nm (Fig. 4b) which was not present in *S. caliendrum* and represents the presence of a fluorescent protein, (confirmed with spectral fluorescence data (data not shown)). However, *S. caliendrum* host absorption was higher than *A. formosa* between 400 and ca. 460 nm.

Zooxanthellae bulk absorption (Fig. 4c) was influenced more by host species than light regime, since between LL and HL for each coral species, there was negligible change in zooxanthellae absorption (m^2 mg chl a^{-1}). Zooxanthellae isolated from *S. caliendrum* had higher absorption than *A. formosa* zooxanthellae (Fig. 4c).

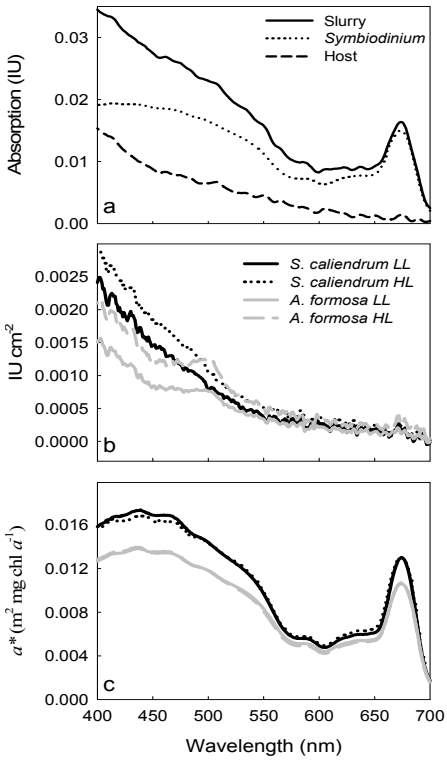

Figure 4: Example absorption characteristics (Instrument Units) of *S.caliendrum* tissue (slurry) and its constituents removed from the coral skeleton (a). Absorption of host tissue (IU cm^{-2}) from *S. caliendrum* and *A. formosa* from LL and HL conditions (b) and of isolated zooxanthellae (m^2 mg chl a^{-1} (c))

Discussion

Substantial variability was observed both in and between algal types in their n and σ-type photoacclimation strategies, consistent with previous studies (Iglesias-Prieto and Trench 1994, Hennige et al. 2009). The variability in bio-physical signatures such as F_v/F_m^{ST} was often larger between types than between environmental conditions. Consequently, if F_v/F_m^{ST} is used to assess photoacclimation across environmental gradients, *Symbiodinium* type must be accounted for to prevent an adaptation of the algal community being misidentified as photoacclimation.

Bio-optical signatures were conserved between types and varied according to pigment concentrations. Since *a** consists of photosystem II and I absorption,

$$a^*(\text{optical}) = a^*\,\text{PSII} + a^*\,\text{PSI} \qquad [1]$$

and optical absorption is controlled by both PSU size and number, we can consider *a**(optical) as

$$a^*(\text{optical}) = [\sigma \cdot n]\text{PSII} + [\sigma \cdot n]\text{PSI} \qquad [2]$$

Since *a** is conserved across *Symbiodinium* types, and n-type strategies are dominant across types, changes in σ between photosystems must therefore balance

changes in n. The predominant n-type strategy in *Symbiodinium* suggests the role of a generalist algal strategy when subject to varying light intensities, which are not nutrient limited (Six et al. 2008). Some types (A1.1, B1's and F2) also exhibited a σ-type strategy in addition to n (Table 3), which may confer additional benefits (Hennige et al. 2009).

Results here enabled direct comparison between zooxanthellae isolated from different coral hosts; the *internal* light environment for *S. caliendrum* was lower than that in *A. formosa.* Increased host absorption by *S. caliendrum* between 400 and 500 nm may have contributed to this. A lower light environment would promote the observed increase in algal absorption to optimise photosynthesis (Fig. 3c). These differences between host absorption could be attributed to increased host tissue volume per unit area as noted by Anthony et al. (2003), or by differences between host pigment content. The presence of a possible green fluorescent protein is noted at ca. 510 nm in *A. formosa* (Fig. 4b). However, skeletally enhanced light (not measured), may also have differed between host species and contributed to internal light variability (Enriquez et al. 2005).

In summary, both parts of the holobiont; the *Symbiodinium* and the host, are crucial to overall coral acclimation. However, the contribution of both fractions to acclimation is still not fully quantified. Importantly, bio-physical approaches need additional genetic identification (as opposed to bio-optical approaches) of the *Symbiodinium* to assess photoacclimation, consequently meaning that grouping algal clades as 'eco-types' may not be suitable. The host modification of internal light environment also differs between coral species, and in some cases may cause as much variability between species as between external light environments. Consequently, future photoacclimation studies will have to account for both *Symbiodinium* and host contribution to unravel coral photoacclimation.

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