Title: *Symbiodinium* migration mitigates bleaching in three octocoral species

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ABSTRACT: Coral reefs are increasingly threatened by bleaching, a breakdown of the mutualism between coral hosts and symbionts (*Symbiodinium* spp.). Symbiont movement within a host may mitigate the effect of environmental stressors that trigger bleaching. Octocorals represent an important component of reef ecosystems, and the alcyonacean taxa *Phenganax parrini*, *Sarcothelia* sp., and *Sympodium* sp. were experimentally perturbed. In colonies subject to elevated temperature (incubated at 30 – 32 °C to a maximum temperature of 31.5 – 33.5 °C) and illumination, the number of *Symbiodinium* decreased in the tissue but increased in the gastrovascular system, with only a small proportion of symbionts expelled. Following within-colony symbiont migration, the three octocoral species retained high densities of symbionts in the coenenchyme. Nevertheless, variable mortality and retention occurred (≈85, 0, and 53% of the initial number of *Symbiodinium* were calculated to have died and ≈15, 100, and 45% were calculated to have been retained by *P. parrini* [maximum 33.5 °C, 24 h], *Sarcothelia* sp. [maximum 31.5 °C, 16 h], and *Sympodium* sp. [maximum 31.5 °C, 24 h],

respectively). The perturbation responses could be due to genetic differences between the hosts or symbionts, or to a host-symbiont interaction. Mitochondrial (mtMutS and COI) and nuclear (28S rDNA) gene sequences demonstrated that *Sympodium* sp. and *Sarcothelia* sp. are closely related xeniids but are only distantly related to *P. parrini*. Conversely, internal transcribed spacer 2 ribosomal DNA regions showed that *P. parrini* and *Sarcothelia* sp. hosted *Symbiodinium* types D4-5 and D4-5-9, respectively, while *Sympodium* sp. had a type C1 variant. Architectural features of the host colonies may also be relevant, with runner-like features of *P. parrini* possibly limiting symbiont retention due to high surface/low volume coenenchyme. Despite these differences, all three alcyonaceans exhibited symbiont migration and symbiont retention when perturbed. Further research on additional taxa will be necessary to determine the relative role of host-*Symbiodinium* interactions and host architectural features in modulating the mitigation of bleaching via migration.

Keywords: coral, *Phenganax parrini*, *Sarcothelia* sp., *Sympodium* sp., thermal stress, zooxanthellae

1. Introduction

Many cnidarian species form a mutualistic relationship with photosynthetic symbionts (*Symbiodinium* spp.) (Muscatine and Porter, 1977). Bleaching is the breakdown of this relationship, whereby symbionts either exit the colony or die, resulting in the loss of symbionts and/or photosynthetic pigments such that the host appears pale or “bleached.” Coral bleaching often occurs in response to elevated temperature and light (Yamashita et al., 2011; Downs et al., 2013; Hawkins et al., 2013; Paxton et al.,

2013; Tolleter et al., 2013), although bleaching can follow a cold thermal event (Kemp et al., 2011), exposure to sedimentation (Philipp and Fabricius, 2003) or other stressors. Bleaching can have dire consequences for the symbionts, the host, or both. Consequently, there is considerable interest in elucidating factors that mitigate the effects of bleaching. Ultimately, such insight can usefully be applied to coral reefs ecosystems in which octocorals are a significant component (McFadden et al., 2010).

During bleaching the *Symbiodinium* may encounter several possible fates (Gates et al., 1992). They can die (apoptosis, necrosis) and/or be removed from the host tissue (exocytosis, pinching off, and host cell detachment). Symbionts released from the host tissue can travel in the lumen of the gastrovascular system of the host, and may either be expelled or retained. Working with the anemone *Aiptasia pulchella*, Gates et al. (1992) suggested a two-phase process of host cell release. During the first phase, *Symbiodinium*-bearing host cells detach from the endoderm, accumulating in the coelenteron as either pellets or loose cells. In the second phase, symbionts leave the host via the mouth. Solitary organisms such as *A. pulchella* may not accurately represent symbiont movement in colonial cnidarians. Many coral colonies possess connections between polyps allowing for symbiont migration and retention in the coenenchyme (octocorals) or the coenosarc (hexacorals).

In many bleaching studies, symbionts in the host and those expelled were examined by tissue maceration and symbiont counting (Hoegh-Guldberg et al., 1987) or by utilizing quantitative polymerase chain reaction (qPCR) (Mieog et al., 2009). These methods, however, cannot resolve the location of symbionts within the host, such as what compartment (e.g., tentacles, polyp body, coenenchyme) they are found in and

whether they are in the tissue or in the lumen of the gastrovascular system. Understanding symbiont migration is important because even in stressed colonies, retained *Symbiodinium* may continue to photosynthesize at high rates (Netherton et al., 2014). Therefore, the current study investigated the extent to which symbiont migration can mitigate the effects of bleaching in three species of alcyonacean octocorals, *Phenganax parrini*, *Sarcothelia* sp. and *Sympodium* sp.

2. Materials and Methods

2.1. Study Species

Octocorals exhibit remarkably little variation in conventional genetic markers (McFadden et al., 2011), and much of the biodiversity of small, cryptic species remains undescribed despite recent progress (e.g., Fabricius and Alderslade, 2001). To control for genotypic variability, we confined our investigations to one clone of each species for *P. parrini*, *Sarcothelia* sp., and *Sympodium* sp. Small colonies of each species were explanted onto 15 mm diameter cover glass for experimentation. Control and stock colonies were maintained in aquaria at 27°C with a natural light cycle supplemented by fluorescent and metal halide lights (12 h dark, 8 h illumination at 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 4 h illumination at 110 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) using standard culture conditions (Netherton et al., 2014).

2.2. Phylogenetics of host species and *Symbiodinium*.

2.2.1. Affinities of host taxa

Sarcothelia sp. and *Sympodium* sp. were sequenced for one nuclear (28S rDNA) and two mitochondrial (mtMutS, COI) markers to confirm their generic identity and phylogenetic affinity to other species. Sequences for *P. parrini* have been previously published (McFadden and Ofwegen, 2012). Extraction of DNA from live material, amplification of genes using PCR, and DNA sequencing followed published protocols (e.g. McFadden et al. 2011, McFadden and Ofwegen, 2012). Sequences were aligned to a reference database of other xeniid taxa using MAFFT (Katoh et al., 2005). MEGA5 (Tamura et al., 2011) was used to calculate pairwise genetic distances (Kimura 2-parameter) between taxa.

2.2.2. Affinities of *Symbiodinium*

Using the Wizard® genomic DNA purification kit (Promega, USA), DNA was extracted from two samples of one colony of *P. parrini* and two colonies each of *Sarcothelia* sp., and *Sympodium* sp. The internal transcribed spacer 2 (ITS2) region of ribosomal DNA was amplified following the protocol of LaJeunesse (2002) with the primers ITSintfor2, ITS-Reverse, and ITS2CLAMP. The PCR products were separated using denaturing gradient gel electrophoresis (DGGE) with a 45-80% denaturing gradient and run at 120 V for 13 h. In the resulting unique banding profiles, diagnostic bands were excised, the DNA was re-amplified and then sequenced using an Applied Biosystems 3730XL automated sequencer at the DNA Laboratory at Arizona State University. The resulting sequences were analyzed using Geneious (version 5.5.8) and compared to GenBank submissions.

2.3. Host colony morphology and gastrovascular architecture

The gastrovascular architectures of *P. parrini* and *Sarcothelia* sp. have been described (Parrin et al., 2010; Harmata et al., 2013; Netherton et al., 2014). Here we further quantified the colony morphology of these species while also adding a third species, *Sympodium* sp. Using a Diagnostic Instruments 3.2.0 camera (Life Technologies, Grand Island, New York) attached to a Leica MZ75 dissecting microscope (Leica, Wetzlar, Germany), both control and perturbed colonies were imaged (*P. parrini*, n = 8 control, 7 perturbed; *Sarcothelia* sp., n = 5, 3; *Sympodium* sp., n = 7, 12). From these images, the components of a colony, including dimensions of pinnules, tentacles, polyps, stolons, and in the case of *Sympodium* sp., the coenenchyme diameter, were measured using Image Pro Plus v. 6.3 (Media Cybernetics, Silver Spring, MD, USA).

2.4. *In vivo* studies in the host *Sympodium* sp.

Symbiodinium migration in *Sympodium* sp. was quantified as previously done in the other host species (Parrin et al., 2012). In a 300 ml finger bowl at 27°C, perturbed colonies (n = 7) were placed in an incubator set at 30°C and illuminated at 140 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on a 12:12 light/dark cycle. Perturbation was initiated in the morning (0900 h) and continued for 12 h. Because of thermal output from the light source, actual water temperature reached 31.5°C after 3 h and remained constant for the remainder of the experiment. For simplicity, we refer to this as treatment at 30°C, even though the temperature varied during treatment and ultimately reached a maximum of 31.5°C. Every 3 h, for 12 h, the gastrovascular lumen of the coenenchyme was imaged at 3 locations per colony with a Zeiss Axiovert 135 inverted microscope (Carl Zeiss, Jena, Germany) and a Hamamatsu Orca-100 cooled CCD camera (Hamamatsu

Photonics, Hamamatsu City, Japan). Control colonies ($n = 10$) were maintained under standard culture conditions and imaged on a similar schedule. Symbiont counts were analyzed using multivariate analysis of variance (MANOVA) of natural log-transformed data, which accounted for the repeated measures of each colony. Each time period was separately analyzed with analysis of variance (ANOVA). Analysis was done separately for moving and non-moving symbionts. The dependent variables were counts for the 5 times and the treatment (perturbed and control) was the class or source variable.

2.5. Symbiodinium counts in fixed specimens of three host species

Because polyps and tentacles continuously move and project out of the focal plane, *in vivo* techniques can only examine the symbionts in the coenenchyme. To understand concomitant changes in the polyp and tentacle compartments, specimens were fixed and sectioned according to standard methods (Parrin et al., 2012). Thick sections ($\sim 1 \mu\text{m}$) were mounted on microscope slides and examined under light microscopy. Images of symbionts in the tissue and in the lumen of the gastrovascular system were acquired using Image Pro Plus, and symbionts were counted from sections of tentacles and polyp tip (the area around the mouth), the polyp body (the remainder of the polyp, minus the tip), and the coenenchyme. The *Symbiodinium* that were counted all appeared intact and healthy (see Results), and this concords with previously obtained physiological data (Netherton et al., 2014).

Images of perturbed colonies were acquired from two different experiments. In the first, colonies from all three species were exposed to the same elevated temperature

and light treatment (incubated at 30°C illuminated at 140 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on a 12:12 light/dark cycle) for the same duration (24 h). As described in section 2.4, incubation at 30°C entails gradual warming from 27°C, ultimately stabilizing at a maximum of 31.5°C due to thermal output from the light source. In the second experiment, the perturbation was tailored to the specific symbioses. Because *P. parrini* is highly resistant to thermal perturbation, and *Sarcothelia* sp. is highly sensitive (Parrin et al., 2012; Netherton et al., 2014), while, based on preliminary data, *Sympodium* sp. is intermediate in sensitivity, colonies of the three species were perturbed differentially. Colonies of *Sarcothelia* sp. were incubated at 30°C for 16 h, while colonies of *P. parrini* were incubated at 32°C for 24 h. The same data for *Sympodium* sp. (30°C, 24 h) were used for comparison. Illumination was 140 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ on a 12:12 light/dark cycle. As with incubation at 30°C, incubation at 32°C resulted in gradual warming with temperature stabilizing at a maximum of 33.5°C due to thermal output from the light source. In both experiments, the perturbed colonies were compared to control colonies. Sample sizes for the number of colonies and compartments used in each experiment are found in the Supplementary material Tables 1A-C.

Data were analyzed separately for each of the three host compartments (tentacles and polyp tip, polyp body, and coenenchyme). Analyzing compartments separately avoids three-way interactions, which are difficult to interpret. Additionally, if no symbionts died or left the colony, dependent variables can be correlated in whole-colony analyses (e.g., if more are in the tissue, then there must necessarily be fewer in the lumen). Examining each compartment separately removes these statistical concerns. Sample sizes differed among compartments because of damage or loss

during fixation. With counts of symbionts in the tissue and in the lumen as the dependent variables and species, treatment (perturbed and control), and species-by-treatment interaction as the classification or source variables, MANOVA examined how the outcomes respond together, while ANOVA analyzed them separately. Since data were natural log-transformed, for any zero counts, a random number between 0-1 was inserted prior to standardization for area and transformation. The only zero counts were for lumens of control colonies; hence, this is a conservative approach that tends to decrease between-treatment effects (which are nevertheless substantial, see Results). In some cases, more than one polyp or stolon per colony was sectioned. Physiological studies show that within-colony variance is as great as that between colonies (Netherton et al., 2014); as expected, no significant within-colony effects were found, and the data were pooled.

An estimate of the number of symbionts lost by a colony during perturbation can be obtained by comparing the perturbed *Symbiodinium* numbers to the control numbers across all compartments. These data, however, do not take into account the different surface areas available in the three compartments nor do they discriminate between *Symbiodinium* that left the colony versus those symbionts that died. To provide an estimate of these quantities, an additional experiment was carried out (Fig. 1). Living colonies were imaged and then perturbed differentially for each species (*P. parrini*, 9 colonies perturbed at 32°C for 24 h; *Sarcothelia* sp., 12 colonies perturbed at 30°C for 16 h; *Sympodium* sp., 7 colonies perturbed at 30°C for 24 h). Individual colonies were placed in microscope chambers with 5 ml filtered (to 0.2 µm) seawater. At 9 h, colonies were rinsed with filtered seawater and the number of symbionts expelled (E) into the

seawater was counted; colonies were then returned to fresh filtered seawater. From 9 h until 16 – 24 h (depending on the species), no additional symbionts were expelled into the seawater. To calculate the initial number (I), the morphometric data (see Results) and images of these colonies were used to estimate the available symbiont-inhabitable surface area in the compartments of each colony. Each component of each compartment (e.g., pinnule, tentacle, polyp body, coenenchyme) was modeled as a cylinder. This calculated surface area and the symbiont densities (see Results) of control colonies were used to calculate the initial number (I). The symbiont densities were based on sections that were 1 micron thick, so after standardizing the diameter, the number of sections that could fit in a given cylinder was calculated. A similar procedure was used to calculate the final number (F) of symbionts retained by the colony, using images of the perturbed colonies to calculate surface area and symbiont densities of perturbed colonies (see Results). The initial number minus the final number and the number expelled provides an estimate of the number that died, i.e., $D = I - F - E$. D, F, and E can be standardized for the different species by dividing by I. These data were not analyzed statistically because the fixed total of symbionts in a colony results in an association between sub-totals, e.g., if many symbionts die then necessarily fewer are retained.

3. RESULTS

3.1. *Phylogenetics of host species and Symbiodinium.*

3.1.1. *Affinities of host taxa*

Partial sequences of mtMutS (729 nt), igr1 + COI (774 nt) and 28S rDNA (752 nt) were obtained for *Sarcothelia* sp. and *Sympodium* sp. (GenBank accessions: xxxxxxxx-xxxxxxx). The closest reference taxon to *Sarcothelia* sp. was *Sarcothelia edmondsoni*, from which it differed by a genetic distance (Kimura 2-parameter) of 0.2%; *Sympodium* sp. differed from an accession of *Sympodium caeruleum* from the Red Sea by 0.5%. Both of these genetic distances fall within the range of overlap between intraspecific and interspecific values typical of octocorals (McFadden et al., 2011; McFadden et al., 2014), thus it is not possible from these data alone to determine if the clones used in this study belong to the same species as their closest congeners.

Representatives of *Phenganax parrini*, *S. edmondsoni* and *S. caeruleum* appeared in a previously published, comprehensive phylogenetic analysis of Octocorallia using the same three genes (McFadden and Ofwegen, 2012). *Sarcothelia* and *Sympodium* both belong to the well-supported, monophyletic family Xeniidae, within which they are distinct but closely related genera (Supplementary material, Fig. 1). In contrast, *P. parrini* belongs to a clade of stoloniferan octocorals that includes representatives of four different families (Acrossotidae, Arulidae, Clavulariidae, Tubiporidae). Although the relationships among the family-level clades within Octocorallia are poorly resolved, Xeniidae and this stoloniferan clade are as different from one another phylogenetically as most of the other families of soft corals and gorgonians that fall within the large Holaxonia-Alcyoniina division of octocorals.

3.1.2. Affinities of *Symbiodinium*

The *Symbiodinium* in *P. parrini* and *Sarcothelia* sp. matched the previously described *Symbiodinium* ITS2 types D4-5, and D4-5-9, respectively (LaJeunesse et al., 2010). *Sympodium* sp. hosted a C1 variant type with sequences from an excised band from the DGGE profile matching GenBank accession no. HM130504 (Reimer et al., 2010), while another diagnostic band differed by 1bp from that sequence.

3.2. Host colony morphology and gastrovascular architecture

Colonies of *P. parrini* were runner-like with many narrow stolons and widely spaced polyps, while those of *Sarcothelia* sp. had broad stolons, and the coenenchyme of *Sympodium* sp. colonies was not divided into stolons at all. The relative proportions of surface area reflected these morphologies (Fig. 2). *P. parrini* exhibited the highest proportion of coenenchyme surface area (34%), due to its small stolons with high surface area to volume.

3.3. In vivo studies of the host *Sympodium* sp.

Elevated temperature and light levels significantly increased both the moving (Fig. 3a) and non-moving (Fig. 3b) symbiont-containing cells in the coenenchyme of colonies of *Sympodium* sp. (Supplementary material, Table 2).

3.4. *Symbiodinium* counts in fixed specimens of the three host species

When colonies from all three species were exposed to the same perturbation of 24 h of incubation at 30°C, there were strong treatment and species effects, and species-by-treatment interactions (Fig. 4, Supplementary material, Table 3A-C). *Sarcothelia* sp. colonies were the most sensitive to the perturbation and those of *P. parrini* were the

least sensitive. A clearer picture of the process of symbiont migration was obtained by differentially perturbing colonies of the three species (*P. parrini*, incubation at 32°C for 24 h, *Sarcothelia* sp., 30°C for 16 h, and *Sympodium* sp., 30°C for 24 h). When colonies were differentially perturbed, strong treatment effects remained, but species effects and species-by-treatment interactions were relatively weak or non-existent (Fig. 4, Supplementary material, Table 4A-C). Overall, when colonies were perturbed, counts of symbionts in the tissue significantly declined, while *Symbiodinium* counts in the lumen of the gastrovascular system significantly increased (Fig. 4).

During the thermal and light stress, the total number of symbionts expelled (i.e., exiting the colony) per mm² colony area (mean ± SE) differed significantly among species ($F_{2,22} = 13.6$, $P < 0.001$): *P. parrini*, 0.37 ± 0.33 ; *Sarcothelia* sp., 18 ± 6.1 ; *Sympodium* sp., 115.6 ± 37.7 . Orthogonal contrasts showed that *P. parrini* differed from *Sarcothelia* sp. ($F_{1,22} = 9.7$, $P < 0.01$), and *Sarcothelia* sp. differed from *Sympodium* sp. ($F_{1,22} = 8$, $P < 0.01$). Nevertheless, in each case the number of *Symbiodinium* expelled represented only a small proportion of the initial numbers. For the 9 colonies of *P. parrini*, with an average surface area of 123.6 mm², an average of 2.07×10^5 *Symbiodinium* (15.4%) remained, 1.14×10^6 (84.6%) died, and 25.3 (≈0%) were expelled. For the 12 colonies of *Sarcothelia* sp., with an average surface area of 208 mm², 5.91×10^5 (99.9%) remained, 0 (0%) died, and 2893.5 (0.005%) were expelled. For 7 colonies of *Sympodium* sp., with an average surface area of 178.9 mm², 4.15×10^5 (45.4%) remained, 5×10^5 (52.7%) died, and 1.6×10^4 (1.9%) were expelled. While the remaining *Symbiodinium* have shifted their location for the most part, they nevertheless appear healthy (Fig. 5). This is consistent with previous studies of oxygen

metabolism that show high rates of photosynthesis in similarly stressed colonies of *P. parrini* and *Sarcothelia* sp. (Netherton et al., 2014).

4. DISCUSSION

As with other experimental studies of bleaching (Philipp and Fabricius, 2003; Abrego et al., 2008; Hill and Ralph, 2008; Buxton et al., 2012; Agostini et al., 2013; Bhagooli, 2013; Hawkins et al., 2013; Paxton et al., 2013; Sammarco and Strychar, 2013; Tolleter et al., 2013; Fujise et al., 2014; Lehnert et al., 2014; Lutz et al., 2014; Tanaka et al., 2014), lower levels of illumination and lower temperatures were used here rather than the maximal values recorded during bleaching events in the field (Supplementary material, Table 5). Extreme conditions typically result in high levels of mortality, which is not particularly illuminating experimentally. Indeed, a recent field study (Graham et al., 2015) suggests that the factors which best predict the recovery of a reef—structural complexity and water depth—may provide some relief from maximum temperatures and illumination. Structural complexity may provide shade while deeper water allows both cooler temperatures and lower illumination. Thus while experimental studies of bleaching may not use extreme conditions, they may still provide insight into the factors that mitigate bleaching and allow resilience and recovery of reef ecosystems in the field.

In the alcyonacean octocorals examined, *Sarcothelia* sp. and *Sympodium* sp. are two closely related xeniids and *Phenganax parrini* is more distantly related. *Sarcothelia* sp. and *P. parrini* hosted *Symbiodinium* types within clade D, D4-5-9 and D4-5, respectively, while *Sympodium* sp. hosted a type within clade C, a variant of C1 (the affinities of *P. parrini* symbionts was previously misstated as clade B, Netherton et al.,

2014). When perturbed by temperature and light, colonies of *Sarcothelia* sp. and *P. parrini* exhibit an accumulation of symbiont-containing cells in the coenenchyme (Parrin et al., 2012). In the current study, we found the same pattern in living colonies of *Sympodium* sp., although this species hosts a different *Symbiodinium* type (a C1 variant versus D4-5 or D4-5-9). Sections of fixed colonies of all three species showed a general exodus of symbionts from the host tissue and an accumulation in the lumen of the gastrovascular system. Using transmission electron microscopy, previous studies have shown that symbionts in the lumen are contained in host cells or at least surrounded by a host membrane (Parrin et al., 2012; Harmata et al., 2013). Our techniques enabled us to document this symbiont shift, a spatial shift that cannot be detected by techniques involving tissue maceration and counting or qPCR.

The similar sequence of events in these three alcyonaceans was more pronounced when the intensity and duration of the perturbation were adjusted to reflect the different thermal sensitivities of each species, with a shorter perturbation duration for *Sarcothelia* sp. (incubated at 30 °C for 16 h, with a maximum temperature of 31.5 °C), greater intensity for *P. parrini* (incubated at 32 °C for 24 h, with a maximum temperature of 33.5 °C) and intermediate conditions for *Sympodium* sp. (incubated at 30 °C for 24 h, with a maximum temperature of 31.5 °C). Adjusting the stress duration and magnitude generated a more homogeneous stress response among the three species (Fig. 4). Perturbation of these octocorals consistently resulted in large-scale movement of symbionts from the host extremities, the polyps, to within the host colony, and from the tissue to the lumen of the gastrovascular system. The three species examined provide three-fold replication for the phenomenon of symbiont migration and suggest that it is

widespread in the octocorals. Further studies should focus on broadening the existing phylogenetic sample and on greater replication of results from the sampled taxa.

Symbiont movement may be a protective mechanism, akin to moving the symbionts from the 'line of fire' to the bunker, and may be elicited by either the host or the symbionts. The *Symbiodinium* movement may entail protection from detrimental effects of elevated temperatures such as increased production of toxic oxygen radicals by damaged photosystem II reaction centers (Lesser et al., 1990) and may explain why following considerable symbiont movement within the colonies, the three octocoral species retained high densities of symbionts in the coenenchyme. Such symbionts could subsequently be broken down, providing the host with a nutritional boost, and/or could multiply and repopulate the colony. This reservoir of symbionts in the coenenchyme may be a factor in the resilience that octocorals display in response to environmental stress (Ruzicka et al., 2013).

Although all three octocoral species exposed to a temperature perturbation exhibited the same within-colony *Symbiodinium* migration (Fig. 4), the three alcyonaceans differed in their sensitivity to elevated temperature. Colonies of *P. parrini* were virtually unaffected by incubation at 30°C. *Sarcothelia* sp. colonies actually appeared to be recovering after 24 h of perturbation since large numbers of symbionts had returned to the tissue of the coenenchyme of these colonies after 24 h (Fig. 4e) compared to 16 h (Fig. 4f). Colonies of *Sympodium* sp. exhibited an intermediate response.

The variation in thermal sensitivity could be due to the host (Loya et al., 2001; Baird et al., 2009), the symbiont (Baker, 2003), or the host/symbiont genotypic combination

(Goulet et al., 2005; Sampayo et al., 2008). Symbiont migration was previously detected only in hosts (*P. parrini* and *Sarcothelia* sp.) with *Symbiodinium* type D4-5 and D4-5-9, respectively (Parrin et al., 2012; Netherton et al., 2014). *Symbiodinium* D4-5 has previously been reported from the octocoral *Tubipora musica*, while D4-5-9 has also been documented in the alcyonacean *Cladiella* sp. (LaJeunesse et al., 2010). *Symbiodinium* D4-5 and D4-5-9 fall within clade D that includes types that are referred to as 'heat tolerant symbionts' (Sammarco and Strychar, 2013). Our study suggests that one cannot generalize *Symbiodinium* thermal sensitivity (Tchernov et al., 2004), and that the host/symbiont combination plays a key role in the thermal response of a given symbiosis (Goulet et al., 2005). For example, Sammarco and Strychar (2013) found that a xeniid (*Xenia elongata*) containing clade C symbionts, was more sensitive to perturbation than other octocorals. In our study, a xeniid species (*Sarcothelia* sp.), which contained D4-5-9 symbionts, appeared to be more sensitive than one (*Sympodium* sp.) that hosted a *Symbiodinium* type C1 variant.

Nevertheless, both xeniids reacted to perturbation at temperatures lower than those required to elicit the same response in colonies of *P. parrini*, which contained D4-5 *Symbiodinium*. After 24 h of incubation at 30°C, the perturbed colonies of *P. parrini* did not significantly differ from the control colonies (Fig. 4). On the other hand, the proportion of symbionts remaining in colonies of *P. parrini* after perturbation was much lower than in the xeniid species. Similarly, a greater proportion of symbionts died in colonies of *P. parrini*, although these colonies also exhibited higher initial numbers of symbionts (Fig. 4, see also Alderslade and McFadden, 2011).

Other factors beyond symbiont type may play a role in the stress response. *P. parrini* colonies have narrow, “runner-like” stolons with large surface areas, but small volumes of the gastrovascular lumen. By comparison, colonies of both *Sarcothelia* sp. and *Sympodium* sp. have broad areas of encrusting coenenchyme. Colonies of *P. parrini* may lack the capacity to house a large proportion of the symbiont population in the lumen of the gastrovascular system. Actinarian anemones, which entirely lack a coenenchyme, may represent the extreme in this condition.

5. Conclusion

The current analysis shows that migration of symbionts mitigates the effects of bleaching in three species of octocorals. Migration is protective, but it is not a panacea; survival of symbionts varies across host/*Symbiodinium* combinations. Nevertheless, a high density of symbionts is retained in the coenenchyme of each host. Since symbiont migration mitigates the effects of bleaching, further studies are urgently needed to examine this process in other host/*Symbiodinium* combinations, including those that replicate the ones used here as well as novel combinations.

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Appendix A. Supplementary material

Supplementary material to this article can be found at:

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Figure legends

Fig. 1. *Methods used to estimate initial number of Symbiodinium (I), number of symbionts expelled (E), and final number of symbionts (F) in perturbed colonies.* Living colonies were placed in microscope chambers with 5 ml filtered (to 0.2 μ m) seawater. After thermal and photo-oxidative stress, colonies were rinsed with filtered seawater and the number of symbionts expelled (E) into the seawater was counted. To calculate the initial number (I), the morphometric data (see Fig. 2) and images of these colonies were used to estimate the available symbiont-inhabitable surface area in the compartments of each colony. This calculated surface area and the previously collected symbiont densities of control colonies (see Fig. 4) were used to calculate the initial number (I). A similar procedure was used to calculate the final number (F) of symbionts retained by the colony, using images of the perturbed colonies to calculate surface area and symbiont densities of perturbed colonies. The initial number minus the final number and the number expelled provides an estimate of the number that died, i.e., $D = I - F - E$.

Fig. 2. *Characteristics and relationships of P. parrini, Sarcothelia sp., and Sympodium sp.* Colony morphology was characterized using a series of measures (e.g., polyp height and width, tentacle length and width). Calculated surface areas of the endoderm for each component (tentacles, polyp body, and coenenchyme), and the respective percentage of each component, are depicted from representative colonies of each species (*P. parrini*, n = 8; *Sarcothelia* sp., n = 5; *Sympodium* sp., n = 7). A simplified phylogenetic tree illustrates that *Sarcothelia* sp. and *Sympodium* sp. are sister taxa in the family Xeniidae, while *P. parrini* is a more distantly related stoloniferan (Supplementary material, Fig. 1). Conversely, *P. parrini* and *Sarcothelia* sp. host *Symbiodinium* type D4-5, and D4-5-9 while *Sympodium* sp. have a variant of type C1.

Fig. 3. *In vivo* counts of *Symbiodinium* in *Sympodium* sp. over a 12-hour period.

Symbiodinium counts (mean $\pm$ SE) in moving (a) and non-moving (b) symbiont-containing cells in the coenenchyme of control and perturbed *Sympodium* colonies.

Fig. 4. *Symbiodinium* density from fixed specimens of *P. parrini*, *Sarcothelia* sp., and

Sympodium sp. *Symbiodinium* mean $\pm$ SE counts in the tissue and lumen are shown

for each of the three compartments: tentacles and polyp tips (T), polyp body (P), and

coenenchyme (C). Top row (a, d, g) present *Symbiodinium* density from control

colonies; middle row (b, e, h) are from colonies incubated at the same temperature

(30°C) and duration (24 h). Bottom row (c, f, i) depicts *Symbiodinium* density from

colonies exposed to perturbations adjusted to match the sensitivity of the different

species (*P. parrini*, incubated at 32°C for 24 h, *Sarcothelia* sp., incubated at 30°C for 16

h, while for *Sympodium* sp., the data from the middle row, incubated at 30°C for 24 h,

are presented again).

Fig. 5. *Micrographs of Symbiodinium* in control and perturbed colonies. Control

colonies (A - D) are compared to perturbed colonies (E - H) of *Sarcothelia* sp. (A, B, E,

F) and *Sympodium* sp. (C, D, G, H). For each species, tentacles (A, E, C, G) and

coenenchyme (B, F, D, H) are shown. After 24 h of incubation at 30°C, colonies show

fewer symbionts in the tissue and more in the lumen (compare to micrographs of *P.*

parrini, Parrin et al., 2012). Nevertheless, all symbionts appear healthy. Dashed

arrows indicate representative symbionts in the lumen, while solid arrows indicate

representative symbionts in the tissue. Scale, 40 μ m.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5