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The Adaptive Bleaching Hypothesis: Experimental Tests of Critical Assumptions

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Abstract. Coral bleaching, the loss of color due to loss of symbiotic zooxanthellae or their pigment, appears to be increasing in intensity and geographic extent, perhaps related to increasing sea surface temperatures. The adaptive bleaching hypothesis (ABH) posits that when environmental circumstances change, the loss of one or more kinds of zooxanthellae is rapidly, sometimes unnoticeably, followed by formation of a new symbiotic consortium with different zooxanthellae that are more suited to the new conditions in the host's habitat. Fundamental assumptions of the ABH include (1) different types of zooxanthellae respond differently to environmental conditions, specifically temperature, and (2) bleached adults can secondarily acquire zooxanthellae from the environment. We present simple tests of these assumptions and show that (1) genetically different strains of zooxanthellae exhibit different responses to elevated temperature, (2) bleached adult hosts can acquire algal symbionts with an apparently dose-dependent relationship between the concentration of zooxanthellae and the rate of establishment of the symbiosis, (3) and finally, bleached adult hosts can acquire symbionts from the water column.

Introduction

Coral bleaching has increased in frequency, intensity, and geographical extent over the last two decades (Huppert and Stone, 1998). Concern is being expressed both by reef scientists (Brown, 1990, 1997; Goreau and Hayes, 1994) and the general public (Brown and Ogden, 1993; Wilson, 1999) about the future of the planet's reefs. Bleaching is a general term describing the loss of color in corals and other symbiotic reef invertebrates, usually due to the loss of their intracellular dinoflagellate symbionts, colloquially termed zooxanthellae. A reduction in the density or pigment content of symbiotic algae can be elicited by a variety of environmental factors including low (Goreau, 1964) and high (Nakano *et al.*, 1997) salinity, low and high levels of illumination—especially ultraviolet radiation (Banaszak and Trench, 1995; Lesser and Shick, 1989), disease (Kushmaro *et al.*, 1996; Rosenberg and Loya, 1999), and high (Iglesias-Prieto *et al.*, 1992; Warner *et al.*, 1996) and low (Steen and Muscatine, 1987; Kobluk and Lysenko, 1994) temperatures, as well as combinations of these factors. Although any of these may act on a local scale, widespread bleaching has generally been linked to high water temperatures at larger geographic scales, particularly those associated with El Niño-Southern Oscillation (ENSO) events (Glynn, 1984, 1991; Coffroth *et al.*, 1988). Most recently, the high sea surface temperatures (SSTs) of the summer of 1998 resulted in massive mortality of reefs in Okinawa, Tahiti, and Belau (ISRS, 1998; Hoegh-Guldberg, 1999; Tsuchiya, 1999). Such events may be becoming more frequent, and it has been suggested that they will become even more common if global warming persists and summer SSTs increase (Glynn, 1991). The loss, in association with such events, of zooxanthellae from a host may be partial or essentially complete. In the latter case recovery frequently does not occur, and the subsequent death of large numbers of symbiotic reef invertebrates can drastically alter the composition of the reef community (Goreau, 1992). Thus, the potential for increased incidence of bleaching may have negative effects on these ecosystems. Here we present results from experiments

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relating zooxanthellar diversity to potential recovery from loss of symbionts.

Our original view of the symbiotic system of reef invertebrates and their algal symbionts was based on the idea that, at least in cnidarians, there was a single algal partner, *Symbiodinium microadriaticum* (Freudenthal) (Taylor, 1974). The elucidation of the complexities of coral and zooxanthellar systematics and taxonomy has made the picture more complex (Rowan and Powers 1991a; Knowlton *et al.,* 1992; Rowan, 1998). About a dozen named dinoflagellate taxa are currently known to associate with temperate and tropical invertebrates (Blank and Trench, 1985; Banaszak *et al.,* 1993; McNally *et al.,* 1994), but the diversity of the algae is probably much higher than this (Rowan, 1998; Darius *et al.,* 1998; Carlos *et al.,* 1999; Baillie *et al.,* 2000). A broad classification of zooxanthellae, based on sequence differences in the small subunit of the ribosomal nuclear genes (ssrDNA) has been proposed (Rowan and Powers, 1991a). This region of the genome contains a range of domains that have evolved at different rates; these domains are useful in characterizing phylogenetic relationships and have been used to characterize populations of zooxanthellae (Rowan, 1991; Rowan and Powers, 1991a, b; Sadler *et al.,* 1992; McNally *et al.,* 1994; Zardoya *et al.,* 1995; Carlos *et al.,* 1999). Analysis of restriction fragment length polymorphism (RFLP) of the ssrDNA has shown that zooxanthellae can be divided into at least four clades (Rowan and Powers, 1991a, b; Rowan, 1998), each probably including many species (Rowan, 1998). In addition, we now know that although some invertebrates may host a single algal clade, others can harbor at least three distinctly different types of zooxanthellae (Rowan and Knowlton, 1995). When more than one clade occurs, the balance among the population densities and the location of the clades within a host coral colony can be altered by changes in microscale environmental conditions (Rowan and Powers, 1991a; Rowan and Knowlton, 1995; Rowan *et al.,* 1997; Buddemeier, 1997). Furthermore, evidence suggests that the consortium may readily alter membership (Davy *et al.,* 1997; Hill and Wilcox, 1998; Belda-Baillie *et al.,* 1999; Bates, 2000). Thus, this symbiosis, which—at least in scleractinians—has persisted since the late Triassic (Stanley and Swart, 1995), appears to be both complex and mutable.

This combination of persistence over geological time scales (Kinzie and Buddemeier, 1996; Buddemeier *et al.,* 1997) and the apparently multifaceted array of symbiotic partnerships that may occur in some hosts (Rowan and Powers, 1991a; Baker and Rowan, 1997; Rowan, 1998) has given rise to the "adaptive bleaching hypothesis" (ABH) (Buddemeier and Fautin, 1993). This hypothesis posits that different algal partners within hosts shift in space—over a range of scales—and time, resulting in a symbiotic complex with many potential combinations, some being more functional under certain environmental conditions than others. According to the ABH, as environmental conditions change, the makeup of the symbiotic unit responds, tracking environmental changes by shuffling the algal membership of the symbiotic consortium. This hypothesis, presented 7 years ago, was explicitly stated so as to be experimentally testable, but despite substantial work both in the field and in laboratories around the world on causes and mechanisms of coral bleaching, surprisingly little effort has been devoted to such tests. Here we present the results of experimental tests of some fundamental assumptions underlying the ABH.

One assumption of the ABH is that the various algal types have physiological differences that are related to the etiology of bleaching. High temperatures are known to negatively affect zooxanthellae through degradation of photosystem II (Iglesias-Prieto *et al.,* 1992), but there has been little study of temperature effects on different zooxanthellar types. It has been known for some time that different zooxanthellae differ in their ability to grow in different hosts (Schoenberg and Trench, 1980) and to support growth and reproduction of the animal hosts (Kinzie and Chee, 1979; Fitt, 1985). Additionally, algae differ in their photosynthetic abilities (Chang *et al.,* 1983) and in their production of mycosporine-like amino acids in response to ultraviolet irradiance (Banaszak *et al.,* 2000).

A second assumption of the ABH is that bleached adult hosts can obtain new symbionts from the reef habitat and reestablish a symbiosis; that is, secondary acquisition of algae is possible. Many studies have demonstrated initial acquisition of newly settled aposymbiotic juveniles (Kinzie, 1974; Fitt and Trench, 1981; Muller-Parker and D'Elia, 1996; Benayahu *et al.,* 1989) or freely swimming planulae (Schwarz *et al.,* 1999). Numerous symbiotic reef invertebrates utilize horizontal transmission (i.e., each generation obtains its algal symbionts from the environment rather than maternally), showing that there must be substantial populations of potential zooxanthellae partners in reef waters (Goulet and Coffroth, 1997). These potential symbiotic partners exist outside of hosts and may occur in fish feces (Muller-Parker, 1984), be released from corals (Stimson and Kinzie, 1991; Hoegh-Guldberg *et al.,* 1987), or occur free living (Carlos *et al.,* 1999). Direct study of free-living zooxanthellae on reefs is difficult because we currently have very little data on their density, spatial distribution, or temporal behavior. We wished to determine whether bleached hosts could acquire symbiotic algae from populations of zooxanthellae in more natural situations. Although bleached adults have been shown to take up zooxanthellae under laboratory conditions, these experiments either used extremely high concentrations of zooxanthellae or the algae were injected into the coelenteron. The ability of adult cnidarians, once they have become bleached, to re-acquire symbionts from populations at concentrations likely to occur *in situ* is less studied (Franzisket, 1970).

Because understanding of the symbiotic unit is based on

knowledge about the individual partners (Iglesias-Prieto *et al.,* 1992), we examined the algal partner under culture conditions to test the first assumption of the ABH. Although certain physiological traits of zooxanthellae can change in culture, (Stochaj and Grossman, 1997), use of well-characterized and controlled partners in isolation provides strong experimental control. In testing the other assumptions we used bleached hosts and naturally occurring populations of zooxanthellae.

Materials and Methods

Algal cultures

We refer to the zooxanthellae used in our experiments as "isolates," referencing the host from which they were isolated rather than a dinoflagellate species because the systematics and nomenclature of the entire group are in formative stages (Lee *et al.,* 1995). The isolates used are from a range of hosts (Table 1) and have been in culture for 4 to 20 years. Each culture was initiated with zooxanthellae from a single host. Because some hosts may harbor more than a single zooxanthellar genotype (Rowan and Powers, 1991a; Rowan and Knowlton, 1995; Rowan *et al.,* 1997; Buddemeier, 1997; Goulet and Coffroth, 1997; Carlos *et al.,* 2000), the isolates could have been initiated from more than a single genetic strain of zooxanthellae. In addition, the culturing process itself may exert strong selection on which genotypes thrive. To determine the number and types of clades present in the isolates as well as those that colonized bleached hosts, zooxanthellar genotypes were characterized using RFLP analysis of zooxanthellar ssrDNA (Rowan and Powers, 1991a). Cladal membership of the stock cultures was determined before the start of the experiment and one month after the experiments were terminated. The experimental cultures were analyzed at the end of the experiment.

Table 1

Isolates used in temperature tolerance experiments

Isolate designation	Clade	Host	Source
Cassiopea xamachana	A	Scyphozoan	Caribbean (*Symbiodinium microadriaticum*)
Montipora verrucosa	C	Scleractinian	Hawaii (*Symbiodinium kawagutii*)
Pocillopora damicornis	B	Scleractinian	Hawaii
Cassiopea KB8	A	Scyphozoan	Hawaii
Zoanthus sociatus	A	Zoanthid	Caribbean (*Symbiodinium pilosum*)
Tridacna gigas	A	Giant clam	Central Pacific
Aiptasia pulchella	B	Anemone	Hawaii (*Symbiodinium pulchrorum*)

Specific names in parentheses are species names given to zooxanthellae from these species (Blank and Trench, 1985, and Banaszak *et al.,* 1993).

The resultant RFLP groups were classified by comparison with known zooxanthellae (*i.e., Symbiodinium* clades A, B, and C, Rowan and Powers, 1991b). Each stock culture contained a single algal clade (*sensu* Rowan and Powers, 1991a; Table 1). In the analysis, algal DNA extraction followed the protocols of Coffroth *et al.* (1992) and Goulet and Coffroth (1997), and RFLP analysis followed the protocols of Rowan and Powers (1991b).

Stock cultures were grown in f/2 medium (Guillard and Ryther, 1962) held at 25°C under 160 μE m^{-2} s^{-1} (160 μmol m^{-2} s^{-1}) on a 12:12 light-dark cycle. Cultures were transferred to new medium monthly. Periodically, cultures were treated with antibiotics (Polne-Fuller, 1991) but were not axenic. As discussed below, even though some isolates have been in culture for more than two decades, they retain their ability to infect their normal host.

Temperature sensitivity of different algal isolates

Aliquots from each cultured isolate were diluted to 1×10^4 cells ml^{-1} with sterile f/2 medium and put into twelve 150-ml culture tubes. Four tubes for each isolate were randomly assigned to one of the three temperature treatments. Zooxanthellae were exposed to temperatures of 27, 29, and 31°C over a 2-week period during which growth rates of the cultures were determined fluorometrically. Temperature treatments consisted of 28 tubes (4 per isolate and 7 isolates) in a test tube rack immersed in a 10-l water bath with an aquarium heater; an aquarium pump kept the water mixed. The three water baths were placed by an east-facing window that provided natural light. Solar radiation within the culture tubes (measured with a BioSpherical quantum meter, model QS100 with a 4π sensor inserted into a tube) ranged from 800 μE m^{-2} s^{-1} in the morning to 100 μE m^{-2} s^{-1} (100 μmol m^{-2} s^{-1}) in the afternoon. Variability within a tube, from the top of the culture medium to the bottom of the tube, was about 30%. Each day, when tubes were read, the positions of the three water baths were rotated, and the positions of the tubes in the racks within each bath were shuffled. Aside from the temperature effects, growth was probably light-limited (unpubl. data) because we wished to reduce the potential for interaction between radiation stress and the experimental treatment. Growth was followed using *in vivo* fluorescence with a Turner Designs model 10 fluorometer. Fluorescence was measured daily starting at 0830. Racks were held in the dark at the experimental temperature for 30 min before fluorescence was read. Just before the reading, cultures in the tubes were dispersed with a vortex mixer to ensure even suspensions. Readings were taken from day 0, when inoculation took place, until day 13, when several of the cultures became difficult to disperse. At the termination of the experiment, 1-ml samples were preserved in Lugol's solution for cell enumeration with a Spiers-Levi eosinophil hemacytometer. Growth was measured as daily

increase in fluorescence. We used growth rate as a measure of each strain's ability to tolerate high temperatures because faster growing zooxanthellae would directly correlate with a coral's recovery from bleaching. Population growth rate is generally considered to be a primary measure of fitness (Cole, 1954). At the end of the experiment, the cell counts were used to convert fluorescence to cell density. Specific growth rate (cell cell^{-1} d^{-1}) was determined from regressions of cell density over time. Growth rate was determined as the slope of the natural log of fluorescence over time. Subsequent analysis indicated that growth was logarithmic over this time period.

Ability of bleached hosts to acquire new symbionts

To demonstrate that secondary acquisition of algal symbionts by bleached adults can occur at low concentrations of zooxanthellae, we used the anemone *Aiptasia pulchella* as a model organism. This symbiotic anthozoan can be bleached completely and maintained indefinitely in this condition, making it particularly useful for reinfection studies. *Aiptasia* polyps were held individually in 50-ml beakers with 0.45-μm-filtered seawater (FSW) in the dark and fed *Artemia* nauplii once a week. After at least one year in the dark, bleached *Aiptasia* were moved to the light (natural sunlight through north-facing windows), and held in 0.22-μm FSW with weekly feeding of *Artemia* nauplii. No anemones were used in experiments unless they remained aposymbiotic in the light for at least 5 months. We have kept bleached *Aiptasia* for more than a year with no signs of zooxanthellae.

Bleached *Aiptasia* polyps were exposed to algal symbionts isolated from *A. pulchella* at concentrations that ranged from 10^5 to 10^0 cells ml^{-1}. Stock culture of the *A. pulchella* isolate (in culture for >20 years) was diluted to 1×10^5 cells ml^{-1} (verified by direct hemacytometer counts), then diluted to the different experimental concentrations with 0.22-μm FSW. The seawater in the beakers with the *Aiptasia* was replaced with the algal suspensions ($n = 3$ per treatment). Anemones remained in the suspension for 48 h, after which the suspension was removed and replaced with 0.22-μm FSW. Anemones were maintained in 0.22-μm FSW with the same light and feeding conditions, and were checked daily. We measured the time required for the zooxanthellae to proliferate to the point that the first brown color was visible. We termed this "visible infection." None of the controls ever showed signs of establishment of symbiosis with algae.

Existence of suitable strains of zooxanthellae in natural waters

To test the possibility that bleached adult hosts can acquire algal symbionts from natural waters, we exposed bleached *Aiptasia* to unfiltered seawater in three situations: on a seawater table in the laboratory, in the lagoon, and on the coral reef (Table 2). The anemones used in these treatments were randomly selected from the set of bleached *Aiptasia* described above. In the laboratory, 9 anemones were placed in individual uncovered 50-ml beakers that were then immersed in running unfiltered seawater (from the laboratory's system) on a water table that had a population of naturally occurring symbiotic *Aiptasia* polyps. For the field exposures, *Aiptasia* were placed in plastic tubes (*ca.* 50 ml) with coarse (5-mm-mesh) netting over the ends. A tube with 3 anemones was suspended at a depth of 0.5 m in the lagoon at a site that had moderate densities of symbiotic *Aiptasia.* A tube with 10 anemones was suspended 1 m above the reef at a site where no *Aiptasia* were visible. The anemones were held in these treatment conditions for 1 to 7 days, then returned to 0.22-μm FSW and kept in the conditions described in the previous section.

Table 2

Exposure and infection of bleached Aiptasia pulchella *polyps*

Treatment	Number of *Aiptasia*	Number reinfected	Time to infection (days) Mean	Time to infection (days) Range
Water table	9	6	41.2	5–76
Lagoon	3	3	54	41–61
Coral reef	10	3	28	18–46

Results

Temperature sensitivity of different algal isolates

All seven isolates showed positive growth at all three temperatures, even at 31°C, which is often associated with bleaching events (Hoegh-Guldberg and Salvat, 1995; Davies *et al.,* 1997; Drollet *et al.,* 1995) (Fig. 1). Although all the zooxanthellae were able to grow at these three temperatures, there were significant differences among the isolates (Table 3). Clade B zooxanthellae showed decreasing growth at higher temperatures, whereas the single clade C isolate showed increasing growth rates at higher temperatures. Among the clade A isolates tested, the response to temperature was variable. Clade A isolates included the fastest growing isolate, which showed increasing growth rates with increasing temperatures, and the slowest growing isolate, which showed decreased growth rates at the higher temperatures. RFLP analysis of ssrDNA of the stock cultures both before and after the experiments verified that each stock culture contained a single algal clade. RFLP analysis of the experimental cultures at the termination of the experiment revealed that several of the treatments showed low levels of contamination with a second clade. Because the cladal type of the stock cultures did not change during this time and the growth rates of replicate isolates were similar, we conclude

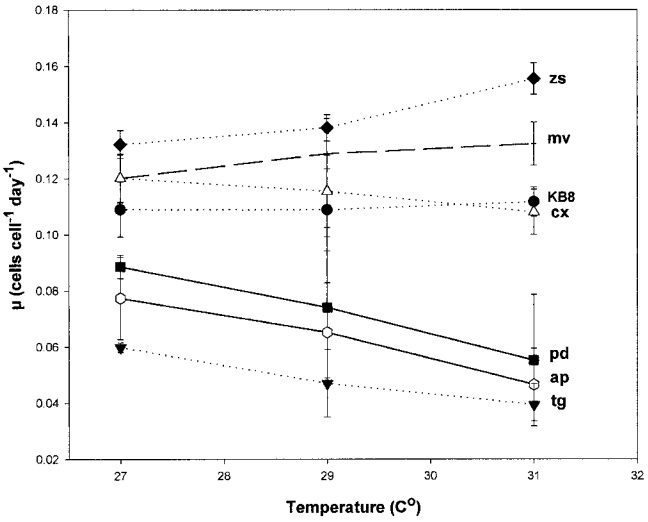

Figure 1. Growth rates of zooxanthellae isolated from 7 hosts at three temperatures. Points represent means; error bars represent ±1 SD ($n = 4$). Data were obtained as increase in fluorescence over the 13-day growth period. Growth was logarithmic during this time. Fluorescence units were converted to cell density at the end of the growth period. Host: zs—*Zoanthus sociatus,* mv—*Montipora verrucosa,* cx—*Cassiopea xamachana,* KB8—*Cassiopea* KB8, pd—*Pocillopora damicornis,* ap—*Aiptasia pulchella,* tg—*Tridacna gigas.* The dotted lines represent algae in clade A, the solid lines algae in clade B, and the dashed line the alga in strain C.

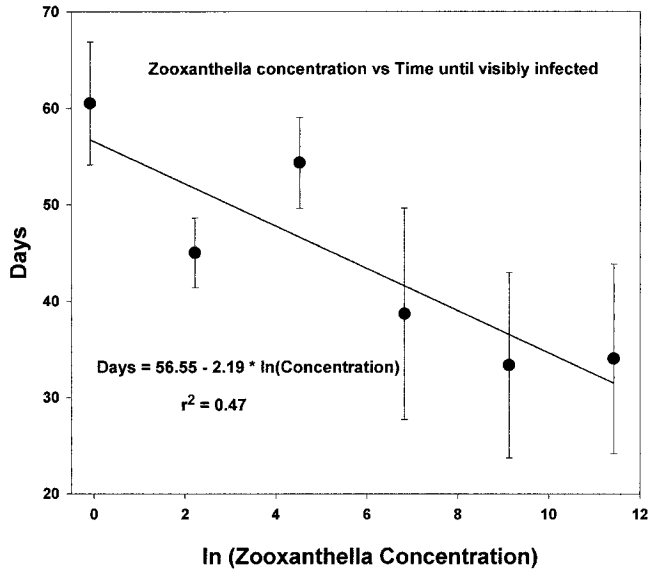

Figure 2. Time to infection of bleached *Aiptasia pulchella* polyps *vs.* concentration of zooxanthellae isolated from *Aiptasia pulchella.*

that the contamination was introduced at the termination of the experiments when samples were being processed for chlorophyll *a* measurements, cell counts, and photosynthetic rate studies, which was also when samples were taken for cladal identification of the experimental cultures.

Ability of bleached hosts to acquire new symbionts at low zooxanthellae concentrations

Symbiosis was successfully established at concentrations as low as 10 cells ml^{-1} (Fig. 2). Anemones exposed to higher concentrations became visibly infected sooner than those exposed to lower concentrations, suggesting that repopulation was due to multiple infection events during the 48-h exposure period and that the time to visible infection is dose-dependent. Although it is not known whether recovery following natural bleaching events comes from a residual population within the host or from free-living populations in reef waters, the minimum effective dose we demonstrated of 10 cells ml^{-1} implies that the concentrations of zooxanthellae repopulating bleached hosts could be almost undetectably low, yet still be sufficient for the bleached host to recover.

Table 3

Analysis of variance of growth rates by temperature for the seven zooxanthella isolates

Source	DF	SS	MS	*F*	*P*
Isolate	6	0.089	0.015	101.03	<0.0001
Temperature	2	0.001	0.000	3.32	0.043
Isolate × Temperature	12	0.006	0.000	3.31	0.0009

Existence of infective forms of zooxanthellae in natural waters

Two-thirds of the bleached anemones exposed to unfiltered seawater in the water table became infected, as did one-third of the bleached anemones suspended above the reef and all of the bleached anemones suspended in the lagoon (Table 2). All *Aiptasia* that became infected when exposed to natural seawater were found to harbor clade B zooxanthellae, which is the zooxanthellar clade normally found in this anemone. We emphasize that in both experiments, in which *Aiptasia* did regain zooxanthellae, none of the control anemones showed any sign of infection at the termination of the experiments, and the same polyps remain aposymbiotic 10 months later. There was no correlation between the time in the treatment and the time to infection.

Discussion

Temperature sensitivity of different algal isolates

The algal isolates we studied responded differently to the different temperatures. Some showed lowered growth rate with increasing temperature, some showed the same growth rate at all three temperatures, and two showed higher growth rates with increasing temperatures. Given that temperatures of 30°C, even when applied for short periods (2 days), can

cause breakdown in the photosynthetic machinery of some zooxanthellae (Iglesias-Prieto *et al.,* 1992; Iglesias-Prieto, 1997; Warner *et al.,* 1996, 1999), the ability of these isolates to grow at 31°C, and for some to apparently show their highest growth rate at this temperature, is surprising. Also unexpected is the apparent lack of relationship between the habitat of the host and the temperature tolerance of the zooxanthellae. For example, algae from *Montipora verrucosa,* isolated from a coral from Hawaii, from relatively cool waters, showed increased growth rates at higher temperatures, whereas an isolate from the giant clam *Tridacna gigas,* typically found in shallow, more equatorial waters, was one of the most sensitive to high temperatures.

When the data are examined in terms of zooxanthellar clade, several trends are apparent. First, clade A zooxanthellae, for which we tested the largest number of isolates, had the widest range of growth rates. This is consistent with previous characterizations of clade A zooxanthellae as "weedy" (Rowan, 1998). Some clade A zooxanthellae showed increased growth with increasing temperatures (*e.g., Zoanthus sociatus*), others showed decreasing growth (*Tridacna gigas*), and isolates from the two species of *Cassiopea* showed little change in growth rate over the experimental temperature range. Clade B zooxanthellae, for which we tested only two isolates, showed less variability in response to temperature, with both isolates growing slower at higher temperatures. Growth rates in the single clade C isolate tested showed a slightly higher growth rate at higher temperatures and was the second fastest growing of all the isolates we tested. The results from clade A zooxanthellae demonstrate that, at least in some algal groups, the variability in physiological response within a clade may be as great as or greater than between clades. Further, these data suggest that the potential for selection for the most ecologically suitable taxon or genotype could occur within a clade. If this proves to be generally true, it might be impossible to detect ecologically significant shifts in the algal complement of an animal host by using the identification techniques we employed in this study.

Ability of bleached hosts to reestablish symbiosis at low zooxanthellae concentrations

Even though the isolate used in these experiments has been in culture for more than two decades, it retained the ability to successfully reestablish a symbiosis with its normal host, even when at exceedingly low densities. Thus, although some physiological changes may occur in the course of the culturing process (Stochaj and Grossman, 1997), the basic mechanisms of recognition and entry to host cells appear to remain intact. The dose-response relationship demonstrated here is ecologically important because it suggests that the chances of secondary acquisition, such as may occur during recovery from bleaching, will be greater at sites where zooxanthellae are abundant in the waters overlying the reef (and presumably their hosts). This implies an effect of spatial scale and community composition in the probability of recovery from different sorts of bleaching events. The experiments described here were done in closed containers, so the concentration at which a 48-h exposure results in infection may not easily translate to concentrations in the water overlying the reef.

Existence of infective forms of zooxanthellae in natural waters

On the reef, water motion is an important factor in ameliorating the effects of low concentration of nutrients on algal growth (Larned and Atkinson, 1997). The same phenomenon is likely to be important in exposing bleached animals to free-living zooxanthellae, potentially resulting in reestablishing the symbiosis even when zooxanthellae are at very low concentrations. It is noteworthy that on the reef at the Hawaii Institute of Marine Biology pier where water motion is greatest, the density of *Aiptasia* is much lower than in the calm lagoon, and yet the mean time to establishment of the symbiosis was the shortest in this location. However, the percentage of polyps that secondarily acquired zooxanthellae was lowest at the reef site. These apparently conflicting results could mean that in open waters, free-living zooxanthellae are sometimes at very low densities and perhaps patchily distributed. Because all 10 anemones were within the same tube, it is also possible that only one took up algae from the water column and the rest somehow obtained their zooxanthellae from this individual.

Conclusion

In summary, we have provided experimental tests of fundamental assumptions of the ABH. The differential growth rates of the isolates in response to increasing temperatures suggest that different responses to a factor suspected as a cause of recent large-scale bleaching events do exist among zooxanthellae. Furthermore, the variability in this response can be as great within a clade as between zooxanthellae from different clades. The uptake of zooxanthellae *in situ* by bleached hosts demonstrates that secondary acquisition can occur, and the results of the laboratory studies show that new symbionts can be acquired when the alga concentrations are very low. These findings address important aspects of the ABH. The assumptions tested here are necessary but not sufficient conditions of the ABH. In addition, the lack of clear cladal patterns in the temperature response demonstrates that the ability to withstand high temperatures is not correlated with membership in a specific clade and implies that finer genetic differentiation will be required to understand the dynamics of these symbionts. A clearer understanding of the significance of the different

temperature responses and the bearing these differences have on the ABH will require studies of intact symbioses.

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