Reference: *Biol. Bull.* **201:** 360–373. (December 2001)

Repopulation of Zooxanthellae in the Caribbean Corals *Montastraea annularis* and *M. faveolata* following Experimental and Disease-Associated Bleaching

W. W. TOLLER[1,3], R. ROWAN[2,*], AND N. KNOWLTON[1,3]

[1]*Marine Biology Research Division 0202, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0202;* [2]*University of Guam Marine Laboratory, Mangilao, Guam 96923; and* [3]*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama*

Abstract. Caribbean corals of the *Montastraea annularis* species complex associate with four taxa of symbiotic dinoflagellates (zooxanthellae; genus *Symbiodinium*) in ecologically predictable patterns. To investigate the resilience of these host-zooxanthella associations, we conducted field experiments in which we experimentally reduced the numbers of zooxanthellae (by transplanting to shallow water or by shading) and then allowed treated corals to recover. When depletion was not extreme, recovering corals generally contained the same types of zooxanthellae as they did prior to treatment. After severe depletion, however, recovering corals were always repopulated by zooxanthellae atypical for their habitat (and in some cases atypical for the coral species). These unusual zooxanthellar associations were often (but not always) established in experimentally bleached tissues even when adjacent tissues were untreated. Atypical zooxanthellae were also observed in bleached tissues of unmanipulated *Montastraea* with yellow-blotch disease. In colonies where unusual associations were established, the original taxa of zooxanthellae were not detected even 9 months after the end of treatment. These observations suggest that zooxanthellae in *Montastraea* range from fugitive opportunists and stress-tolerant generalists (*Symbiodinium* A and E) to narrowly adapted specialists (*Symbiodinium* B and C), and may undergo succession.

Received 9 February 2000; accepted 5 July 2001.

* To whom correspondence should be addressed. E-mail: rrowan@uog9.uog.edu

Abbreviations: RFLP, restriction fragment length polymorphism; srDNA, small subunit ribosomal RNA gene; YBD, yellow-blotch disease.

Introduction

Scleractinian reef-building corals are obligate, mutualistic symbioses involving heterotrophic coral animals (hosts) and phototrophic dinoflagellate endosymbionts in the genus *Symbiodinium* (commonly called zooxanthellae). Scleractinian corals (Wells, 1956; Veron, 1995; Cairns, 1999) and zooxanthellae (Trench, 1997; Rowan, 1998) are both taxonomically diverse groups. Their symbioses, however, are restricted to a small and specific subset of the myriad combinations that theoretically might exist (Trench, 1988, 1993). Presumably this host-symbiont specificity is shaped by natural selection, which favors those combinations that perform well and can perpetuate themselves effectively (Trench, 1988; Rowan and Powers, 1991a; Buddemeier and Fautin, 1993). Hypotheses about coral-zooxanthellar specificity were originally shaped by the belief that corals (as individuals or as species) associate with only one species of *Symbiodinium* (Trench, 1988, 1993; see Buddemeier and Fautin, 1993). Accordingly, any direct interactions among different species of *Symbiodinium* were thought to result in one species of zooxanthella consistently "winning" and therefore specifically and exclusively populating its host (Fitt, 1985a; Trench, 1988, 1993).

In contrast to this view, we found that individual colonies of coral in the *Montastraea annularis* species complex often contain more than one taxon of *Symbiodinium* (Rowan and Knowlton, 1995; Rowan *et al.*, 1997; Toller *et al.*, 2001). At Aguadargana reef in the San Blas Islands of Panama (see fig. 1 in Toller *et al.*, 2001), colonies of *M. annularis* host *Symbiodinium* B (or rarely, *Symbiodinium* A) in tissues exposed to high irradiance, and they host *Symbiodinium* C

in tissues exposed to low irradiance. Colonies of *M. faveolata* exhibit a similar pattern except that *Symbiodinium* A and B are both common at high irradiance in these corals (Rowan and Knowlton, 1995; Rowan *et al.,* 1997). Nearby at Río Cartí (a near-shore habitat; see fig. 1 in Toller *et al.,* 2001), members of the *Montastraea annularis* complex host *Symbiodinium* E in tissues exposed to high irradiance and host *Symbiodinium* C otherwise (Toller *et al.,* 2001). Thus on these two reefs, corals at shallower depths, which experience both high (on the colony top, exposed to downwelling irradiance) and low (on colony sides) irradiance, typically host both high- (*Symbiodinium* A and/or B or *Symbiodinium* E) and low- (*Symbiodinium* C) irradiance-associated zooxanthellae simultaneously. (On another offshore reef, *Symbiodinium* E also occurs in some of the deepest colonies of *M. franksi,* possibly as a result of sediment-associated stress [Toller *et al.,* 2001]).

Several observations suggest that interactions among different taxa of *Symbiodinium* within one colony of *Montastraea* may be dynamic. First, coral growth causes slow changes in irradiance microenvironments (*e.g.,* corallites moving from tops to sides of *M. annularis* columns), and the specificity of different zooxanthellae for different irradiance environments (above) implies that zooxanthellar communities will change in response to these irradiance changes. Second, experimental manipulations of irradiance gradients within colonies of *M. annularis* hosting *Symbiodinium* B and C resulted in changes in the distribution of these zooxanthellae (Rowan *et al.,* 1997). Finally, the proportions of *Symbiodinium* A, B, and C in *Montastraea* changed during a coral bleaching event (Rowan *et al.,* 1997).

The present study tested the ability of zooxanthellar symbioses in *M. annularis* and in *M. faveolata* to reestablish typical patterns of association after being disturbed. Because zooxanthellae in unmanipulated corals have such environmentally predictable patterns of distribution (above), we hypothesized that disturbed zooxanthellar populations would re-establish the same patterns of association, directly. To disturb zooxanthellae, we treated corals with low light (*e.g.,* Franzisket, 1970) or with high light (*e.g.,* Dustan, 1979), both of which caused corals to lose zooxanthellae (to bleach). Corals were then allowed to recover. We also studied the zooxanthellar communities of unmanipulated corals that exhibited yellow-blotch disease and associated reductions in zooxanthellar numbers.

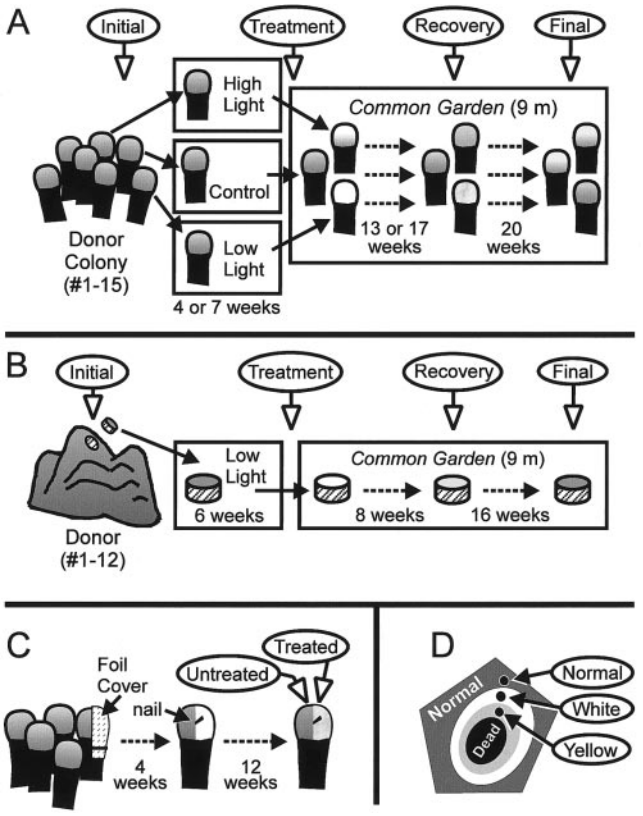

Figure 1. Experimental and sampling designs. (A) Experiment I, in which three columns (gray is live tissue; black is nonliving base) from each of 15 donor colonies of *Montastraea annularis* living at 9–10 m depth (#1–15, corresponding to Corals 1–15 in Figs. 3 and 4) were transplanted to 1 m depth (box labeled High Light), to 9 m depth (Control), and to a cave (Low Light) for treatment for a period of 4 (Colonies 11–15) or 7 (Colonies 1–10) weeks; corals were then transplanted to common gardens at 9 m depth. Samples of zooxanthellae (Initial, Treatment, Recovery, Final) were taken at the times indicated (open arrows); longer times apply to Colonies 1–10; shorter times to Colonies 11–15. (B) Experiment II, in which cores cut from 12 colonies of *M. faveolata* (#1–12, corresponding to Corals 1–12 in Fig. 5) were transplanted to caves (Low Light) for 6 weeks, and then to a common garden at 9 m depth. Samples of zooxanthellae (Initial, Treatment, Recovery, Final) were taken (open arrows) at the times indicated. (C) Experiment III, in which one column on each of 14 colonies of *M. annularis* was half-covered with aluminum foil for 4 weeks, then uncovered for 12 weeks (nail marks the treatment boundary), and then sampled on the top of each half (open arrows, Untreated and Treated; data in Fig. 6). (D) Schematic of yellow-blotch disease (YBD) on *Montastraea* (polygon), showing concentric halos of yellow (light gray color) and bleached (white color) tissue around dead skeleton (Dead), and surrounded by normally pigmented tissue (Normal). Black dots and arrows indicate places where zooxanthellae were sampled (Normal, White, Yellow; data in Fig. 7).

Materials and Methods

Experimental manipulations

Experiments were conducted between October 1997 and October 1998 at Aguadargana reef, San Blas Archipelago, Republic of Panama (see fig. 1 in Toller *et al.,* 2001). Time courses of experiments (not always optimal) were dictated by the imminent closure of the Smithsonian Institution's field station. Experiments used parts of apparently healthy, large colonies of *Montastraea annularis* (Experiments I and III) and *M. faveolata* (Experiment II), as described below and in Figure 1. These large donor coral colonies were separated from one another by more than 5 m. Coral tissues

were sampled with a #6 hole punch, which yields a sample (small core) with about 0.24 cm^2 of coral tissue, or with a #12 hole punch (*ca*. 1.3 cm^2 of tissue). These samples were wrapped in aluminum foil and frozen in a cryogenic dry shipper (chilled with liquid nitrogen) in the field, and then stored in the laboratory at −80 °C until analysis.

Experiment 1 (Fig. 1A). In December 1997, three columns of similar size (*ca*. 7–10 cm diameter) were collected from each of 10 colonies of *M. annularis* living at a depth of 9–10 m. (Colonies of *M. annularis* consist of clusters of columns, each of which is covered distally with living tissue; see Fig. 1A.) The columns were broken off at their nonliving bases, labeled, and a sample (#6 hole punch) was taken from the top of each one (Initial samples). The three columns from each colony were then distributed among three treatments: one was transplanted to an open site on the reef crest at a depth of about 1 m (high-light treatment; High Light in Fig. 1A); one was transplanted to a cave at 14 m (low-light treatment; Low Light in Fig. 1A); and one was transplanted to an open site at 9 m (treatment control; Control in Fig. 1A). The cave was a crevice (*ca*. 2.5 m deep, *ca*. 1.5 m wide, and <1 m high) that was completely shaded from downwelling irradiance, largely shaded from other irradiance, and lacked conspicuous photosynthetic organisms. For low-light treatment, coral columns were mounted upright on PVC posts set in blocks of concrete, using nylon cable ties to secure the columns at their nonliving bases. These blocks then were placed in the back of the cave. Control and high-light-treated coral columns were affixed in an upright position to wire grids using cable ties, and these grids were secured to the reef by wedging them into substrate and covering them with rubble.

After 7 weeks of treatment, all coral columns were collected, assessed visually, and sampled (#6 hole punch; Treatment samples). They were then mounted on wire grids in an upright position and placed in a common garden at 9 m depth, with unobstructed irradiance, for the remainder of the experiment. There were six grids, each with five columns arranged analogously to a Latin square with respect to treatment. After 17 weeks, all columns were assessed and sampled again (#6 hole punch; Recovery samples). Nine of the 30 columns were assessed and sampled once more (#6 hole punch; Final samples) after a total of 37 weeks in the common garden; the other 21 columns had been lost to vandals by that time. All samples (Initial, Treatment, Recovery, and Final) were taken from the tops of columns, within an area (*ca*. 7 cm^2) over which zooxanthellar identities do not vary much or at all in unmanipulated columns of *M. annularis* (Rowan *et al.*, 1997).

A second experiment was done (starting in January 1998) at a different location (*ca*. 0.5 km away). The second experiment differed from the first one only as follows: five colonies (15 coral columns) of *M. annularis* were used, treatment was for 4 rather than 7 weeks, Recovery samples were obtained after 13 rather than 17 weeks, and Final samples were obtained (from all columns) after a total of 33 rather than 37 weeks.

Experiment II (Fig. 1B). In October 1997, one core was removed from each of 12 large (> 1.5 m tall and wide) colonies of *M. faveolata* living at depths of 1–9 m, using a pneumatic drill fitted with a 44-mm hole saw (resulting cores had *ca*. 12.6 cm^2 of live tissue and were *ca*. 5 cm in height). At this time, tissue samples (Initial samples) were taken immediately adjacent to the coring sites with a steel hole punch (#12). Coral cores were then transplanted among three small caves (Low Light, as above) on the reef (7–11 m depth), where they were secured with plastic cable ties to masonry nails pounded into reef framework. Cores occupied the back (darkest) portion of the caves and were mounted upside-down on the cave ceilings.

After 6 weeks of low-light treatment in caves, coral cores were collected and assessed visually; tissue samples were taken from each coral core at a haphazardly selected location away from the core's perimeter (#6 hole punch; Treatment samples). Cores were then attached to cleared reef substrate at 9 m depth with epoxy putty (Z-Spar Splash Zone, Kop-Coat, Inc., Pittsburgh, PA), facing upward under unobstructed natural irradiance. After 8 weeks in this common garden and then again after another 16 weeks, coral cores were assessed and sampled again (#6 hole punch; Recovery samples and Final samples, respectively). In the latter case (Final samples), only eight coral cores were sampled—the four others were lost.

Experiment III (Fig. 1C). In January 1998, individual columns of *M. annularis* were each half-covered with a shield of aluminum foil. This treatment bisected each column vertically into two morphologically equivalent halves, one of which was covered by foil and therefore low-light treated (Treated) and the other of which was exposed to natural irradiance (Untreated). Each shield was molded to its column, lifted off slightly (<0.5 cm), and secured to the column's nonliving base with nylon cable ties. A shield was placed on one column of each of 15 colonies living at depths of 2–4 m (shallow group), and on one column of each of 15 colonies living at depths of 7-9 m (deep group). Shields were removed after 4 weeks, at which time treatment boundaries were marked by gently tapping two small steel nails into opposite sides of each column. After another 12 weeks, columns were assessed visually and a pair of tissue samples was taken from the top of each, 2 cm apart and on either side of the treatment boundary (#6 hole punch; Treated and Untreated samples, Fig. 1C).

Yellow-blotch disease

In October 1997 and January 1998 at Cayos Limones, San Blas (see fig. 1 in Toller *et al.*, 2001), we found a number of colonies of *Montastraea* that appeared to have

"yellow-blotch disease" (YBD; Santavy *et al.,* 1999). Some colonies had only one or two small lesions (*ca*. 10–30 cm wide), which usually consisted of a patch of exposed skeleton surrounded by a halo (typically *ca*. 1–3 cm wide) of yellow living tissue, which in turn was surrounded by a halo (typically ≤2 cm wide) of white (bleached) tissue; lesions were surrounded by apparently healthy tissue (see Fig. 1D). Other colonies were mostly dead, in which case a patch of normal tissue was surrounded by a band of bleached tissue inside a band of yellow tissue.

Using a steel hole punch (#12), we took samples from five colonies of *M. franksi* (one lesion per colony), from six colonies of *M. faveolata* (one or two lesions per colony), and from one colony of *M. annularis* (two lesions). Two samples were taken at every lesion—one of normally pigmented tissue and one of yellow tissue nearby (≤3 cm apart; Normal and Yellow, respectively; see Fig. 1D). At five lesions we also sampled the white tissue that was between yellow and normal tissue (White; see Fig. 1D).

Progression of YBD was monitored in 12 colonies of *M. faveolata*. On 25 January 1998, two small nails were driven into the bare skeleton next to one YBD lesion in each colony. The two nails defined a line parallel to the lesion edge, and the distance between that line and the lesion edge (living, yellow tissue) was measured with a pair of calipers. We also measured the distance to normally pigmented tissue, along the same vector. These measurements were repeated 5 months later (28 May 1998).

Laboratory methods

Zooxanthellae were isolated from frozen samples as described previously (Rowan and Powers, 1991b; Rowan and Knowlton, 1995), except that skeletal cores or fragments, after being stripped of tissue, were broken apart with a steel spatula and then washed with isolation buffer. That wash was combined with the tissue that had been stripped from the sample previously. At this point, one-tenth of each sample was fixed in 10% formalin and stored at 4 °C for cell counts, which were obtained from eight subsamples of each sample by hemacytometry. The rest of each sample was used to prepare DNA as described previously (Rowan and Powers, 1991b; Rowan and Knowlton, 1995).

Zooxanthellae in each sample were identified by restriction fragment length polymorphism (RFLP) genotypes of small ribosomal subunit RNA genes (srDNA), as described previously (Rowan and Powers, 1991b; Toller *et al.,* 2001). Each sample was analyzed at least twice—once by srDNA amplification with universal PCR primers (ss3 and ss5; Rowan and Powers, 1991b), and once by srDNA amplification with host-excluding PCR prim (ss3Z and ss5; Rowan and Powers, 1991b; Toller *et al.,* 2001). All amplified srDNAs were digested with *Dpn* II and with *Taq* I, and then compared to standard srDNA genotypes of *Symbiodinium* A, B, C, and E (srDNA clones A^0, B^0, C^0, and E^{0-1}, amplified and digested the same way; see Toller *et al.,* 2001). RFLP genotypes C2 (in two samples) and C (in 12 samples) were compared in greater detail using a total of 12 restriction enzymes: *Alu* I, *Bst*N I, *Bst*U I, *Dpn* II, *Hae* III, *Hha* I, *Hinf* I, *Mbo* I, *Mse* I, *Msp* I, *Sau*96 I, and *Taq* I.

Samples that contained more than one zooxanthellar RFLP genotype were compared to a series of synthetic mixtures of cloned srDNAs (srDNA clones A^0, B^0, C^0, and E^{0-1} and srDNA clone $C2^{0-1}$, see below) to estimate the relative abundance of each genotype in the sample (Rowan *et al.,* 1997; Toller *et al.,* 2001). For graphical presentation, these estimates were multiplied by the sample's total cell number (see above) to estimate the number of cells of each genotype in the sample, and these values were then converted to numbers of cells per square centimeter of live coral surface (number of zooxanthellae/cm^2 in Figs. 3–7).

Samples with low numbers of zooxanthellae ($<4 \times 10^5$ cells/cm^2 of coral) yielded little zooxanthellar srDNA when srDNAs were PCR-amplified in the usual manner. To obtain more srDNA from such samples in Experiment I, we used two rounds of amplification (Roux, 1995) as follows. Sample srDNAs were amplified with host-excluding PCR primers over 34 cycles of the PCR. Aliquots (10 μl) of those amplifications were electrophoresed on agarose gels (1.0% Nuseive GTG; FMC BioProducts, Rockland, ME), and faint bands of srDNA were excised and added to 100 μl of water. These gel-purified srDNAs were heated to 65 °C for 2 min, and then 1 μl of each one was PCR-amplified with host-excluding primers in the same manner. The resulting re-amplified srDNAs were then analyzed as described above.

srDNA with an RFLP genotype distinct from *Symbiodinium* A^0, B^0, C^0, and E^{0-1}, here called C2, was cloned and sequenced using methods described previously (Toller *et al.,* 2001). It was amplified with host-excluding PCR primers from a colony of *M. annularis* in Experiment III (clone $C2^{0-1}$) and from an unmanipulated colony of the coral *Siderastrea siderea* (clone $C2^{0-2}$). DNA sequences were deposited in GenBank [http://www.ncbi.nlm.nih.gov/; accession numbers AF238259 ($C2^{0-1}$), AF238260 ($C2^{0-2}$)].

Results

RFLP genotypes of zooxanthellae in experimental corals

Using the restriction enzymes *Dpn* II and *Taq* I, we scored six different RFLP genotypes of srDNA in samples of zooxanthellae (Fig. 2). As explained below, genotypes A, B, C, C2, and E represent the taxa *Symbiodinium* A, B, C, C2, and E. RFLP genotype N (Fig. 2, lane N^{0-1}) is not a taxon of *Symbiodinium* and is instead related to protozoa of the phylum Apicomplexa (Toller *et al.,* in press). Using our methods (above), genotype N was observed only in six corals—all of these from Experiment I (low-light treatment) and only in samples taken immediately after treatment (see

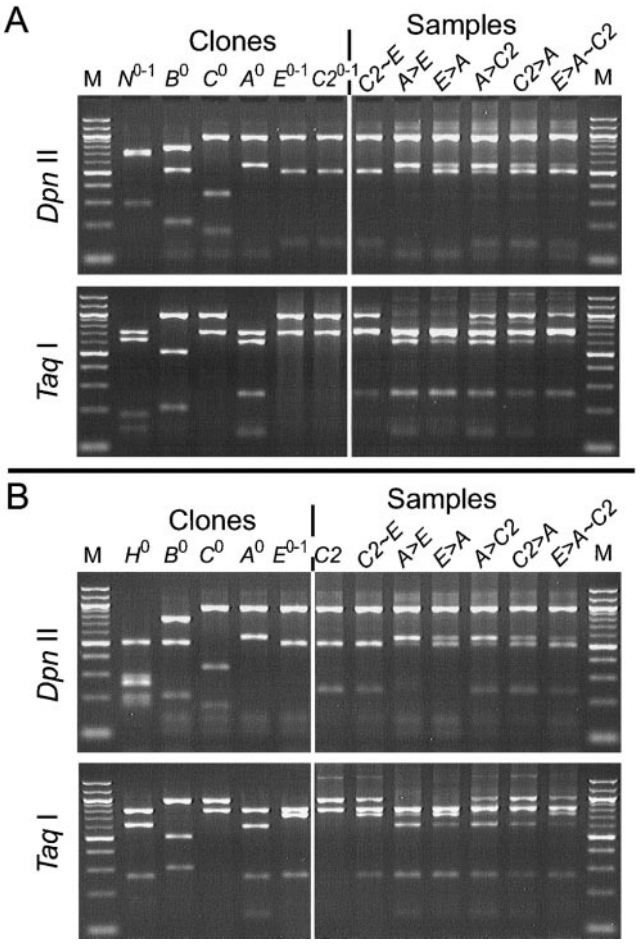

Figure 2. RFLP genotypes of *Symbiodinium*. The same srDNAs were amplified with host-excluding PCR primers (A) and with universal PCR primers (B), and then digested with *Dpn* II (upper panels) and with *Taq* I (lower panels). Clones are srDNA standards for genotype *N* (clone N^{0-1}; see Results), *Montastraea annularis* (clone H^0), *Symbiodinium* B (clone B^0), *Symbiodinium* C (clone C^0), *Symbiodinium* A (clone A^0), *Symbiodinium* E (clone E^{0-1}), and *Symbiodinium* C2 (clone $C2^{0-1}$). Samples are zooxanthellae from *Montastraea* scored as *Symbiodinium* C2 (B; C2), *Symbiodinium* C2 and *Symbiodinium* E in approximately equal amounts (A and B; C2~E), more *Symbiodinium* A than *Symbiodinium* E (A and B; A > E), more *Symbiodinium* E than *Symbiodinium* A (A and B; E > A), more *Symbiodinium* A than *Symbiodinium* C2 (A and B; A > C2), more *Symbiodinium* C2 than *Symbiodinium* A (A and B; C2 > A), and *Symbiodinium* A and C2 in approximately equal amounts with more *Symbiodinium* E (A and B; E > A~C2). Lane M contains DNA fragment size standards of (top to bottom) 1500 base pairs (bp), 1200 bp, and then 1000 bp to 100 bp in 100-bp increments.

below). Further observations on genotype N are presented elsewhere (Toller *et al.*, in press).

The srDNA genotypes of *Symbiodinium* A, B, C, and E are represented by cloned srDNAs A^0, B^0, C^0, and E^{0-1} (respectively), and these genotypes differ from one another in both *Dpn* II and *Taq* I digests (Toller *et al.*, 2001; Fig. 2A, B). RFLP genotype C2, represented in Figure 2A by a cloned srDNA ($C2^{0-1}$; below) and in Figure 2B by a sample of zooxanthellae (C2), has not been found in unmanipulated colonies of *Montastraea annularis* and *M. faveolata* (Rowan and Knowlton, 1995; Rowan *et al.*, 1997; Toller *et al.*, 2001; this study and unpubl. obs.). Genotype C2 is distinguished from genotypes C and E only when both *Dpn* II and *Taq* I digests are examined together (Fig. 2A, B).

srDNA of genotype C2 appears to lack a *Dpn* II restriction site relative to srDNA of genotype C (Fig. 2). Defined by this character, genotype C2 was found previously in various other species of host (R. Rowan and W. Toller, unpubl. obs.). Cloned srDNAs C^0 (which represents *Symbiodinium* C; Toller *et al.*, 2001), $C2^{0-1}$ (genotype C2 from Experiment III), and $C2^{0-2}$ (genotype C2 from *Siderastrea siderea*, collected nearby) differed from one another in nucleotide sequence by about 0.9% (not shown; see also Toller *et al.*, 2001). That amount of srDNA sequence difference could imply that these three clones represent three species of *Symbiodinium* (*e.g.*, McNally *et al.*, 1994), or one species of *Symbiodinium* in which srDNA is heterogeneous (see Toller *et al.*, 2001).

We further compared the two samples from which clones $C2^{0-1}$ and $C2^{0-2}$ were obtained to 12 samples of *Symbiodinium* C (samples in fig. 9 in Toller *et al.*, 2001) by digesting srDNAs with 12 restriction enzymes (listed in Materials and Methods). The two samples of genotype C2 were indistinguishable and differed from *Symbiodinium* C only in *Dpn* II (above) and *Mse* I digests (not shown). This analysis also showed that srDNA was heterogeneous in all samples, which means that zooxanthellae in the samples cannot be described precisely by sequences of cloned srDNA (*i.e.*, clones C^0, $C2^{0-1}$, and $C2^{0-2}$; see above and Toller *et al.*, 2001). Nevertheless, RFLP data indicate that genotype C2 represents a taxon of zooxanthella, *Symbiodinium* C2, that is distinct from the *Symbiodinium* C that occurs commonly in *M. annularis* and *M. faveolata*.

Examples of RFLP genotypes that we interpreted as mixtures of taxa of *Symbiodinium* are shown on the right side of Figure 2 (Samples). The figure compares data obtained by amplifying srDNAs with host-excluding (Fig. 2A) *versus* universal (Fig. 2B) PCR primers because, using *Dpn* II and *Taq* I, both sets of data are needed to distinguish mixtures of genotypes A and C2 (*e.g.*, Fig. 2, A > C2 and C2 > A) from mixtures of genotypes A, C2, and E (*e.g.*, Fig. 2, E > A~C2). Universal PCR primers also amplify coral host srDNA (Fig. 2B, clone H^0), but none was detected in the samples of zooxanthellae shown in Figure 2B.

Experiment I

The experimental units were individual coral columns taken from 15 donor colonies of *M. annularis* (see Fig. 1A). Below, a column is identified by the colony from which it came and by its treatment group (*e.g.*, Colony 1, High Light). Columns 1–10 were in the first experimental group;

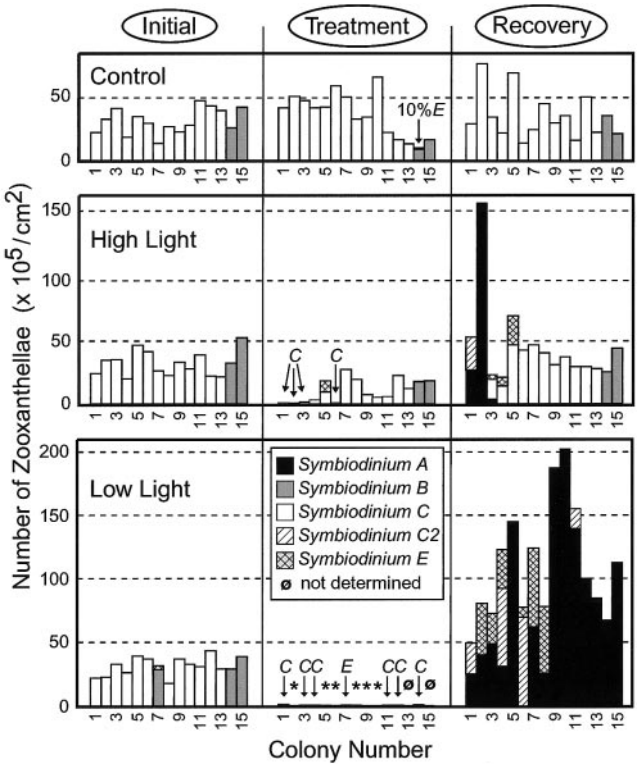

Figure 3. Zooxanthellae observed in *Montastraea annularis* in Experiment I. Panels labeled Control (top), High Light (middle), and Low Light (bottom) present data from corals in the treatments labeled as such in Figure 1A. Panel divisions labeled Initial, Treatment, and Recovery (in ovals at top) present data from samples labeled as such in Figure 1A (ovals with open arrows). Colony Number (horizontal axes) identifies data obtained from different coral columns; data with the same Colony Number within a panel are different samples from the same column; data with the same Colony Number in different panels are samples from different columns taken from the same donor coral colony (see Fig. 1A). Colonies 1–10 are from the first experimental group, and Colonies 11–15 are from the second experimental group (see Methods). Bars indicate the taxa of zooxanthellae (by shade, according to the key in the middle panel) and the number of zooxanthellae (by height, normalized to 1 cm^2 of coral surface) observed in each sample. Where bars are too short to be legible, zooxanthellar identities are given by the arrows labeled C (*Symbiodinium* C), E (*Symbiodinium* E), and 10% E (together with 90% *Symbiodinium* B). Samples in which zooxanthellae were not identified are indicated with a theta (ø). Samples that contained RFLP genotype N (see text) are indicated with asterisks (*).

Columns 11–15 were in the second group (see Materials and Methods). Before treatment, samples from most coral columns contained *Symbiodinium* C (Fig. 3, Initial). Exceptions were two donor colonies that yielded only *Symbiodinium* B (Fig. 3, Initial; Colonies 14 and 15 in all treatment groups), and one column that yielded *Symbiodinium* B with a small amount of *Symbiodinium* E (Fig. 3, Initial; Colony 7, Low Light). Initial zooxanthellar numbers in the three treatment groups (Control, High Light, and Low Light; Fig. 3) were indistinguishable ($31.5 \pm 10.1 \times 10^5$, $31.9 \pm 9.80 \times 10^5$, and $31.4 \pm 7.10 \times 10^5$ zooxanthellae/cm^2 of coral, respectively).

Zooxanthellar numbers decreased after treatment. Samples from high-light-treated coral columns (Fig. 3, High Light, Treatment) had, on average, 29% as many zooxanthellae as did samples from controls ($10.7 \pm 9.0 \times 10^5$ *versus* $36.6 \pm 17.5 \times 10^5$ zooxanthellae/cm^2 [means $\pm$ standard deviations]; Wilcoxon signed rank test, $P < 0.001$). Two high-light-treated columns (Colonies 14 and 15; with *Symbiodinium* B before treatment) appeared normal; the other 13 (with *Symbiodinium* C before treatment) were pale or bleached, but only on their tops and south-facing (sun-facing) sides. All low-light-treated columns (Fig. 3, Low Light, Treatment) were white, and samples from them had, on average, only about 2.5% as many zooxanthellae as did samples from controls ($0.90 \pm 1.4 \times 10^5$ *versus* $36.6 \pm 17.5 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank test, $P < 0.001$). Zooxanthellar identities did not change in samples from the tops of coral columns in the control group after 4 or 7 weeks (Fig. 3, Control, Treatment *versus* Initial), with one exception. That exception was Colony 14, which yielded only *Symbiodinium* B initially but yielded *Symbiodinium* B with a small amount of *Symbiodinium* E 4 weeks later. In high-light-treated columns one change was observed (Fig. 3, High Light, Treatment *versus* Initial): Colony 5 initially yielded *Symbiodinium* C but yielded roughly equal parts of *Symbiodinium* C and *Symbiodinium* E immediately after treatment.

Identities of zooxanthellae were difficult to determine in low-light-treated coral columns at the end of treatment, presumably because these columns contained so few zooxanthellae (above). Two rounds of PCR amplification (see Materials and Methods) allowed 13 samples to be analyzed (Fig. 3, Low Light, Treatment); no srDNA was obtained from the other two samples. Six samples contained *Symbiodinium* C, one contained *Symbiodinium* E (Colony 7, which contained some *Symbiodinium* E initially), and six yielded only a non-*Symbiodinium* RFLP genotype (genotype N; see above).

After a total time of 17 or 24 weeks, zooxanthellar numbers and RFLP genotypes in samples from the control group were similar to initial conditions; Colony 14 once again yielded only *Symbiodinium* B (Fig. 3, Control; Initial, Treatment, Recovery). High-light-treated columns, which had then spent 13 or 17 weeks in their original, deeper environment (Fig. 3, High Light, Recovery) regained coloration (11 normal, 4 pale on tops only) and zooxanthellar numbers (Recovery *versus* Treatment: $45.4 \pm 34.2 \times 10^5$ *versus* $10.7 \pm 9.0 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank test, $P < 0.001$). At this time, zooxanthellar numbers in samples from high-light-treated columns were similar to those in samples from controls ($45.4 \pm 34.2 \times 10^5$ *versus* $35.2 \pm 18.5 \times 10^5$ zooxanthellae/cm^2, respectively; Wilcoxon signed rank test, $P > 0.1$).

Thirteen or 17 weeks after the end of treatment, 10 of 15 high-light-treated coral columns had the same taxa of *Symbiodinium* that they contained before treatment, but 5 of 15 coral columns apparently contained different taxa of *Symbiodinium* than they began with (Fig. 3, High Light, Recovery). One of these taxonomic differences had been observed at the end of treatment (Colony 5). The other four changes (Fig. 3, High Light, Initial versus Recovery) were *Symbiodinium* C to *Symbiodinium* A and C2 (Colony 1); *Symbiodinium* C to *Symbiodinium* A (Colony 2); *Symbiodinium* C to *Symbiodinium* C, A, and E (Colony 3); and *Symbiodinium* C to *Symbiodinium* C and E (Colony 4). These four columns represented four of the five columns with the lowest numbers of zooxanthellae following treatment.

All low-light-treated coral columns experienced major changes in zooxanthellar populations after 13 or 17 weeks back in their original environment (Fig. 3, Low Light, Recovery). Zooxanthellar numbers were about 100-fold higher than after treatment (Recovery *versus* Treatment: $111 \pm 45.2 \times 10^5$ *versus* $0.90 \pm 1.4 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank test, $P < 0.001$), and were about 3-fold higher than in the control group ($35.2 \pm 18.5 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank test, $P < 0.001$). Only Colony 2 appeared normal; the other 14 columns, despite their large numbers of zooxanthellae, were still pale at this time (May 1998).

No low-light-treated coral column contained the same zooxanthellae that it had originally (Fig. 3, Low Light, Recovery *versus* Initial). Eight of them contained mixtures of taxa, and *Symbiodinium* A was predominant, followed by *Symbiodinium* E and C2; *Symbiodinium* C and B were not detected (Fig. 3, Low Light, Recovery). The predominance of *Symbiodinium* A was observed primarily in Colonies 11–15 (sampled 13 weeks after treatment); among Colonies 1–10 (sampled 17 weeks after treatment) only four samples contained more than 50% *Symbiodinium* A.

Twenty-four of the above coral columns ($n = 8$ colonies) were sampled again, for the last time, 33 or 37 weeks after treatment. At this time (18 October 1998), many unmanipulated colonies of *M. annularis*, *M. faveolata*, and *M. franksi* living at depths of 8–14 m at our study site were pale or bleached on their upper surfaces. Weekly mean sea-surface temperatures near our study site ranged between 29.4 °C and 29.9 °C from 26 August to 7 October (9.5 °N, 78.5 °W; data from Integrated Global Ocean Services System, http://ingrid.ldgo.columbia.edu/SOURCES/.IGOSS). Historically, temperatures this high are associated with coral bleaching at our study site (see fig. 3e in Rowan *et al.*, 1997). Moreover, most of the summer of 1998 was unusually warm: from the first week of May through the first week of October (23 weeks) in the years 1981 to 1997 (but excluding 1983 and 1995, when corals bleached), there were an average of 3.5 weeks of average sea-surface temperature at or above 29.0 °C near our study site; for this period in 1998, there were 16 such weeks (data from IGOSS, as above). However, bleaching appeared to be less severe than in October 1995 (Rowan *et al.*, 1997; pers. obs.) in terms of the number of colonies of *Montastraea* affected, the number of species of coral affected, and the extent to which individual corals were bleached.

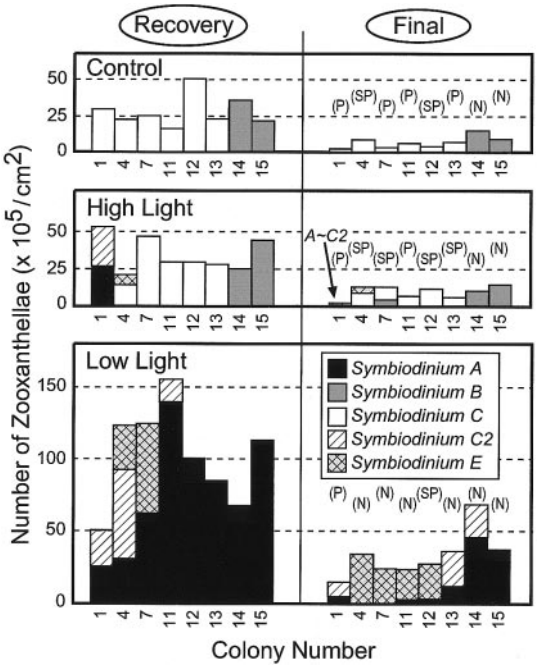

Figure 4. Zooxanthellae observed in *Montastraea annularis* at the last two sampling times of Experiment I. Data are presented as in Figure 3, using the same system to number colonies. Data under Recovery (oval, above) are the same data from Figure 3, and data under Final (oval, above) are from samples from the same corals 20 weeks later (see Fig. 1A). When Final samples were collected, corals were scored as normal (N), slightly pale (SP), or pale (P).

Results are presented in Figure 4 (Final), in comparison to Recovery samples (13 or 17 weeks after treatment) from the same columns (data from Fig. 3). When final samples were collected, six of eight control coral columns appeared pale or slightly pale [labeled (P) and (SP), respectively, in Fig. 4] on top, as were six of eight high-light-treated columns (Fig. 4, Final); the other two columns in each group hosted *Symbiodinium* B and appeared normal [labeled (N)]. Twenty weeks earlier (Recovery) only one of these 16 columns appeared pale (Colony 1, High Light) and all others appeared normal. As suggested by the increase in numbers of pale colonies, average zooxanthellar numbers decreased and were about 5-fold lower than in the previous samples (Final *versus* Recovery: control columns, 6.7 ± 4.0 *versus* $35.2 \pm 18.5 \times 10^5$ zooxanthellae/cm^2; high-light-treated columns, 9.4 ± 4.3 *versus* $45.4 \pm 34.2 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank tests, $P = 0.01$).

In contrast, only two low-light-treated coral columns (Colonies 1 and 12) appeared pale or slightly pale in October, and six appeared normal (Fig. 4, Low Light, Final). All

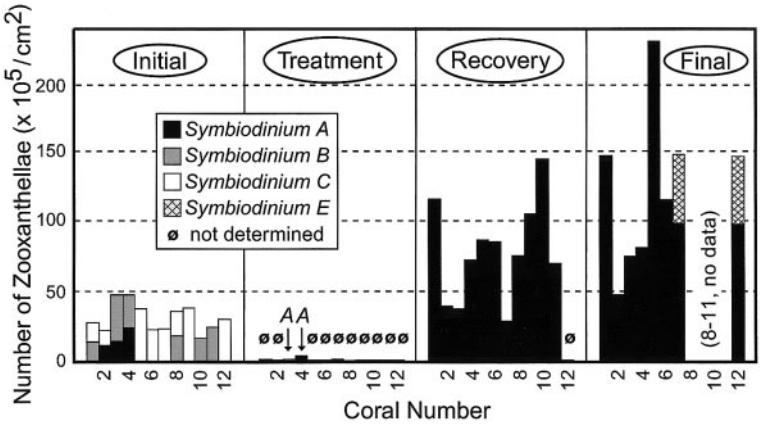

Figure 5. Zooxanthellae observed in *Montastraea faveolata* before and after low-light treatment (Experiment II). The data are presented as in Figures 3 and 4, for the experiment diagrammed in Figure 1B; there was no control group in this experiment. Corals 8–11 were lost before Final samples were collected (8–11, no data).

eight of these columns were pale 20 weeks earlier. Contrary to expectation, the overall increase in pigmentation was accompanied by an average decrease of about 3-fold in zooxanthellar number (Final versus Recovery: 33.6 ± 16.1 *versus* $111 \pm 45.2 \times 10^5$ zooxanthellae/cm^2; Wilcoxon signed rank test, $P < 0.02$), to numbers comparable to those in control, normally pigmented columns at previous sampling times (*e.g.*, Fig. 4, Low Light, Final *versus* Control, Recovery).

Final samples from control columns contained the same taxa of *Symbiodinium* that were observed previously in those columns (Fig. 4; Control, Recovery). This was also true for most high-light-treated columns, although in one of these *Symbiodinium* B was found with *Symbiodinium* C (Fig. 4, High Light, Final; Colony 7), whereas only *Symbiodinium* C was detected in that column previously. In contrast, we found different zooxanthellae (relative to Recovery) in six of eight low-light-treated columns (Fig. 4, Low Light, Final *versus* Recovery). In final samples from low-light-treated corals overall, *Symbiodinium* A declined, *Symbiodinium* E increased to become predominant, and *Symbiodinium* C2 appeared in different columns, compared to samples taken 20 weeks earlier. *Symbiodinium* C and B were not detected.

Experiment II

Cores taken from colonies of *M. faveolata* (see Fig. 1B; these cores are hereafter referred to as "corals") living at depths between 1 and 9 m contained a variety of zooxanthellar taxa before treatment (*Symbiodinium* A, B, C; Fig. 5, Initial), as expected based on earlier surveys (Rowan and Knowlton, 1995; Rowan *et al.*, 1997). After low-light treatment, all 12 corals were white and contained, on average, about 2.6% as many zooxanthellae as they began with (Fig. 5, Treatment *versus* Initial; $0.8 \pm 1.0 \times 10^5$ versus $30.8 \pm 10.2 \times 10^5$ zooxanthellae/cm^2, respectively; Wilcoxon signed rank test, $P = 0.002$). Zooxanthellae were identified in only two samples; both contained *Symbiodinium* A, and were from corals that had mixtures of A and B before treatment (Corals 3 and 4; Fig. 5).

Eight weeks after the end of treatment, zooxanthellar numbers were about 2-fold higher than before treatment (Fig. 5, Recovery *versus* Initial; $71.6 \pm 40.5 \times 10^5$ *versus* $30.8 \pm 10.2 \times 10^5$ zooxanthellae/cm^2, respectively; Wilcoxon signed rank test, $P = 0.01$). Corals 1 and 6 appeared normal, Coral 12 was bleached (and had very few zooxanthellae; Fig. 5, Recovery), and the other eight corals appeared pale. Only *Symbiodinium* A was detected at this time (Fig. 5, Recovery), in contrast to the typical pattern for *M. faveolata* in this habitat, which host *Symbiodinium* C (Rowan and Knowlton, 1995).

Eight corals were sampled after a further 16 weeks in their common garden (24 weeks total time after the end of treatment, at the end of May 1998, prior to the bleaching event noted above). Six of them appeared normal and two (Corals 5 and 6) were pale. Zooxanthellar numbers remained high on average ($111.1 \pm 55.6 \times 10^5$ zooxanthellae/cm^2). Samples from six corals contained only *Symbiodinium* A; samples from the other two corals contained *Symbiodinium* A and E (Fig. 5, Final).

Experiment III

When the foil treatment shields (see Fig. 1C) were removed, all treated tissues were white. Adjacent tissues that had not been covered appeared normal, and borders between the white (treated) tissue and the normal (untreated) tissue were sharp. Many corals had suffered partial mortality in covered areas during treatment; further observations were made only on those in which more than 50% of the treated tissue appeared healthy ($n = 5$ in the shallow group; $n = 9$

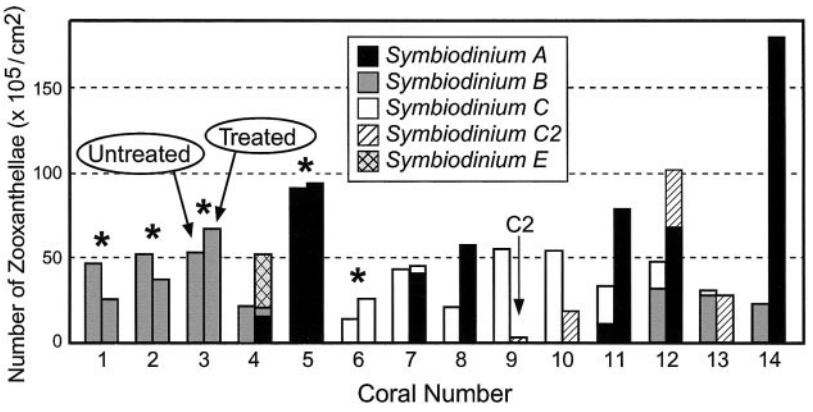

Figure 6. Zooxanthellae observed in untreated and in treated parts of the same column of *Montastraea annularis* (Experiment III; see Fig. 1C). Corals 1–5 lived at 2–4 m depth, Corals 6–14 lived at 7–9 m depth. Data from untreated (Untreated) and from treated (Treated) parts of the same coral column are paired (left bar and right bar, respectively, as shown for Coral 3). Asterisks (*) identify coral columns in which samples from both treated and untreated tissue appeared normal (see text). Otherwise, data are presented as in Figures 3–5.

in the deep group). To avoid additional stress to the corals, no samples were taken immediately after treatment.

The corals had different appearances 12 weeks after treatment. In four shallow corals and one deep coral (Fig. 6, asterisks; Corals 1, 2, 3, 5, and 6) it appeared that normal pigment had spread from untreated into treated tissue by about 2–3 cm, so that treatment boundaries were no longer apparent. In these corals, samples taken from either side of the treatment boundary (see Fig. 1C, Treated and Untreated) were normally pigmented; they also had similar numbers of zooxanthellae, of the same taxon of *Symbiodinium*. The taxa were those expected in shallower (Corals 1, 2, 3, 5; *Symbiodinium* B or A) and deeper (Coral 6; *Symbiodinium* C) colonies of *M. annularis* at this location (Rowan and Knowlton, 1995; Rowan *et al.*, 1997).

In the other nine corals (Fig. 6; Corals 4, 7–14) treatment boundaries were still obvious 12 weeks after treatment. Untreated tissues appeared normal, whereas treated tissues were unevenly pigmented and pale overall, and samples taken from either side of the treatment boundary had different taxa of *Symbiodinium*. Untreated halves contained the expected taxa (*Symbiodinium* B, C, or, rarely in deeper water, some A); treated halves contained, in order of decreasing occurrence, *Symbiodinium* A, C2, E, and B or C (Fig. 6; Corals 4, 7–14). In samples from three of these nine corals (Corals 4, 7, and 11), the taxon of *Symbiodinium* found in the untreated half was also found in the treated half, but—except where that taxon was *Symbiodinium* A (Colony 11)—it was relatively minor in the treated tissue. Zooxanthellar numbers were variable among samples from treated halves (Fig. 6); overall, there was no significant difference in zooxanthellar numbers in samples from treated *versus* untreated halves of corals.

Disease-associated disturbance of zooxanthellae

We marked YBD lesions in 12 colonies of *M. faveolata* and observed that mortality progressed by 11 ± 6 mm (mean ± standard deviation) during 5 months. Yellow and white halos (see Fig. 1D) progressed in concert with mortality. Thus, as YBD spreads across a coral, it appears that tissue first loses zooxanthellae (white), then partially recovers zooxanthellae (yellow), and then dies. Average numbers of zooxanthellae in samples of normal ($31.8 \pm 10.1 \times 10^5$ zooxanthellae/cm^2), white ($3.7 \pm 1.8 \times 10^5$ zooxanthellae/cm^2), and yellow ($24.6 \pm 16.1 \times 10^5$ zooxanthellae/cm^2) tissues confirmed that hypothesis.

With one exception (Fig. 7; Colony 9), normal and yellow samples from the same lesion contained different taxa of *Symbiodinium*. Samples of normal tissues contained the taxa that are common in unaffected corals at these depths (Rowan and Knowlton, 1995; Rowan *et al.*, 1997; Toller *et al.*, 2001)—predominantly *Symbiodinium* C—and yellow tissues contained predominantly *Symbiodinium* A (estimated at ≥ 50% of the total in samples from 12 of 15 lesions; Fig. 7). Yellow tissue also often (9 of 15 samples; Fig. 7) contained the zooxanthellae found in the adjacent normal tissue. We could identify zooxanthellae in two samples of white tissue (Colony 3 and Lesion 7-1, Fig. 7); they contained mixtures of the taxa found in the adjacent normal (*Symbiodinium* C) and yellow (*Symbiodinium* A) tissues.

Discussion

Taxonomic identities of zooxanthellae

In laboratory studies of establishment or re-establishment of symbiosis between *Symbiodinium* and host animals, sources of zooxanthellae are under full experimental control

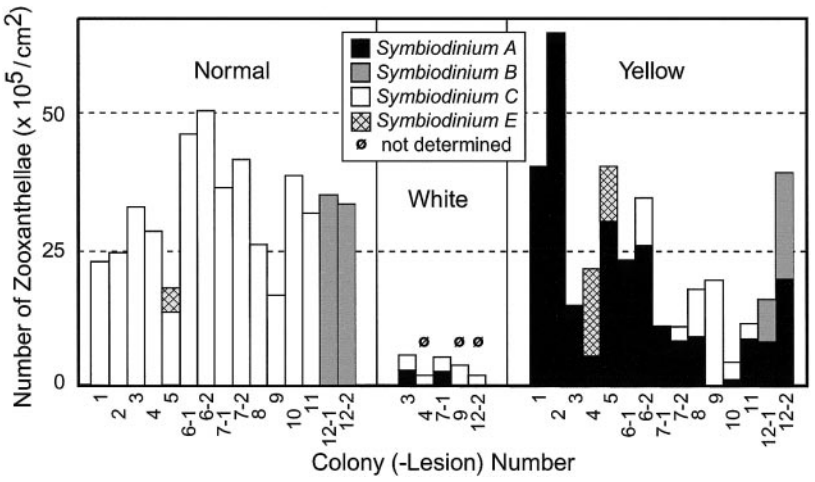

Figure 7. Zooxanthellae associated with yellow-blotch disease (YBD). Lesions of YBD were sampled in 12 affected coral colonies (Colony Number, 1–12), one lesion per colony (most colonies) or two lesions per colony (Colonies 6, 7, and 12 only; Colony-Lesion Numbers 6-1 and 6-2, 7-1 and 7-2, 12-1 and 12-2). At every lesion, samples were taken from Normal and Yellow tissues as diagrammed in Figure 1D. Samples of White tissue (see Fig. 1D) were obtained from five of the lesions only. Each bar represents data from one sample, presented as in Figures 3–6. Coral species were *Montastraea franksi* (Colonies 1–5), *M. faveolata* (Colonies 6–11), and *M. annularis* (Colony 12).

(*e.g.*, Kinzie and Chee, 1979; Schoenberg and Trench, 1980; Colley and Trench, 1983; Davy *et al.*, 1997). In contrast, corals in our field experiments were exposed to uncharacterized natural populations of *Symbiodinium*. Here, the identities of zooxanthellae in re-established symbioses are certain only to the extent that zooxanthellar taxonomy is certain.

Our study compared zooxanthellae that were identified by *Dpn* II- and *Taq* I-generated RFLP genotypes of srDNA, and this method does not discriminate all species of *Symbiodinium* (Rowan, 1998; Toller *et al.*, 2001). For example, three known species of *Symbiodinium* A—*S. microadriaticum* (GenBank accession number M88521), *S. pilosum* (X62650), and *S. corculorum* (L13717)—would be indistinguishable in this analysis. Nevertheless, where the same zooxanthellar RFLP genotype was detected in a coral both before and after treatment (*e.g.*, Fig. 3, High Light, Colonies 6–15), parsimony argues that the coral hosted the same species of *Symbiodinium* throughout the experiment. On the other hand, no taxonomic uncertainty affects our observation that many re-established symbioses involved changes; when compared samples of zooxanthellae differed with respect to RFLP genotypes A, B, C, or E (*e.g.*, Fig. 3, Low Light; Fig. 5), it is clear that the samples contained different species of *Symbiodinium* (Rowan, 1998; Toller *et al.*, 2001).

Disturbance and re-establishment of zooxanthellar symbioses

Where our experiments were conducted, *Symbiodinium* B predominates in *Montastraea annularis* at higher irradiance, *Symbiodinium* A and B predominate in *M. faveolata* at higher irradiance, and *Symbiodinium* C predominates in both species of coral at lower irradiance (Rowan and Knowlton, 1995; Rowan *et al.*, 1997). This predictable pattern suggests that host-symbiont specificity is defined largely by the interaction of each host-zooxanthella combination with its environment (*sensu* Buddemeier and Fautin, 1993). This led us to hypothesize that, under a constant environment, host-symbiont specificity should be directly re-established following acute disturbance.

We found some evidence for this in Experiment I, in that most (10 of 15) of high-light-treated columns of *M. annularis* were repopulated with the same zooxanthellae that existed prior to treatment (*Symbiodinium* C or B; Fig. 3, High Light, Colonies 6–15). However, the host-zooxanthella specificity was not re-established in the other high-light-treated columns, which contained at least some different zooxanthellae (*Symbiodinium* A, E, and C2; Fig. 3, High Light, Colonies 1–5) after recovery. In these five corals, treatment had led to significantly fewer zooxanthellae than in the other 10 corals (5.0×10^5 cells/cm^2 *vs.* 13.6×10^5 cells/cm^2, respectively; $P < 0.05$, Mann-Whitney test). This suggests that coral-zooxanthella associations may or may not be re-established following disturbance, depending on the magnitude of zooxanthellar depletion.

This conclusion is supported by the results of Experiment I, in which none of the previously observed coral-zooxanthella associations were re-established in low-light-treated *M. annularis*. Zooxanthellae were severely depleted in these corals during treatment (to *ca.* 1×10^5 cells/cm^2 on aver-

age), and all corals were repopulated by completely different zooxanthellae (*Symbiodinium* A, E, and C2; Fig. 3, Low Light, Recovery), even 9 months after treatment (Fig. 4). A similar result was obtained by low-light treatment of *M. faveolata* in Experiment II, although in that experiment the re-establishment of symbioses was not tested under a constant environment; after being treated with low light, most corals were also transplanted to a new environment. In that new environment (9 m depth), unmanipulated *M. faveolata* host *Symbiodinium* C (Rowan and Knowlton, 1995; unpubl. obs.), whereas re-established symbioses involved *Symbiodinium* A or A and E (Fig. 5).

We hypothesized that new taxa (*Symbiodinium* A, E, or C2) following severe bleaching (Experiments I and II) would not become established if untreated zooxanthellae (resident *Symbiodinium* B or C populations) were abundant near bleached tissues (zooxanthellae are thought to be translocated within colonies, among coral polyps, *via* their gastrovascular systems [*e.g.*, Gladfelter, 1983; Gateño *et al.*, 1998]). In Experiment III, the results from 5 of the 14 half-bleached columns were consistent with this hypothesis: bleached tissues were repopulated with zooxanthellae that apparently originated from untreated tissues (Fig. 6, Corals 1, 2, 3, 5, 6). Together with the observations on the spread of pigmentation (Fig. 6, Results), this indirect evidence suggests that zooxanthellae are translocated into bleached tissues in some cases. However, in the majority of tested cases (9 of 14), new zooxanthellae did become established: *Symbiodinium* A, E, and/or C2 repopulated treated tissues (Fig. 6, Corals 4, 7–14), despite the proximity ($\leq$7 polyps away; see Weil and Knowlton, 1994) of untreated zooxanthellae (*Symbiodinium* C, with one exception). When new zooxanthellae became established, they were observed more frequently in the deeper habitat (8 of 9 columns in the deep group *vs.* 1 of 5 in the shallow group), and when resident zooxanthellae were *Symbiodinium* C (rather than *Symbiodinium* B)—our data do not resolve which factor had the greater influence. Nevertheless, these observations clearly show that a reservoir of adjacent zooxanthellae, whether *Symbiodinium* C or B, is not sufficient to prevent the establishment of new host-zooxanthella associations in bleached tissues.

We do not know where the new *Symbiodinium* in re-established symbioses came from. For most experimental corals, the fact that these zooxanthellae were not detected initially or after treatment is not good evidence that they were truly absent. This is because an srDNA genotype must be at least 5% of the total to be detected reliably (*e.g.*, for values of *ca.* 12%, see fig. 4 in Toller *et al.*, 2001; fig. 2B in Rowan *et al.*, 1997). Thus, corals that had *ca.* 1×10^5 cells/cm^2 of *Symbiodinium* C after low-light treatment (Fig. 3, Low Light, Treatment; also see Results) also may have contained up to about 5×10^3 cells/cm^2 of *Symbiodinium* A, E, or C2 that went undetected. With a hypothetical doubling time of 5 days (*e.g.*, Wilkerson *et al.*, 1988), 5×10^3 zooxanthellae/cm^2 become 150×10^5 zooxanthellae/cm^2 after only 8 weeks. Thus, even where only *Symbiodinium* C was detected right after treatment (Fig. 3, Low Light, Treatment; Colonies 1, 3, 4, 11, 12, and 14), there might have been enough *Symbiodinium* A, E, and/or C2 present to found the established symbioses observed 13 or 17 weeks later.

On the other hand, no data show that treated corals did not acquire *Symbiodinium* A, E, and C2 for the first time during recovery. Free-living *Symbiodinium* may be attracted specifically to hosts lacking zooxanthellae (Fitt, 1985b). Juveniles of host species that do not transmit zooxanthellae vertically (*e.g.*, *Montastraea*; Szmant, 1991) must be colonized, and the ability of adult hosts to pick up *Symbiodinium* from the environment has been documented for bleached anemones (Kinzie *et al.*, 2001) and for juvenile giant clams originally inoculated with cultured zooxanthellae (Belda-Baillie *et al.*, 1999; also see Fitt, 1984).

Regardless of where *Symbiodinium* A, E, and C2 came from, they fared well compared to any *Symbiodinium* C or B that remained in corals after low-light treatment. For example, the about 1×10^5 cells/cm^2 of *Symbiodinium* C that six corals in Experiment I contained after treatment (Fig. 3, Low Light, Treatment; Colonies 1, 3, 4, 11, 12, and 14) would have been observed in re-established symbioses if they had doubled only three or four times during 13 or 17 weeks (doubling times of 23–29 days; a slow rate of growth for zooxanthellae in general [Wilkerson *et al.*, 1988]). Thus, the identities of re-established symbioses in these six corals resulted not only from the proliferation, acquisition, or both of *Symbiodinium* A, E, or C2, but also from the failure of *Symbiodinium* C to proliferate.

Competition and succession in zooxanthellar communities

In general, the first phototrophs to colonize disturbed habitat are transient and eventually replaced by competitively superior species that dominate thereafter. This process is called succession (Odum, 1969; Connell and Slatyer, 1977; Huston and Smith, 1987), and it might eventually have led from *Symbiodinium* A, E, and C2 to *Symbiodinium* C or B, and thus restored the host-symbiont specificity observed in nature. We did not observe this hypothetical succession of zooxanthellae. However, the only corals we followed for more than 17 weeks after treatment (Fig. 4, Final; 33 or 37 weeks after treatment) experienced a natural bleaching event (Results) that apparently reduced populations of *Symbiodinium* C by about 80% in control and high-light-treated *M. annularis*; *Symbiodinium* B may have been affected also (Fig. 4, Final *vs.* Recovery). It seems unlikely that *Symbiodinium* C or B would have proliferated in low-light-treated corals during the same period of time.

Thus, unfavorable conditions might explain why succession was not observed.

Hypotheses on the mechanisms of plant succession invoke genetic differences in the abilities of species to compete for resources such as water, light, and nutrients, supplies of which decrease as succession proceeds (*e.g.*, Huston and Smith, 1987; Tilman, 1988; Wilson, 1999). Zooxanthellae in unmanipulated corals cannot be water-limited, nor can they extensively shade one another (Drew, 1972), but they probably are nutrient-limited (Rees, 1991; Falkowski *et al.*, 1993). In contrast, severely bleached corals may be nutrient-rich zooxanthellar habitats because the waste products of coral heterotrophy go largely unutilized (*e.g.*, Szmant-Froelich and Pilson, 1977; Muscatine and D'Elia, 1978); competition among zooxanthellae for nutrients may be minimal in this case. Competition should increase, however, as zooxanthellar biomass increases, and the zooxanthellar genotype that competes for nutrients best should ultimately prevail, regardless of its rate of growth in the absence of competition or its initial abundance (*e.g.*, Huston and Smith, 1987; Tilman, 1988).

In *M. annularis* and *M. faveolata* living at 9 m depth at our study site, those efficient, specialized, but comparatively slowly growing zooxanthellae might be *Symbiodinium* C and B. In contrast, *Symbiodinium* A, E, and C2 seem to have played the role of early successional, rapidly proliferating opportunists in our experiments. In Experiment II, *Symbiodinium* A reached large numbers in only 8 weeks (Fig. 5, Recovery; see also Fig. 3, Low Light). Opportunistic behavior by *Symbiodinium* A has also been observed in *M. annularis* and *M. faveolata* during a natural coral bleaching event (Rowan *et al.*, 1997) and in mixed *in vitro* cultures of *Symbiodinium* (Rowan, 1998; Carlos *et al.*, 1999). Our observation of *Symbiodinium* E in *Montastraea* living in a marginal habitat near our study site (Río Cartí; see Toller *et al.*, 2001) also suggests a weed-like ecology. Similarly, the unusual association of *Symbiodinium* C2 with *M. annularis* (Results) suggests that our experimental treatment enabled this zooxanthella to exploit a host species with which it does not commonly associate. We note, however, in the cases of both *Symbiodinium* A and E, it is unknown whether different observations involved one or several species of zooxanthella (see discussion on taxonomy, above).

Stability of zooxanthellar communities and coral bleaching

During natural episodes of coral bleaching (reviewed in Brown, 1997), even severely bleached colonies of *M. annularis* retain at least 10% of their pre-bleaching population of zooxanthellae (Porter *et al.*, 1989; Fitt *et al.*, 2000), which represents at least four times as many zooxanthellae as our low-light-treated corals had. Thus, the dramatic changes in species of *Symbiodinium* that we observed following low-light treatment of *M. annularis* and *M. faveolata* are unlikely to be common in nature. Mild coral bleaching or seasonal fluctuations in numbers of zooxanthellae in normal years (Stimson, 1997; Fagoonee *et al.*, 1999; Fitt *et al.*, 2000) involve lesser depletions of zooxanthellae, comparable to those observed in high-light-treated *M. annularis* in which communities of *Symbiodinium* did not change (Fig. 3, High Light, Colonies 6–10). Nevertheless, two high-light-treated corals that retained about 15% and 20% (Fig. 3, Colonies 4 and 5, respectively) of their zooxanthellae after treatment apparently did acquire detectable amounts of *Symbiodinium* E as a result of treatment. This suggests that severe natural bleaching episodes might modify communities of *Symbiodinium* in *M. annularis*, at least in part.

If infrequent, natural bleaching events at our study site (*e.g.*, Lasker *et al.*, 1984; Rowan *et al.*, 1997) do allow *Symbiodinium* E or C2 to proliferate in *M. annularis* and *M. faveolata* from time to time, the effect must be transient or slight. These host-zooxanthella combinations are rarely encountered in unmanipulated corals from this reef (Rowan and Knowlton, 1995; Rowan *et al.*, 1997; this study), despite occasional coral bleaching. On the other hand, at a nearby coastal site (Río Cartí), where stresses that can induce coral bleaching may be severe and frequent (Toller *et al.*, 2001), disturbance appears to have a widespread effect on host-zooxanthella association. In high-irradiance habitats, *M. faveolata* and *M. annularis* at Río Cartí associate predominantly with *Symbiodinium* E—an opportunistic taxon of zooxanthella (above) that may also be stress-resistant (Toller *et al.*, 2001). In this environment, these host-zooxanthella associations resemble the persistence of early-to-mid successional phototrophs under conditions of chronic disturbance (Odum, 1969; Horn, 1974; Huston and Smith, 1987).

Buddemeier and Fautin (1993) proposed that bleaching allows corals to replace their zooxanthellae with different, better ones (see also Baker, 1999, 2001; Kinzie *et al.*, 2001). According to this "adaptive bleaching hypothesis," such replacements are driven by environmental change, which simultaneously makes some host-zooxanthella combinations less well adapted and other combinations better adapted than they had been. Baker (2001) found that the mortality of corals challenged with prolonged environmental change (transplantation) was reduced when they acquired new zooxanthellae, but these new host-zooxanthella associations were only established after coral bleaching. Similarly, our experiments indicate that severe bleaching allowed corals to associate with new zooxanthellae: *Symbiodinium* A, E, or C2 replaced *Symbiodinium* C or B in *Montastraea* (see above). However, in our experiments, environmental change was not a prerequisite; instead, *Symbiodinium* A, E, or C2 proliferated in the very environment that *Symbiodinium* C or B apparently thrive in (Figs. 3 and

5; Recovery). Although our experiments did not include prolonged environmental change and therefore was not a direct test of the adaptive bleaching hypothesis, they do show that testing this hypothesis may not be straightforward-severe coral bleaching may favor zooxanthellar replacements, irrespective of environmental change.

Disease-related disturbance of zooxanthellar symbioses

Symbiodinium A especially and also *Symbiodinium* E proliferated in the bleached tissues of corals with yellow-blotch disease (Fig. 7, Yellow). These zooxanthellae apparently gave many lesions of YBD their distinctive yellow color, and they were fugitives in the strict sense because their habitat was ephemeral. Our measurements suggest that their habitat lasted an average of about 5 months. Nonetheless, continuous progression of YBD across a coral would provide a large amount of habitat for these fugitive zooxanthellae to occupy.

Our findings explain the "yellow" in YBD, but they do not address the cause of the pathology. Most colonies we encountered were in two clusters that were surrounded widely by unaffected corals, which suggested an infectious agent with limited dispersal. YBD may have more than one etiology: our experimental results imply that it would arise when anything spread through a coral and disturbed stress-sensitive communities of zooxanthellae (*Symbiodinium* B or C) without actually killing the host immediately. One agent might even be a "rogue" (parasitic) genotype of *Symbiodinium* A that prospered at the expense of its coral host.

Acknowledgments

We thank the Kuna Nation and the Republic of Panama (Autoridad Nacional del Ambiente, Departamento de Cuarentena Agropecuaria del Ministerior de Desarrollo Agropecuario, and Recursos Marinos) for permission to collect and export specimens. Many thanks to Javier Jara for tireless field assistance and cell counts. Ursula Anlauf, Ralf Kersanach, Mike McCartney, and Suzanne Williams provided valuable advice. Mick Wilson and Dave Wilson assisted with field manipulations. R. R. thanks Chris Hein and Uma Narayan for hospitality in California. This research was supported by the Andrew W. Mellon Foundation, the Smithsonian Tropical Research Institute, the National Institutes of Health, and the Scripps Institution of Oceanography.

Literature Cited

Baker, A. C. 1999. Symbiosis ecology of reef-building corals. Ph.D. dissertation, University of Miami. 120 pp.

Baker, A. C. 2001. Reef corals bleach to survive change. *Nature* **411:** 765–766.

Belda-Baillie, C. A., M. Sison, V. Silvestre, K. Villamor, V. Monje, E. D. Gomez, and B. K. Baillie. 1999. Evidence for changing symbiotic algae in juvenile tridacnids. *J. Exp. Mar. Biol. Ecol.* **241:** 207–221.

Brown, B. E. 1997. Coral bleaching: causes and consequences. *Coral Reefs* **16:** S129– S138.

Buddemeier, R. W., and D. G. Fautin. 1993. Coral bleaching as an adaptive mechanism. *Bioscience* **43:** 320–326.

Cairns, S. D. 1999. Species richness of recent Scleractinia. *Atoll Res. Bull.* **459:** 1–12.

Carlos, A. A., B. K. Baillie, M. Kawachi, and T. Maruyama. 1999. Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. *J. Phycol.* **35:** 1054–1062.

Colley, N. J., and R. K. Trench. 1983. Selectivity in phagocytosis and persistence of symbiotic algae by the scyphistoma stage of the jellyfish *Cassiopeia xamachana. Proc. R. Soc. Lond. B* **219:** 61–82.

Connell, J. H., and R. O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.* **111:** 1119–1144.

Davy, S. K., I. A. N. Lucas, and J. R. Turner. 1997. Uptake and persistence of homologous and heterologous zooxanthellae in the temperate sea anemone *Cereus pedunculatus* (Pennant). *Biol. Bull.* **192:** 208–216.

Drew, E. A. 1972. The biology and physiology of alga-invertebrate symbioses. II. The density of symbiotic algal cells in a number of hermatypic corals and alcyonarians from various depths. *J. Exp. Mar. Biol. Ecol.* **9:** 71–75.

Dustan, P. 1979. Distribution of zooxanthellae and photosynthetic chloroplast pigment of the reef-building coral *Montastrea annularis* Ellis and Solander in relation to depth on a West Indian coral reef. *Bull. Mar. Sci.* **29:** 79–95.

Fagoonee, I., H. B. Wilson, M. P. Hassell, and J. R. Turner. 1999. The dynamics of zooxanthellae populations: a long-term study in the field. *Science* **283:** 843–845.

Falkowski, P. G., Z. Dubinsky, L. Muscatine, and L. R. McCloskey. 1993. Population control in symbiotic corals. *Bioscience* **43:** 606–611.

Fitt, W. K. 1984. The role of chemosensory behavior of *Symbiodinium microadriaticum,* intermediate hosts and host behavior in the infection of coelenterates and molluscs with zooxanthellae. *Mar. Biol.* **81:** 9–17.

Fitt, W. K. 1985a. Effect of different strains of the zooxanthella *Symbiodinium microadriaticum* on growth and survival of their coelenterate and molluscan hosts. *Proc. Fifth Int. Coral Reef Congr. (Tahiti).* **6:** 131–136.

Fitt, W. K. 1985b. Chemosensory responses of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Dinophyceae). *J. Phycol.* **21:** 62–67.

Fitt, W. K., F. K. McFarland, M. E. Warner, and G. C. Chilcoat. 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol. Oceanogr.* **45:** 677–685.

Franzisket, L. 1970. The atrophy of hermatypic reef corals maintained in darkness and their subsequent regeneration in light. *Int. Rev. Hydrobiol.* **55:** 1–12.

Gateño, D., A. Israel, Y. Barki, and B. Rinkevich. 1998. Gastrovascular circulation in an octocoral: evidence of significant transport of coral and symbiont cells. *Biol. Bull.* **194:** 178–186.

Gladfelter, E. H. 1983. Circulation of fluids in the gastrovascular system of the reef coral *Acropora cervicornis. Biol. Bull.* **165:** 619–636.

Horn, H. S. 1974. The ecology of secondary succession. *Annu. Rev. Ecol. Syst.* **5:** 25–37.

Huston, M., and T. Smith. 1987. Plant succession: life history and competition. *Am. Nat.* **130:** 168–198.

Kinzie, R. A., III, and G. S. Chee. 1979. The effect of different

zooxanthellae on the growth of experimentally reinfected hosts. *Biol. Bull.* **156:** 315–327.

Kinzie, R. A., III, M. Takayama, S. R. Santos, and M. A. Coffroth. 2001. The adaptive bleaching hypothesis: experimental tests of critical assumptions. *Biol. Bull.* **200:** 51– 58.

Lasker, H. R., E. C. Peters, and M. A. Coffroth. 1984. Bleaching of reef coelenterates in the San Blas Islands, Panama. *Coral Reefs* **3:** 183–190.

McNally, K. L., N. S. Govind, P. E. Thomé, and R. K. Trench. 1994. Small-subunit ribosomal DNA sequence analyses and a reconstruction of the inferred phylogeny among symbiotic dinoflagellates (Pyrrophyta). *J. Phycol.* **30:** 316–329.

Muscatine, L., and C. F. D'Elia. 1978. The uptake, retention, and release of ammonium by reef corals. *Limnol. Oceanogr*. **23:** 725–734.

Odum, E. P. 1969. The strategy of ecosystem development. *Science* **164:** 262–270.

Porter, J. W., W. K. Fitt, H. J. Spero, C. S. Rogers, and M. W. White. 1989. Bleaching in reef corals: physiological and stable isotope responses. *Proc. Natl. Acad. Sci. USA* **86:** 9342–9346.

Rees, T. A. V. 1991. Are symbiotic algae nutrient deficient? *Proc. R. Soc. Lond. B* **243:** 227–234.

Roux, K. H. 1995. Optimization and troubleshooting in PCR. Pp. 53–62 in *PCR Primer: A Laboratory Manual*, C. W. Dieffenbach and G. S. Dveksler, eds. Cold Spring Harbor Laboratory Press, New York.

Rowan, R. 1998. Diversity and ecology of zooxanthellae on coral reefs. *J. Phycol.* **34:** 407–417.

Rowan, R., and N. Knowlton. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc. Natl. Acad. Sci. USA* **92:** 2850–2853.

Rowan, R., and D. A. Powers. 1991a. A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. *Science* **251:** 1348–1351.

Rowan, R., and D. A. Powers. 1991b. Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). *Mar. Ecol. Prog. Ser.* **71:** 65–73.

Rowan, R., N. Knowlton, A. Baker, and J. Jara. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* **388:** 265–269.

Santavy, D. L., E. C. Peters, C. Quirolo, J. W. Porter, and C. N. Bianchi. 1999. Yellow-blotch disease outbreak on reefs of the San Blas Islands, Panama. *Coral Reefs* **18:** 97.

Schoenberg, D. A., and R. K. Trench. 1980. Genetic variation in *Symbiodinium* (*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *S. microadriaticum. Proc. R. Soc. Lond. B* **207:** 445–460.

Stimson, J. 1997. The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus). *J. Exp. Mar. Biol. Ecol.* **214:** 35–48.

Szmant, A. M. 1991. Sexual reproduction by the Caribbean corals *Montastrea annularis* and *M. cavernosa. Mar. Ecol. Prog. Ser.* **74:** 13–25.

Szmant-Froelich, A., and M. E. Q. Pilson. 1977. Nitrogen excretion by colonies of the temperate coral *Astrangia danae* with and without zooxanthellae. *Proc. Third Int. Coral Reef Symp.* **1:** 418–423.

Tilman, D. 1988. *Plant Strategies and the Dynamics and Structure of Plant Communities.* Princeton University Press, Princeton, NJ.

Toller, W. W., R. Rowan, and N. Knowlton. 2001. Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol. Bull.* **201:** 348–359.

Toller, W. W., R. Rowan, and N. Knowlton. In press. Genetic evidence for a protozoan (phylum Apicomplexa) associated with corals of the *Montastraea annularis* species complex. *Coral Reefs.*

Trench, R. K. 1988. Specificity in dinomastigote-marine invertebrate symbioses: an evaluation of hypotheses of mechanisms involved in producing specificity. Pp. 325–346 in *Cell to Cell Signals in Plant, Animal and Microbial Symbiosis*, S. Scannerini, D. C. Smith, P. Bonfante-Fasolo, and V. Gianninazzi-Pearson, eds. NATO Advanced Research Workshop on Cell to Cell Signals in Plant, Animal, and Microbial Symbiosis (1987: Turin, Italy). Springer-Verlag, Berlin.

Trench, R. K. 1993. Microalgal-invertebrate symbioses: a review. *Endocytobiosis Cell Res.* **9:** 135–175.

Trench, R. K. 1997. Diversity of symbiotic dinoflagellates and the evolution of microalgal-invertebrate symbioses. *Proc. Eighth Int. Coral Reef Symp.* **2:** 1275–1286.

Veron, J. E. N. 1995. *Corals in Space and Time: The Biogeography and Evolution of the Scleractinia.* UNSW Press, Sydney, Australia.

Weil, E., and N. Knowlton. 1994. A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Gregory, 1895). *Bull. Mar. Sci.* **55:** 151–175.

Wells, J. W. 1956. Scleractinia. Pp. 328–444 in *Treatise in Invertebrate Paleontology, Part F*, R. C. Moore, ed. Geological Society of America and University of Kansas Press, Lawrence, KS.

Wilkerson, F. P., D. Kobayashi, and L. Muscatine. 1988. Mitotic index and size of symbiotic algae in Caribbean reef corals. *Coral Reefs* **7:** 29–36.

Wilson, S. D. 1999. Plant interactions during secondary succession. Pp. 611–632 in *Ecosystems of the World. Vol. 16: Ecosystems of Disturbed Ground*, L. R. Walker, ed. Elsevier, New York.