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Highly stable symbioses among western Atlantic brooding corals

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Abstract The reproductive mode of corals largely determines how zooxanthellae (*Symbiodinium* spp.) are acquired. Typically, broadcast spawning corals obtain symbionts from the surrounding environment, whereas most brooders transfer symbionts from maternal parent to offspring. Brooding corals are therefore predicted to harbor stable communities of *Symbiodinium*. This study documents the associations between *Symbiodinium* spp. and brooding corals in response to seasonal environmental fluctuations. Between March 2002 and December 2005, endosymbiont identity was determined seasonally from replicate colonies ($n = 6$) of three brooding species, *Agaricia agaricites*, *Porites astreoides* and *Siderastrea radians*, from shallow environments (1–4 m) of the Florida Keys and Bahamas. Symbionts were identified via denaturing gradient gel electrophoresis (DGGE) of the internal transcribed spacer 2 (ITS2) region. No change was detected in the *Symbiodinium* communities harbored within these brooding colonies. Additionally, no change in symbiosis was observed through a moderate bleaching event, thereby demonstrating that some bleached corals recover without changing symbionts.

Keywords Brooding corals · Coral bleaching · *Symbiodinium* · Vertical transmission · Zooxanthellae

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Introduction

Reef-building corals commonly associate in mutualisms with dinoflagellates of the genus *Symbiodinium* (Trench 1993; Rowan 1998). Phylogenetic studies based on the nuclear ribosomal RNA genes (e.g., Rowan and Powers 1991; LaJeunesse 2001; van Oppen et al. 2001) and chloroplast large subunit (cp23S)-rDNA genes (Santos et al. 2002) reveal that *Symbiodinium* consists of eight major sub-generic clades (A–H) and numerous genetically distinct types based on the rDNA Internal Transcribed Spacers (ITS). A coral colony typically harbors only one detectable type of *Symbiodinium* spp. type (e.g., LaJeunesse 2002). Heterogeneous populations consisting of several *Symbiodinium* types within a colony are infrequently detected (e.g., Rowan et al. 1997). However, many coral species are capable of associating with more than one symbiont type; these different host–symbiont combinations usually depend heavily upon a colony's geographic location and depth distribution (e.g., Rowan and Knowlton 1995; LaJeunesse 2002; Iglesias-Prieto et al. 2004).

The mechanism of symbiont acquisition typically depends on the reproductive strategy of the coral host (Richmond and Hunter 1990). Most corals broadcast spawn aposymbiotic gametes into the water column (Richmond and Hunter 1990). In such cases symbionts are normally "horizontally" acquired after settlement from the surrounding environment, thereby creating the possibility for new host-symbiont combinations with each new generation (Richmond and Hunter 1990). In contrast, corals that internally brood their larvae usually transfer symbionts "vertically" from the maternal parent to offspring (Richmond and Hunter 1990). Because of the maternal transmission of zooxanthellae, symbioses in brooding corals are hypothesized to be more specific than those of broadcast spawners (e.g., Loh et al. 2001). Across a wide biogeographic gradient, LaJeunesse et al. (2004) found environment and latitude determined symbiont associations in broadcast spawning corals whereas

brooding corals associated with a number of closely related symbionts specialized exclusively on a single host genus. Similarly, Barneah et al. (2004) observed that symbiont clade correlated with mode of acquisition in Red Sea soft corals. Loh et al. (2001) reported a higher degree of regional divergence in the symbionts of the brooding *Seriatopora hystrix* compared to the broadcasting *Acropora longicyathus* suggesting regional host-symbiont coevolution and specialization in brooding corals. However, van Oppen (2004) found that mode of symbiont acquisition does not affect the overall level of symbiont diversity in Indo-Pacific acroporid corals, suggesting that brooding corals are more open to exogenous symbiont infection than previous hypothesized.

Few previous studies have examined multi-year host-symbiont relationships. While available data predominantly shows stable symbioses (Goulet and Coffroth 2003; Thornhill et al. 2006), fluctuations in *Symbiodinium* spp. populations have been observed in several corals in response to environmental change (Baker 2001; Toller et al. 2001). To further explore the stability of symbiotic associations in brooding corals, this study examined the extent to which three brooding species [*Agaricia agaricites* (Linnaeus), *Porites astreoides* (Lamarck), and *Siderastrea radians* (Pallas)], which acquire their symbionts from the maternal parent, undergo changes in their symbiotic associations over time. If variance in abiotic factors, such as temperature and light, leads to variable symbioses, this pattern should be evident at the northern and southern limits of reef occurrence, where seasonality is most pronounced. Therefore, this study examined symbioses in two northern reef systems, the Bahamas and Florida Keys.

Materials and methods

Between March 2002 and December 2005, coral tissue samples were collected several times per year from three species of brooding scleractinian coral, *A. agaricites*, *P. astreoides*, and *S. radians* from inshore habitats (1–4 m) off Lee Stocking Island, the Bahamas (24°15′N, 76°30′W) and the Upper Florida Keys (24°59′N, 80°22′W). Six replicate colonies were tagged for each available species to ensure that subsequent collection was from the same colony. *S. radians* was the only species not collected in this manner due to the small size of colonies; instead samples were obtained from six distinct colonies from approximately the same area for each sampling period. Approximately 4 cm diameter tissue samples were collected from "boulder" corals via hammer and chisel, with care taken to ensure that the same relative position (i.e., the unshaded colony tops) was sampled each time. For "leaf" colonies of *A. agaricites*, approximately 4 cm diameter leaves were snapped off by hand. Coral fragments were placed in seawater filled, prelabeled plastic bags and transported immediately to the laboratory in an insulated cooler, where they were processed immediately.

Coral tissue was removed from 5 to 25 cm^2 of coral skeleton with a recirculating Water Pik™ using filtered (0.45 μm) seawater. Tissue was removed with this method from equal areas on either side of *A. agaricites* leaves and completely from the tops of *P. astreoides* and *S. radians* fragments. This salt-water "blastate" was pulsed briefly (1–4 s) using a Brinkmann Instruments Tissue Homogenizer™ to disperse mucopolysacharides. *Symbiodinium* spp. cells were isolated from the remaining salt-water blastate via centrifugation in 50 ml tubes at 1,000*g* and the resulting algal pellet was preserved in DMSO buffer (20% dimethyl sulfoxide and 0.25 M ethylene diaminetetraacetic acid (EDTA) in NaCl-saturated water) (Seutin et al. 1991).

Nucleic acids were extracted using the Wizard® (Promega) DNA preparation protocol following the methods of Thornhill et al. (2006). The internal transcribed spacer 2 region (ITS 2) of nuclear ribosomal RNA was used to discriminate molecular types of *Symbiodinium* (LaJeunesse 2001, 2002). This region was amplified from the DNA extract for denaturing-gradient gel electrophoresis (DGGE) using primers "ITS 2 clamp" (5′-CGCCCGCC GC GCCCCGCGCC CGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGT-3′) and "ITSintfor 2" (5′-GAATTGCAGA ACTCCGTG-3′). PCR amplification followed the "touchdown" thermal cycle protocol of LaJeunesse (2002). Samples containing successfully amplified PCR products were subsequently electrophoresed on denaturing gradient gels following the methods outline in LaJeunesse (2002). Because of difficulty working with *P. astreoides* extracts, in addition to DGGE analysis, the genetic identity of *Symbiodinium* was verified via Sanger DNA sequence analysis with an applied Biodsystems ABI Prism 377™ according to the protocol of LaJeunesse (2002).

Mean daily sea-surface temperature was obtained from the Coral Health and Monitoring Program (CHAMP) for North Norman's Reef off Lee Stocking Island in the Bahamas and from the NOAA National Data Buoy Center for Molasses Reef in the Upper Florida Keys. The data was annotated for errors and analyzed using Statstica 6 (Statsoft™).

Results and discussion

The symbiont types detected in replicate colonies ($n = 6$) of *A. agaricites, P. astreoides*, and *S. radians* from March 2002 to December 2005 were compiled in pie charts (Fig. 1). Unless otherwise noted, each pie chart represents one season and a 1/6 wedge of a pie chart designates each colony. Because PCR–DGGE is not a quantitative method, relative abundance above the minimum detection threshold (≥7%, see Thornhill et al. 2006) of a particular symbiont type cannot be precisely determined. As a result, detection of multiple types within a colony is illustrated by splitting a 1/6 wedge between the two types. Each row of pie charts was taken from the same replicate colonies.

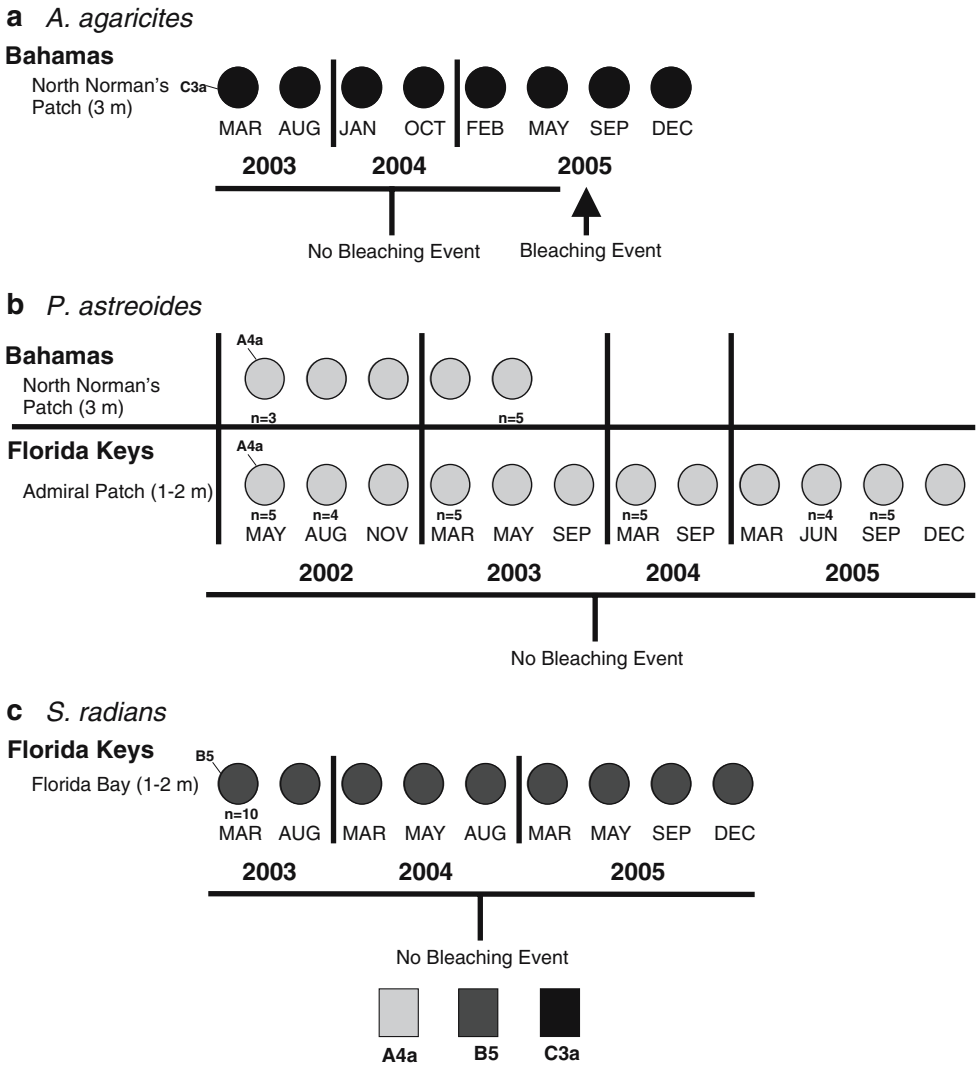

Fig. 1 ITS 2 *Symbiodinium* types detected in colonies from the Bahamas and Florida Keys from March 2002 to December 2005. Colonies ($n = 6$) listed by region, reef type, and depth as rows of pie charts. Symbiont type in each set of colonies is designated with its alphanumeric clade designation. Instances of fewer or greater than six colonies are demarcated by "$n =$ the number of replicates" below a relevant chart. Bleaching history of these colonies is listed below the relevant pie charts. **a** *Agaricia agaricites*. **b** *Porites astreoides*. **c** *Siderastrea radians*

At the detection level of DGGE, colonies of all three brooding coral species were invariably symbiotic with a single dominant *Symbiodinium* type (Fig. 1). This pattern occurred throughout the sampling; no change was observed in relation to seasonal fluctuations. Additionally, during early September 2005 all *A. agaricites* colonies from the Bahamas experienced significant visual bleaching. Despite this, no change in symbiont type was observed during or after this event.

To some extent, this stability exceeds that encountered for most broadcast spawning corals sampled from the same locations and seasonal periods (see Thornhill et al. 2006). Although most broadcast spawning colonies were generally stable in their symbioses, occasionally mixed populations of multiple ITS2 types have been detected, even in the absence visible coral bleaching (Thornhill et al. 2006). No such mixed populations occurred for any of the brooding colonies examined. This modest difference in the stability of the symbiosis may relate to the reproductive mode of the host. Vertical transmission of symbionts between the maternal parent and offspring theoretically limits the opportunity for the intergenerational symbiont change. Hypothetically, this would result in a more tightly coevolved relationship between host and symbiont, thereby reducing the opportunity for symbiotic change (Douglas 1998; Loh et al. 2001). Indeed, *Symbiodinium* types C3a and B5a are considered specialist types found only in their respective host genus (C3a in *Agaricia* spp. and B5a in *Siderastrea* spp.) (LaJeunesse 2002). Similar results have been described for *Symbiodinium* types (C21 and related types) associating exclusively with vertically transmitting *Montipora* spp. corals (LaJeunesse et al. 2004).

The Bahamas and Florida Keys approach the northern limits of reef accretion and are regions where seasonal fluctuations in temperature and light are most pronounced in the Caribbean reef system. Mean daily sea-surface temperature for reefs from these two regions is shown (Fig. 2). In 2005, sea-surface temperature off Lee Stocking Island exceeded 30°C for a total of 63 days out of the year. The duration of this 2005 high temperature markedly exceeded that of preceding years, temperatures were above 30°C for 17 days in 2002, data not available in 2003, and 22 days in 2004. As a result, many

species of corals from Lee Stocking Island bleached in 2005, including all of the *A. agaricites* colonies measured in this study. Despite the magnitude of these seasonal changes and a bleaching event in the Bahamas, no change in symbiont type was observed among any of the brooding corals examined. Barneah et al. (2004) suggested that the coevolved specialist symbionts found in brooding colonies must be able to tolerate a broad range of environmental conditions. The results of this study point to a similar conclusion. Change in the dominant type of *Symbiodinium* is hypothesized to facilitate acclimatization to a broad range of environmental conditions (Rowan and Powers 1991; Buddemeier and Fautin 1993; LaJeunesse 2005). Corals that maintain highly stable symbiotic associations with one dominant type of *Symbiodinium* through a range of environmental conditions must be capable of responding to environmental change through acclimatization on the part of either the coral host and/or the dinoflagellate symbiont.

Porites astreoides is known to be symbiotic with several different ITS types of *Symbiodinium* [including types A4a, A3, B1 (LaJeunesse 2002) as well as a C type (Baker 2001)], however, colonies from this study were detected with only type A4a. It is possible that there are distinct subpopulations of *P. astreoides* and other brooding corals, each associating with a distinct maternally inherited *Symbiodinium* type. Previous analyses of broad biogeographic patterns suggest that this is the case (Loh et al. 2001; LaJeunesse et al. 2004). However, Indo/Pacific Acroporid brooding corals show a similar level of overall symbiont diversity to broadcast spawners (van Oppen 2004). Comparative analysis of the host and symbiont fine scale population structure is necessary to further test this hypothesis.

Baker (2001) found that some *P. astreoides* colonies experimentally transplanted from deep (20–23 m) to shallow (2–4 m) habitats changed their dominant symbiont from clade C to clade A *Symbiodinium* ($n = 5$ out of 8), thereby indicating that *P. astreoides* colonies are not fixed in their symbioses. Transplanting colonies between two such drastically different environmental regimes of light and temperature also elicited a symbiotic change in several other coral species, including both brooding and broadcast spawning corals (Baker 2001). It is apparent that the fluctuations experienced by coral colonies in our study, including seasonal variation in temperature and light, as well as the 2005 temperature induced bleaching event in the Bahamas, was insufficient to drive a similar change in the dominant *Symbiodinium* type. Alternatively, insufficiently availability of alternative *Symbiodinium* types from endogenous or exogenous sources could explain the lack of symbiont change. It is also possible that change in the symbiotic associations of these species was missed due to the limited sample size ($n = 6$ per host species) of this study. Regardless of the explanation for these results, much of the current literature focuses on change in the dominant *Symbiodinium* type in response to bleaching stress (e.g., Buddemeier and Fautin 1993; Baker 2001; Toller et al. 2001; Berkelmans and van Oppen 2006). It is important to recognize that bleached corals sometimes recover without changing symbionts (see also Goulet and Coffroth 2003).

The overall trend of highly stable symbioses (Goulet and Coffroth 2003; Thornhill et al. 2006) appears to be especially true for the brooding corals examined in this study. Whether widespread symbiotic change will occur in brooding colonies through the predicted rise in global sea-surface temperature (Hoegh-Guldberg 1999) remains an open question. In order to predict how coral symbioses will respond to climatic change, future experiments should focus on the conditions required to drive symbiont change as well as the viability and physiological capacity of various host–symbiont combinations.

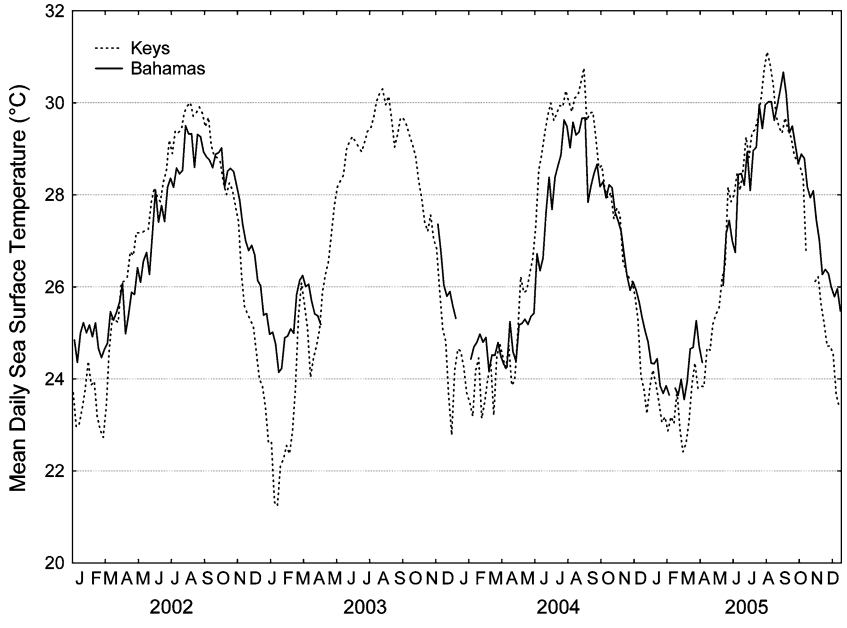

Fig. 2 Mean daily sea-surface temperature (°C) for North Norman's Patch Reef in the Bahamas (*solid line*) and Molasses Reef in the upper Florida Keys (*dashed line*)

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References

Baker AC (2001) Reef corals bleach to survive change. Nature 411:765–766

Barneah O, Weis VM, Perez S, Benayahu Y (2004) Diversity of dinoflagellate symbionts in Red Sea corals: mode of symbiont acquisition matters. Mar Ecol Prog Ser 275:89–95

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. Proc R Soc Lond B Biol Sci [doi:10.1098/rspb.2006.3567]

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. BioScience 43:320–326

Douglas AE (1998) Host benefit and the evolution of specialization in symbiosis. Heredity 81:599–603

Goulet TL, Coffroth MA (2003) Stability of an octocoral–algal symbiosis over time and space. Mar Ecol Prog Ser 250:117–124

Hoegh-Guldburg O (1999) Climate change, coral bleaching, and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc R Soc Lond B Biol Sci 271:1757–1763

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiontic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a "species" level marker. J Phycol 37:866–880

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387–400

LaJeunesse TC (2005) "Species" radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. Mol Biol Evol 22:570–581

LaJeunesse TC, Bhagooli R, Hidaka M, deVantier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004) Differences in relative dominance beween closely related *Symbiodinium* spp. in coral reef host communities across environmental, latitudinal, and biogeographic gradients. Mar Ecol Prog Ser 284:147–161

Loh WK, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. Mar Ecol Prog Ser 222:97–107

van Oppen MJH (2004) Mode of zooxanthellae transmission does not affect zooxanthella diversity in acroporid corals. Mar Biol 144:1–7

van Oppen MJH, Palstra FP, Piquet AT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc R Soc Lond Ser B 268:1759–1767

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the tropical Pacific and the Red Sea. Mar Ecol Prog Ser 60:185–203

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

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

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal–algal symbiosis. Science 251:1348–1351

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

Santos SR, Taylor DJ, Kinzie RA III, Hidaka M, Sakai K, Coffroth MA (2002) Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast subunit (cp23S)-rDNA sequences. Mol Phyl Evol 23:97–111

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analysis. Can J Zool 60:82–92

Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral–algal symbioses reveal prevalent stability or post-bleaching reversion. Mar Biol 148:711–722

Toller WW, Rowan R, Knowlton N (2001) Repopulation of zooxanthellae in Caribbean corals *Montastrea annularis* and *Montastrea faveolata* following experimental and disease induced bleaching. Biol Bull 201:360–373

Trench RK (1993) Microalgal–invertebrate symbiosis: a review. Endocytobiosis and Cell Research 9:135–175