CORRESPONDENCE BETWEEN COLD TOLERANCE AND TEMPERATE BIOGEOGRAPHY IN A WESTERN ATLANTIC Symbiodinium (Dinophyta) LINEAGE

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Many corals form obligate symbioses with photosynthetic dinoflagellates of the genus Symbiodinium Freudenthal (1962). These symbionts vary genotypically, with their geographical distribution and abundance dependent upon host specificity and tolerance to temperature and light variation. Despite the importance of these mutualistic relationships, the physiology and ecology of Symbiodinium spp. remain poorly characterized. Here, we report that rDNA internal transcribed spacer region 2 (ITS2) defined Symbiodinium type B2 associates with the cnidarian hosts Astrangia poculata and Oculina arbuscula from northerly habitats of the western Atlantic. Using pulse-amplitude-modulated (PAM) fluorometry, we compared maximum photochemical efficiency of PSII of type B2 to that of common tropical Symbiodinium lineages (types A3, B1, and C2) under cold-stress conditions. Symbiont cultures were gradually cooled from 26°C to 10°C to simulate seasonal temperature declines. Cold stress decreased the maximum photochemical efficiency of PSII and likely the photosynthetic potential for all Symbiodinium clades tested. Cultures were then maintained at 10°C for a 2-week period and gradually returned to initial conditions. Subsequent to low temperature stress, only type B2 displayed rapid and full recovery of PSII photochemical efficiency, whereas other symbiont phylotypes remained nonfunctional. These findings indicate that the distribution and abundance of Symbiodinium spp., and by extension their cnidarian hosts, in temperate climates correspond significantly with the photosynthetic cold tolerance of these symbiotic algae.

Key index words: Astrangia poculata; biogeography; cold temperature stress; coral; molecular diversity; Oculina arbuscula; Symbiodinium; zooxanthellae

Abbreviations: ITS2, internal transcribed spacer region 2; PAM, pulse amplitude modulated

The structural and trophic foundations for coral reef ecosystems are dependent upon the obligate mutualisms between reef-building corals and their dinoflagellate algal symbionts (genus Symbiodinium). Optimally, intracellular Symbiodinium provides the coral host with 90% or more of its energy needs through photosynthesis (Muscatine 1990). In the past few decades, however, episodes of increased ocean temperatures have led to disruptions of these important symbiotic associations as a result of thermal stress (Hoegh-Guldberg 1999, Hughes et al. 2003). Therefore, considerable attention has been given to the possibility that thermal acclimatization of corals may occur via acquisition of heat-tolerant dinoflagellate symbiont complements (e.g., Buddemeier and Fautin 1993, Baker et al. 2004, Berkelmans and van Oppen 2006, Goulet 2006, Thornhill et al. 2006a,b). An alternative conjecture is that corals might undergo selective adaptation including latitudinal shifts in distribution. This possibility would require host and symbiont resilience to considerable seasonal changes in temperature and
diurnal light exposure compared to the more constant environment in the tropics (Muller-Parker and Davy 2001).

*Symbiodinium* is a genetically diverse group, consisting of eight divergent lineages or clades (designated A through H; Pochon et al. 2006) and numerous more taxonomically specific types (reviewed in Baker 2003, Coffroth and Santos 2005, Stat et al. 2006). However, relatively little is known about the physiological properties that distinguish the various types. Differential tolerance of some symbiont species to high temperature stress has been the subject of many recent reports (e.g., Warner et al. 1996, 1999, Rowan 2004, Tchernov et al. 2004, Berkelmans and van Oppen 2006). Exposure to high temperature stress is now recognized to generally culminate in chronic photoinhibition in the symbionts (Warner et al. 1999). Such thermally induced perturbations are likely triggered by decreased photochemical and nonphotochemical quenching of PSII excitation energy, which can be exacerbated by disabled repair of photodamaged PSII in some symbiont types (Warner et al. 1999). Symbiotic dinoflagellates exposed to cold temperature stress may also undergo chronic photoinhibition (Saxby et al. 2003), corresponding to decreased photosynthetic membrane fluidity, as has been documented for many vascular plant species (Smille et al. 1988, Aro et al. 1990, Greer 1990, Krause 1992). Furthermore, low temperature stress might disrupt the host–symbiont association by mechanisms akin to high-temperature-induced coral bleaching (Steen and Muscatine 1987, Hoegh-Guldberg et al. 2005).

The distribution of coral reefs appears to be primarily limited by a lower thermal threshold of ~18°C, light limitation, and decreased aragonite saturation state in high-nutrient temperate waters (Dana 1843, Vaughan 1919, Birkeland 1988, Crossland 1988, Kleypas et al. 1999), although biotic interactions have also been implicated (Johannes et al. 1983, Miller and Hay 1996). While diversity of symbiotic cnidarians is low outside the tropics, several species of symbiotic corals, anemones, and other cnidarians occur in temperate habitats (Schuhmacher and Zibrowius 1985). Similar to tropical corals, temperate cnidarians hosting *Symbiodinium* benefit from enhanced calcification and growth rates (Jacques and Pinson 1980, Jacques et al. 1983, Miller 1995, Dimond and Carrington 2007), reduced nitrogen loss (Szmant-Froelich and Pinson 1984), and translocation of photosynthetically fixed carbon (Szmant-Froelich 1981, Schiller 1993, Miller 1995). However, unlike the obligate associations occurring in most tropical hosts, temperate symbiotic cnidarians typically associate facultatively with *Symbiodinium* and often survive without symbionts via heterotrophic feeding alone (Jacques et al. 1983, Szmant-Froelich and Pinson 1984, Schuhmacher and Zibrowius 1985, Farrant et al. 1987, Dimond and Carrington 2007). Seasonal fluxes in temperature, light, and nutrients are considerably more pronounced in temperate versus tropical marine habitats (e.g., Muller-Parker and Davy 2001). Despite this, zooxanthellae densities in symbiotic anemones, for example, are apparently more stable and less seasonal in temperate than in tropical hosts (Muller-Parker and Davy 2001 and references within), raising questions about how these temperate symbioses function physiologically.

Tropical and subtropical coral reefs contain complex communities of numerous *Symbiodinium* species (e.g., Rowan and Knowlton 1995, van Oppen et al. 2001, LaJeunesse 2002, LaJeunesse et al. 2003, Thorntill et al. 2006a,b). The diversity of *Symbiodinium* types from temperate habitats is apparently lower than that occurring in the tropics (LaJeunesse and Trench 2000, LaJeunesse 2001, Rodriguez-Lanetty et al. 2001, 2003, Savage et al. 2002, Visram et al. 2006). However, symbiotic diversity in many temperate regions and the physiological properties of these symbionts remain largely unexplored. Here, we examine the biogeography and photosynthetic physiology of *Symbiodinium* spp. associated with two scleractinian hosts from the temperate western Atlantic. The identities of *Symbiodinium* types from the corals *A. pociulata* and *O. arbuscula* were characterized using denaturing gradient gel electrophoresis (DGGE) and sequencing of the ITS2 region of the rDNA.

It has been hypothesized that temperate zooxanthellae should be able to withstand greater ranges of temperatures than their tropical counterparts (Muller-Parker and Davy 2001). Therefore, we tested this hypothesis using PAM fluorometry to characterize the maximum quantum efficiency of PSII ($F_v/F_m$) and relative electron transport rates (ETR) of cultured temperate and tropical *Symbiodinium* types under cold-stress conditions. Such investigations of the relationship between *Symbiodinium* physiology and biogeography provide further insights into the ecology and evolution of this important dinoflagellate group.

**MATERIALS AND METHODS**

**Sample collection and Symbiodinium identification.** In 2005, the symbiotic hosts *A. pociulata* (= *A. danca*, = *A. astreiformis*, Peters et al. 1988) and *O. arbuscula* were surveyed from three coastal locations along the eastern seaboard of the U.S., including a limestone and live-bottom reef near Gray’s Reef National Marine Sanctuary (J Reef, Georgia, USA; 31.601° N, 80.791° W); an open bay site in Fort Wetherill State Park, Narragansett Bay (Rhode Island, USA; 41.478° N, 71.357° W); and a tidal river site in the lower Pettaquamscutt River, Narragansett Bay (Rhode Island, USA; 41.449° N, 71.449° W; Table 1). A map of the estimated distributions of these scleractinian species and sampling locations for this study is provided in Figure 1. Temperatures at these noncoral reef sites are always <18°C during winter months (Table 1; for more details on the temperature and other conditions in Narragansett Bay see Dimond and Carrington 2007), the purported
lower temperature threshold for coral reef accretion (Dana 1843, Vaughan 1919, Kleypas et al. 1999). Coral fragments (<5 cm³) were collected by SCUBA using a hammer and chisel. Fragments were placed into 70% ethanol to preserve DNA during transport back to the laboratory. 

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Identify Symbiodinium ITS2 types (LaJeunesse 2001, 2002). The ITS2 rDNA was amplified from each extract using the primer set “ITS2clamp” and “ITSinfotr2” (LaJeunesse and Trench 2000) with touchdown thermal cycle as described in LaJeunesse et al. (2003). Products from these PCR reactions were electrophoresed for 1,500 volt-hours at 60°C on 8% polyacrylamide denaturing gradient gels (45%-80% formamide/urea wherein 100% of the denaturants are 40% formamide and 7 M urea) using a CBS Scientific DGGE system (DelMar, CA, USA). The diagnostic bands (including dominant and minor bands) in each profile were then excised, reamplified, and bidirectionally sequenced using GenomeLab™ Quick Start Mix (Beckman Coulter, Fullerton, CA, USA) on a Beckman CEQ 8000 Genetic Analysis System (Beckman Coulter) according to the protocol of LaJeunesse (2002). Sequences were deposited in GenBank (accession no. EU315253; Table 1).

As a means of validating the Symbiodinium ITS2 sequences as diagnostic markers for a particular symbiont type, secondary structural analysis was performed by superimposing individual ITS2 sequences onto the secondary structure of the most similar Symbiodinium type reported in Hunter et al. (2007). Areas of high sequence conservation with other Symbiodinium types served as reference points. Less conserved regions were folded by eye to conserve general secondary structure characteristics. Additional details of this method can be found in Hunter et al. (2007) and Thornhill et al. (2007). 

Cold-stress experiment and fluorescence measurements. To investigate the physiological properties of Symbiodinium type B2 relative to other types of symbionts, four clonal Symbiodinium cultures representing Symbiodinium ITS2 types A3, B1, B2, and C2 (sensu LaJeunesse 2001; Fig. 1b) were grown in ASP-8A medium at 26°C and ~60–80 μmol photons·m⁻²·s⁻¹ under cool-white fluorescent lamps at a 10:14 light:dark (L:D) cycle under otherwise standard culturing conditions (Blank 1987; see Table 2 for culture details). Culture stocks have been maintained under these conditions for many years and generations, with bimonthly refreshments of culture media. Experimental cultures (one flask per ITS2 type) were grown from small stock cultures of ~50 mL to a volume of 2 L over ~50 generations. Cultures were selected based on available isolates as representatives of the predominantly temperate ITS2 type B2 (see results below) and several common tropical symbiont types that associate with a range of symbiotic hosts (LaJeunesse 2002, LaJeunesse et al. 2003). Batch cultures were refreshed

Table 1. Geographic and host origin of the cultures used for this experiment.

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Symbiodinium ITS2 type</th>
<th>Geographic origin</th>
<th>Host origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>B1</td>
<td>Jamaica, Caribbean</td>
<td>Cassiopea xamachana (Rhizostomaeae)</td>
</tr>
<tr>
<td>141</td>
<td>B2</td>
<td>Bermuda, W. Atlantic</td>
<td>Oculina diffusa (Scleractinaria)</td>
</tr>
<tr>
<td>203</td>
<td>C2</td>
<td>Palau, W. Pacific</td>
<td>Hippopus hippopus (Bivalvia)</td>
</tr>
<tr>
<td>292</td>
<td>A3</td>
<td>Palau, W. Pacific</td>
<td>Tridacna maxima (Bivalvia)</td>
</tr>
</tbody>
</table>
with new medium (one part fresh medium to two parts medium) 2 d before the beginning of the experiment.

To examine the effect temperature decline has on the photophysiological properties of representative *Symbiodinium* types, an experiment was devised for three phases: graduated temperature decline from 26°C to 10°C, extended low temperature at 10°C, and gradual recovery from 10°C back to 26°C. The cold-stress condition of 10°C was selected as an approximation of the annual minimum temperature experienced at Gray’s Reef National Marine Sanctuary and the mean temperature in Narragansett Bay, RI (Table 1). Additionally, temperatures of 9°C to 12°C have been reported as the minimum experienced by several other temperate zooxanthellate cnidarians (Kevin and Hudson 1979, Farrant et al. 1987, Schiller 1993). Cultures were first subjected to gradual cooling by decreasing the incubation temperature 2°C per day to a final temperature of 10°C. Cultures were maintained at 10°C for a 2-week period and finally warmed by 2°C per day until the temperature returned to 26°C. Control cultures of each symbiont type were maintained at 26°C and ~60–80 μmol photons m⁻² s⁻¹ under otherwise identical conditions. Six hours after the onset of light, repeated measurements of maximum quantum yields of PSII (Fv/Fm) were obtained using a Walz DIVING-PAM fluorometer (Walz, Eichenring, Germany) throughout the 31 d experiment (Warner et al. 1996). Fv/Fm is a measure of the percentage of absorbed light that is converted into biochemical energy, nonphotochemically quenched by thermal decay of excitation energy, or emitted as fluorescence. Five replicate measurements were taken on each replicate of *Symbiodinium* culture (measurements were made on different areas of the flask to confirm physiological homogeneity throughout the culture) daily as temperatures were decreased, every other day as temperatures were maintained at 10°C, and daily again as temperatures were restored to 26°C. Cultures were dark acclimated for 30 min and allowed to settle, and immobile dinoflagellates were analyzed by affixing the fiber optic probe to random regions of the bottoms of culture flasks. This method eliminates stirring artifacts in fluorescence measurements that have been recently noted with the stirring/illumination/detection array of the PHYTO-PAM and WATER-PAM instruments (Cosgrove and Borowitzka 2006). Furthermore, results obtained via this protocol are statistically indistinguishable from those obtained by direct measurement of homogenized cocoid and motile cells (J. McCabe-Reynolds and G. Schmidt, unpublished data), indicating that these results are not biased by preferential measurement of cocoid cell stages at the flask bottom.

In addition, relative ETR versus irradiance measurements were conducted using the Diving-PAM fluorometer according to the manufacturer’s protocol (Walz, DIVING-PAM Manual, http://www.walz.com). Subsamples of homogenized *Symbiodinium* cultures were collected and preserved with 1% formalin, and cell densities were calculated from replicate (n = 6 per culture) hemocytometer counts. Chl a levels were also measured (from 1 to 2 replicates per culture) by centrifuging 10–15 mL of homogenized culture at 8,000 g, removing the supernatant and extracting chl in 1 mL of N,N-dimethylformamide (DMF). Samples were vortexed and centrifuged again at 8,000 g, and absorbance of the resulting supernatant was measured at wavelengths of 664 and 647 nm on a BioRad SmartSpec 3000 spectrophotometer (Bio-Rad Laboratories, Richmond, CA, USA). Chl a concentration was calculated according to Moran (1982).

**RESULTS**

Genotyping from the *Symbiodinium* biogeography surveys is presented in Figure 2 and Table 1. *Symbiodinium* ITS2 type B2 was the only symbiont detected in *A. pocaullata* and *O. arbuscula* coral colonies from the selected temperate habitats. Excision and direct sequencing of dominant and faint minor bands from each DGGE profile recovered only the type B2 sequence, indicating that no other symbiont types were present at the detection limits of this technique (see Thornhill et al. 2006b).

The ITS2 rRNA secondary structure of the sequence recovered for type B2 was also assessed (Fig. 3) to validate this sequence as relevant for biodiversity estimates and not indicative of a nonfunctional rRNA copy or pseudogene (see Thornhill et al. 2007). The deduced secondary structure presented in Figure 3 is consistent with that of other clade B *Symbiodinium* (Hunter et al. 2007) and lacked mutations at any positions that would potentially disrupt the proper folding and processing to generate mature rRNA products. Therefore, we...
interpret this B2 sequence as representative of the
dominant rDNA copy for this symbiont type and
consequently relevant as a marker for ecological
diversity estimates (Thornhill et al. 2007). It should
be noted that this structure is different from that
presented for type B2 in the supplemental materials
of Hunter et al. (2007). Hunter et al. used an
unpublished clade B sequence instead of type B2 in
their proposed secondary structure resulting in a
slightly different estimate of folding of the molecule.

The biogeography of symbionts from temperate
and tropical communities led to a supposition that
Symbiodinium ITS2 type B2 represents a cold-tolerant
lineage able to survive conditions inhospitable to
most other Symbiodinium spp. To test this idea, four
clonal Symbiodinium cultures, representing the
temperate type B2 and three tropical Symbiodinium types
(Fig. 2b and Table 2), were subjected gradual cool-
ning, followed by a prolonged period of cold stress.
As the cultures were cooled, maximum photochemi-
cal efficiency of PSII ($F_v/F_m$) decreased for all four
types concomitant with decreasing temperature
(Fig. 4), indicating diminished electron transport
and/or increased photoinhibition (Schreiber and
Bilger 1987). Interestingly, during the cooling phase
of the experiment, Symbiodinium type C2 from a
tropical host responded more slowly. At 10°C, Symbio-
dinium type B2 $F_v/F_m$ values diminished to $\sim 0.1$;
$F_v/F_m$ values for other Symbiodinium types steadily
decreased to $\sim 0.0$. When restoration to 26°C was
gradually imposed, type B2 readily returned to pre-
stress $F_v/F_m$ values. However, types A1, B1, and C2
did not recover in the subsequent phase of the
experiment (Fig. 4).

Relative ETR versus irradiance curves are pre-
sented in Figure 5. Each Symbiodinium type dis-
played a different, type-specific curve under initial
conditions (Fig. 5a). All four types experienced
steady declines in photosynthesis upon cooling,
with minimal function at 10°C (Fig. 5b), and
remained minimally functional throughout the 2-
week cold-stress treatment at 10°C. However, once
gradual warming to 26°C was initiated, only Symbio-
dinium type B2 returned to prestress levels of
photosynthetic function, whereas the other Symbio-
dinium types remained nonfunctional (Fig. 5c).
At the end of the 31 d experimental period, Symbio-
dinium B2 had returned to prestress levels of
photosynthesis, while the other types remained
photosynthetically inactive. There was little differ-
ence in the overall curves between pretemperature
stress experimental cultures (Fig. 5a) and cultures
kept at control illumination and temperature for
an equivalent 31 d (Fig. 5d). The sustained pho-
synthetic efficiency for all four symbiont types kept
at prestress conditions excludes the possibility that
nutrient depletion or aging of the cultures was
causative in the differential cold responses of the
Symbiodinium types.

Symbiodinium cell densities throughout the exper-
iment are provided for each culture in Figure 6a.
Although cell density varied somewhat between cul-
tures and time points, densities remained approxi-
mately stable or underwent modest declines. Thus,
the cultures were in stationary phase through most
of the experiment, although cultures of tropical
symbionts likely became moribund in response to
the cold treatment. Differences in cell density
between cultures likely relates to cell size and type-
specific nutrient requirements (data not shown).
For instance, type B1 cells were the smallest, and
this culture averaged the highest overall cell density,
whereas type C2 cells were larger and maintained
lower densities overall (see also LaJeunesse 2001).
The greatest increases in cell density were observed
in the early part of the experiment for type A3,
although cell density subsequently decreased during
the temperature decline. This initial growth possibly
relates to the low starting concentrations of type A3
relative to the other cultures. Type B2 experienced
modest declines in cell density through the cold-
stress phase of the experiment, indicating that this
temperate symbiont type was affected to a limited
extent by exposure to low temperatures.

Chl $a$ levels for the four cultures are also pro-
vided in Figure 6b. From these measurements, we
innovated extraction of Symbiodinium cultured cells
with DMF instead of the more conventional acetone
solvent: with the latter reagent, precipitates of cell
debris remained visibly green, whereas DMF yielded
white pellets. Cultures all showed a general trend of
increasing chl $a$ concentration during the first 5 d
of the experiment. Maximum chl $a$ levels varied by
Symbiodinium type: culture C2 reached the highest
chl concentration by day 5, whereas culture B2
reached the lowest relative concentration. Sub-
sequently, chl $a$ levels decreased for types A3, B1,
and C2. Type B2 maintained relatively stable

Fig. 3. Proposed ITS2 secondary structure of Symbiodinium
type B2. ITS2, internal transcribed spacer region 2.
concentrations of chl a as other cultures declined, further suggesting photosynthetic cold tolerance in this symbiont type. However, the low chl sample number and some variance in these data prevented robust statistical comparisons between cultures or time points. It should be noted that Symbiodinium B2 regained photosynthetic efficiency during warming of cold-treated cells without a substantial increase in cellular chl levels. This finding reflects restoration of reaction centers through repair processes that could include recruitment of chl a from residual light-harvesting complexes.

**DISCUSSION**

Symbiodinium type B2 was the only symbiont detected in any of the A. pocculata or O. arbuscula corals from temperate western Atlantic habitats. In contrast to the dominance of type B2 in these temperate corals, B2 is a relatively rare symbiont in tropical and subtropical ecosystems (Lajeunesse 2002, Lajeunesse et al. 2003, Thornhill et al. 2006a,b). Previously, ITS2 type B2 has been reported only from northerly coral reef habitats, including the Florida Keys (Lajeunesse 2001, Santos et al. 2001) and Bermuda (Savage et al. 2002). Furthermore, no Symbiodinium types commonly occurring in the tropics were recovered at any of the temperate habitats (Table 1; D. J. Thornhill and D. W. Kemp, unpublished data). Annually, the environmental conditions at these temperate locations fluctuate considerably; consequently, type B2 must be able to physiologically tolerate a wide range of temperature, light, and other conditions. Here we tested this hypothesis on cold-stressed B2 Symbiodinium and three tropical Symbiodinium types using PAM fluorometry. Our results indicated that type B2 is physiologically cold tolerant and able to rapidly recover from prolonged exposure to low temperatures. Unlike type B2, the tropical Symbiodinium spp. exposed to a prolonged period of cold stress demonstrated no signs of photosynthetic recovery when returned to prestress conditions. Although it is possible that some unknown factor, such as growth phase of the cultures, influenced these data, results from the nontemperature stressed control cultures diminish this possibility. Therefore it seems likely that the cold-tolerant physiology demonstrated by type B2 contributes significantly to the distribution of this symbiont in temperate versus tropical habitats and hosts.

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**Fig. 4.** (a) Maximum photochemical efficiency of PSII (Fv/Fm) of clonal cultures of Symbiodinium types A3 (◇), B1 (□), B2 (●), and C2 (△) throughout the 31 d experiment (five replicates per Symbiodinium type). Data are presented as mean ± 95% confidence intervals. (b) Temperature (°C) levels experienced by cultures for the duration of the experiment.
In a report on the effects of abrupt, short-term cold exposure on symbiotic corals from tropical environments, chronically depressed \( F_v/F_m \) values persisted several hours after temperatures were returned to the original state (Saxby et al. 2003). Irreversible photodamage to the symbionts was concluded to have been inflicted as a consequence of the adverse irradiance and temperature conditions (Saxby et al. 2003). While similar phenomena likely occurred for \textit{Symbiodinium} types A3 (\( \triangle \)), B1 (\( \square \)), B2 (\( \diamond \)), and C2 (\( \triangle \)) (five replicates per \textit{Symbiodinium} type). Data are presented as mean ± 95% confidence intervals. (a) Day 1, 26°C. (b) Day 18, 10°C. (c) Day 31, after return to 26°C. (d) Control cultures day 31, 26°C.

\textit{Symbiodinium} type B2 presumably has either photodamage repair and/or protective mechanisms to enable its ability to withstand prolonged periods of cold. Recent work by Tchernov et al. (2004) documented that reduced membrane fluidity, correlating with higher unsaturated fatty acid composition, partially correlates with high temperature tolerance for some \textit{Symbiodinium} isolates. A reciprocal phenomenon could contribute to low temperature tolerance, since a high proportion of unsaturated lipid membranes could enable certain symbionts to remain photosynthetically functional under moderate to severe cold conditions. However, type B2 exhibits a cold-induced decrease in photosynthetic efficiency that largely parallels that of other \textit{Symbiodinium} clade representatives. In fact, the type that sustains the highest level of photosynthetic activity during gradual decreasing temperature exposure is type C2 isolated from a tropical host. Consequently, we cannot attribute type B2’s cold tolerance to unique fluidity properties of its photosynthetic thylakoid membranes. However, there is a possibility that other membranous components in this symbiont type are endowed with lipid bilayers with a larger proportion of unsaturated fatty acids or sterols, contributing to the cold-stress recovery process.

Type B2 is the first \textit{Symbiodinium} ITS2 type to be identified in temperate environments of the western Atlantic. The cold thermal tolerance apparently provides this symbiont with a survival advantage in temperate habitats over that of other \textit{Symbiodinium} species. It should be noted, however, that other types of \textit{Symbiodinium}, such as types A4, B4 (\textit{S. muscatenei}), E1 (\textit{S. californium}) (LaJeunesse and Trench 2000, LaJeunesse 2001, Muller-Parker et al. 2007), and an additional type of “temperate” clade A (Savage et al. 2002, Visram et al. 2006), have been
documented to occur in other hosts of temperate marine climates. Thus, temperate Symbiodinium ITS2 types likely share a similar underlying basis for cold tolerance of subclade B2, described here as complete photosynthetic recovery after cold thermal stress.

The unusual capacity of Symbiodinium type B2 to rapidly recover photosynthetic efficiency after low temperature exposure possibly contributes to the biogeography of its hosts. Although symbiosis is facultative in A. poculata and O. arbuscula, harboring Symbiodinium leads to enhanced calcification and growth (Jacques et al. 1983, Miller 1995, Dimond and Carrington 2007). Therefore, the physiological tolerance of Symbiodinium spp. may contribute to their hosts’ fitness and distribution, particularly in habitats where coral growth is otherwise limited (i.e., by a lack of available prey, low temperatures throughout much of the year, etc.).

While Symbiodinium type B2 remained only minimally functional at temperatures as low as 10°C, it rapidly recovered upon warming. Assuming a similar pattern probably occurs in hospite, this symbiont probably can remain photosynthetically inactive throughout the colder months of the year, persisting without making a major contribution to the nutrition of the host. In fact, Jacques et al. (1983) found no contribution by Symbiodinium sp. to A. poculata growth or calcification at temperatures below 15°C. During the warm season, as simulated in the recovery phase of this experiment, Symbiodinium B2 likely increases its photosynthetic function and therefore would contribute more actively to its host’s calcification and growth (Jacques et al. 1983, Dimond and Carrington 2007). It is noteworthy that A. poculata and Oculina spp. are known to survive indefinitely without symbionts via heterotrophic feeding (e.g., Jacques et al. 1983, Farrant et al. 1987, Dimond and Carrington 2007). Therefore, the symbioses between Symbiodinium B2 and these hosts may shift seasonally between mutualism in the summer months to commensalism or parasitism during winter when type B2 is photosynthetically inactive. Furthermore, in contrast, the marked seasonal fluctuations recorded in tropical hosts (Muller-Parker 1987, Fagoonee et al. 1999, Fitt et al. 2000), temperate symbiotic cnidarians are known to maintain stable zooxanthellae densities through
spatial gradients (Bythell et al. 1997) and seasonal fluctuations (Dykens and Schick 1984, Farrant et al. 1987) of light and temperature. For instance, zooxanthellae density in temperate scleractinian Plesiastrea urvillei and mitotic index in the temperate anemone Anemonia viridis did not decline when subjected to prolonged periods (48 and 66 d, respectively) of total darkness (Kevin and Hudson 1979, Beever 1996), whereas tropical hosts quickly lose their symbionts under such conditions (Steen and Muscutine 1987). Therefore, seasonal shifts in the nature of symbioses seem likely in many temperate cnidarians—Symbiodinium associations.

In summary, A. pociulata and O. arbescula are two symbiotic cnidarians that commonly occur in the temperate western Atlantic. Both of these corals harbor Symbiodinium type B2, an extremely rare symbiont type in tropical environments. The ability of these symbionts to survive extended periods of cold temperature stress and rapidly return to normal photosynthetic function when permissive conditions recur is undoubtedly critical for their survival in temperate environments and may play a key role shaping temperate coral communities.

This work was funded by NSF (9906976 and 0137007). We would like to thank Daniel F. Gleason, R. Robert Ruzicka, and Keith Goldman for assistance in coral sampling at Gray's Reef National Marine Sanctuary. We are also grateful for the help of James L. Dimond who contributed samples from Rhode Island to the biogeography portion of this study. Kenneth M. Halanych graciously provided use of his laboratory and NSF grant support for sequencing of these samples. Finally, we would like to thank Scott R. Santos, Todd C. LaJeunesse, Clay B. Cook, and two anonymous reviewers for their insightful comments on this manuscript. This is Auburn University Marine Biology Program contribution number 33.


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Symbiodinium


