

IN SITU OBSERVATIONS OF FORAMINIFERAL BLEACHING IN THE MALDIVES, INDIAN OCEAN

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ABSTRACT

Coral reefs are threatened worldwide by a variety of natural and human-induced stressors; anomalous temperatures are presently among the most serious threats by causing extensive coral bleaching. *Amphistegina* spp. exhibit similar bleaching as corals in the presence of photo-oxidative stress induced by either light or temperature, especially during times of maximum solar irradiance. At 11 islands (34 sampling sites) in the North Ari Atoll in the Maldives, bleaching in *Amphistegina* was observed a few weeks before the onset of an extensive El Niño-related coral bleaching that was more severe than expected for this region. Assessment using the *Amphistegina* Bleaching Index (ABI) showed that the proportions of bleached specimens of *Amphistegina* in April–May 2015 can be explained by photo-inhibitory stress associated with temperatures exceeding 30°C during peak seasonal solar irradiance and water transparency. Importantly, the ABI indicates that environmental conditions are suitable for *Amphistegina* and other calcifying symbioses at most of the investigated sites, and that either chronic or relatively recent onset of photo-oxidative stress was present at the time of sampling. The observed bleaching in *Amphistegina* further demonstrates the potential of these unicellular protists to identify stressors in coral reefs; such applications should be considered in future reef-management plans.

INTRODUCTION

Anomalous high temperatures can affect coral reefs worldwide, producing massive bleaching and consequently are among the primary threats to these ecosystems (e.g., Wilkinson et al., 1999; Graham et al., 2015). Climate-induced coral bleaching and subsequent loss of vital coral cover directly impact the economies of coastal regions dependent upon tourism and fisheries, as loss of coral directly compromises the related functions and services that coral reefs provide (Wilkinson et al., 1999).

The term “bleaching”, in the context of organisms that host algal endosymbionts, is usually defined as the temporary or permanent loss of the symbiotic microalgae or the loss of their pigments (Glynn, 1996). Triggers of bleaching include, but are not limited to, high or low temperatures, salinity stress (Jokiel & Coles, 1990), changes in visible or ultraviolet solar irradiance (e.g., Gleason & Wellington, 1993; Glynn, 1996), sedimentation (Hoegh-Guldberg & Smith, 1989), disease, pollution, heavy metals (Jones & Hoegh-Guldberg, 1999), or a combination of these factors. Most reef-dwelling organisms that host algal symbionts are susceptible to bleaching includ-

ing sponges, anemones, stony corals, octocorals, giant clams and larger foraminifers (e.g., Talge & Hallock, 2003; Schmidt et al., 2011). The majority of coral-bleaching events have been associated with anomalous increases in sea-surface temperature (Jokiel & Coles, 1990; Hoegh-Guldberg, 1999). Thermal and associated photo-inhibitory stresses are the main drivers of bleaching because thermal stress increases the susceptibility of the symbionts to photo-inhibition through photo-oxidative reactions, resulting in symbiont loss in corals and larger benthic foraminifers (Coles et al., 1976; Goreau & Hayes, 1994; Glynn, 1996; Hoegh-Guldberg, 1999; Talge & Hallock, 2003). In particular, when an excess of solar energy exceeds the rate of light utilization (photochemistry), production of reactive oxygen species (ROS) can damage both symbionts and the hosting cells (e.g., Lesser & Farrell, 2004; Sheppard et al., 2009). During an acute bleaching episode, a coral host may lose up to 90% of its symbionts, while the remaining symbionts may lose 50–80% of their photosynthetic pigments (Glynn, 1996).

Larger benthic foraminifers (LBF) are unicellular protists having symbiotic relationships with algae analogous to those in corals (Lee & Anderson, 1991; Hallock, 1999). Bleaching in foraminifers was first described in laboratory experiments (Hallock et al., 1986), however, it was unknown in field populations until Hallock et al. (1993) collected several specimens of *Amphistegina gibbosa* that appeared “spotted” during a post-bleaching coral survey in 1988. Since 1991, some bleaching of *A. gibbosa* has been consistently observed in field samples from the Florida Keys during summer months (Hallock et al., 1995; Williams et al., 1997; Mendez-Ferrer et al., 2018). Symbiont loss may affect over 80% of the total adult *Amphistegina* population; individual specimens display unusual color sometimes restricted to a few white spots, or progressing to near absence of symbiont color (Hallock et al., 1995; Mendez-Ferrer et al., 2018). A detailed history of observations in foraminiferal bleaching in the Florida Keys is summarized in Hallock et al. (2006a). Since the first observations, bleaching in *Amphistegina* spp. has been documented on the eastern and western shelves of Australia, the Bahamas, Jamaica, Hawaii, and Micronesia (Hallock, 2000).

Based on many years of laboratory experiments and field observations, Hallock et al. (2006a, and references therein) summarized similarities and differences between bleaching in *Amphistegina* and bleaching in corals. They identified important differences in the modality, timing and causes of bleaching. In particular, they note that mass bleaching in corals occurs primarily by expulsion of symbionts; it requires high light intensity but most strongly correlates with elevated temperatures. In contrast, bleaching in *Amphistegina* occurs by digestion of damaged symbionts (Talge & Hallock, 2003) and, in field studies, has been demonstrated to correlate with the solar cycle that precedes seasonal temperature extremes (Williams, 2002; Hallock et al., 2006a).

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Temperature can also induce bleaching in foraminifers (Talge & Hallock, 2003; Schmidt et al., 2011; Stühr et al., 2017). Schmidt et al. (2011) observed that a 2–3°C increase in temperature can produce rapid bleaching in several diatom-bearing benthic foraminiferal species, including *Amphistegina*, which hosts naked diatom cells lacking silica frustules (e.g., Lee & Anderson, 1991; Talge & Hallock, 2003). Laboratory experiments conducted by Schmidt et al. (2016a, b) demonstrated that reduced photosynthetic activity in *Amphistegina lobifera* occurs at 32°C. The combination of elevated temperatures, nutrients and bleaching in *A. lobifera* from outer-shelf habitats on the Great Barrier Reef, Australia, has been reported by Prazeres et al. (2016, 2017).

Biotic indices are quantitative indicators for ecological water quality based on faunal composition in sediments (e.g., Borja et al., 2011; Alve et al., 2016). Bioindicator species can be used as a measure or a model to characterize an ecosystem or one of its critical components (Jackson et al., 2000). A biotic index, based on abundance and population health state of the bioindicator *Amphistegina* spp., was proposed by Hallock (1995), discussed further by Hallock et al. (2006b), and improved by Ramirez (2008). However, this index has not been tested further outside Florida.

Maldivian coral reefs are some of the most diverse reefs of the Indian Ocean, hosting more than 250 species of corals and 1200 species of fish (Naseer & Hatcher, 2004). Their remote location, combined with a fishery that historically has not been based on reef fish, place them among the reefs across the world with relatively low local anthropogenic disturbances. Despite their isolation, Maldivian reefs have been severely affected by coral bleaching with many reefs losing more than 80% of their coral cover following the 1998 bleaching event (McClanahan, 2000; Morri et al., 2015). Post bleaching recoveries have been variable with respect to rates of recovery of coral cover and return to the original community composition (McClanahan, 2000; Edwards et al., 2001; Morri et al. 2015).

The goals of our study were to quantify occurrences of bleached specimens of *Amphistegina* spp. collected from coral reefs near 11 Maldivian islands in the North Ari Atoll (Fig. 1), during the REGENERATE Cruise in 2015. Importantly, this study also aimed to apply the index proposed for the reefs in Florida by Hallock et al. (2006b) and applied by Ramirez (2008; hereafter termed *Amphistegina* Bleaching Index, ABI) and to test whether the visual response to stressors of *Amphistegina* populations has the potential to serve as a low-cost risk-assessment tool for the Maldivian reefs in view of climate change or local anthropogenic impacts. The ABI was not conceived as an indicator of coral bleaching per se but, if combined with ecological data and physical parameters, can be used to determine the presence of stressors that could induce coral bleaching. The ABI indicates whether water quality supports calcifying symbioses and whether damaging photo-inhibitory stress is present in the environment (Hallock et al., 2006b). This research is the first to provide an ABI data set for the Maldivian coral reefs, and, in addition to data from Florida (summarized in Hallock et al., 2006a), it provides the framework for future application of the ABI to reefs worldwide in the context of climate change.

MATERIALS AND METHODS

The Maldives include 16 complex atolls with >1100 islands extending from the central part of the Chagos-Maldives-Laccadive ridge in the central Indian Ocean, from approximately 7°07'N to 0°40'S in latitude and 72°33'E to 73°45'E in longitude. Eleven islands were surveyed between 22 April–6 May 2015 (Fig. 1) during the International Union for Conservation of Nature (IUCN) REGENERATE Cruise. Surveyed islands included three community islands: Rasdhoo, Feridhoo and Maalhos; four uninhabited islands: Gaathafushi, Alikoirah, Vihamafaru and Madivaru; and four resort islands: Velidhu, Kandholhudhoo, Maayafushi, and Madoogali. At each island reef, three sites at 10 m water depth were randomly chosen along the slope, and nine pieces of coral rubble per sampling sub-site (27 per island) were collected by SCUBA divers to quantify bleaching in *Amphistegina* spp. The positions of each site and sub-site are shown in detail in Pisapia et al. (2016). Each count of living *Amphistegina* was based on nine pieces of reef rubble for each sampling site (a, b and c in Table 1). On the vessel, rubble samples were stored in Petri dishes in the shade for a maximum of two hours before processing. Although strict control of temperature and light was not logistically practical, exposure to bright light and temperature extremes (e.g., >3°C) were avoided, as recommended by Hallock et al. (2006b).

To evaluate the number of bleached *Amphistegina*, biogenic material was removed from the rubble surfaces using a small brush and the resultant sediment-meiobenthos slurries were placed in labeled Petri dishes. Rubble pieces were also scrutinized using a stereomicroscope to record still-attached specimens. Material scrubbed from rubble was left to rest for a few hours and then all specimens belonging to the genus *Amphistegina* showing pseudopodial activity (e.g., when pseudopods were visibly extruded from the test and/or the specimens displayed ability to move) were picked using a small brush, counted and evaluated for the degree of bleaching following Hallock et al. (2006a, b) and Ramirez (2008). The categories evaluated were: Normal = no bleaching observed; partially bleached = < 50% of bleached surface; completely bleached = > 50% of bleached surface. Each piece of rubble was photographed on a gridded paper to estimate area of bottom cover. All images were then processed using the software Image J (<http://imagej.nih.gov/ij>) to quantify the planar areal extent of every rubble piece.

Basic data from each site were a) bottom area of the rubble in cm², b) total number of live *Amphistegina* (adults and juveniles), and c) numbers of normal-appearing, partially, and completely bleached specimens. The *Amphistegina* Bleaching Index was calculated following Ramirez (2008) based on the density of *Amphistegina* and the percent experiencing bleaching (Table 1), to assess whether water quality supports calcifying symbioses and whether damaging photo-inhibitory stress is present in the environment. On the X-axis of the matrix (Fig. 2A, B), the density rank was plotted, which represents the number of live *Amphistegina* standardized for a rubble area of 100 cm² (number of live *Amphistegina*/rubble area in cm²) in three categories (<10¹/100 cm², 10¹–10²/100 cm², >10²/100 cm²). On the Y-axis, the bleaching rank was plotted, which represents the relative abundance of bleached specimens in three categories (>40%, 5–40% and <5%). The

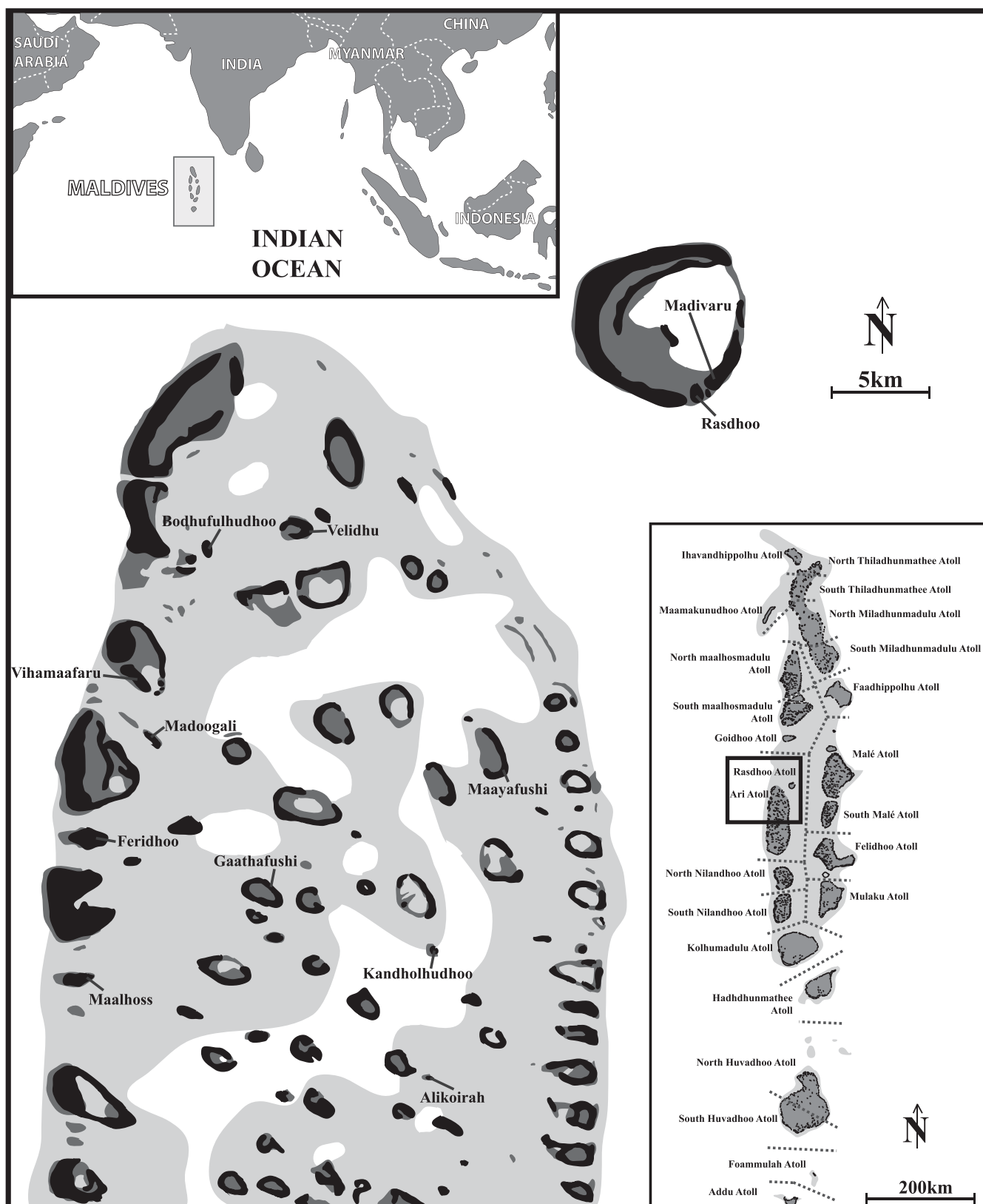


FIGURE 1. Location map of North Ari atoll and the islands investigated in this study. The three community islands are Rasdhoo, Feridhoo and Maalhoss; the four uninhabited islands are Gaathafushi, Alikoirah, Vihamaafaru and Madivaru; and the four resort islands are Velidhu, Kandholhudhoo, Maayafushi, and Madoogali (after Pisapia et al., 2016).

TABLE 1. Average abundances per site of partially bleached, completely bleached and total specimens exhibiting bleaching *Amphistegina* spp., plotted versus their abundance per 100 cm² of rubble surface. PB = Partially bleached; CB = Completely bleached; a, b and c mark the sub-sampling sites.

| Island | Site | Total Living | Normal Spec. | PB Spec. | CB Spec. | TB Spec. | Rubble Area | Living x 100 cm ² | % PB | % CB | % TB |
|-------------------|------|--------------|--------------|----------|----------|----------|-------------|------------------------------|------|-------|------|
| Rasdho (c) | a | 225 | 134 | 82 | 9 | 91 | 93.6 | 240 | 36.4 | 4 | 40.4 |
| | b | 202 | 134 | 66 | 2 | 68 | 86.7 | 233 | 32.7 | 0.99 | 33.7 |
| | c | 73 | 58 | 11 | 4 | 15 | 120 | 60.7 | 15.1 | 5.48 | 20.6 |
| Feridhoo (c) | a | 9 | 8 | 1 | 0 | 1 | 93.5 | 9.63 | 11.1 | 0 | 11.1 |
| | c | 5 | 5 | 0 | 0 | 0 | 104 | 4.79 | 0 | 0 | 0 |
| Maalhos (c) | a | 46 | 28 | 15 | 3 | 18 | 49.5 | 92.9 | 32.6 | 6.52 | 39.1 |
| | b | 14 | 10 | 4 | 0 | 4 | 80.8 | 17.3 | 28.6 | 0 | 28.6 |
| | c | 34 | 30 | 4 | 0 | 4 | 89.2 | 38.1 | 11.8 | 0 | 11.8 |
| Alikoirah (u) | a | 8 | 5 | 3 | 0 | 3 | 73.9 | 10.8 | 37.5 | 0 | 37.5 |
| | b | 47 | 31 | 11 | 3 | 14 | 87.0 | 54.0 | 23.4 | 6.38 | 29.8 |
| | c | 58 | 48 | 10 | 0 | 10 | 71.6 | 81.0 | 17.2 | 0 | 17.2 |
| Vihamafaru (u) | a | 71 | 64 | 7 | 0 | 7 | 90.9 | 78.1 | 9.86 | 0 | 9.86 |
| | b | 117 | 96 | 21 | 0 | 21 | 111 | 105 | 17.9 | 0 | 17.9 |
| | c | 27 | 18 | 9 | 0 | 9 | 89.7 | 30.1 | 33.3 | 0 | 33.3 |
| | ab | 84 | 69 | 12 | 3 | 15 | 80.5 | 104 | 14.3 | 3.57 | 17.9 |
| | bb | 97 | 60 | 18 | 19 | 37 | 70.6 | 137 | 18.6 | 19.59 | 38.1 |
| Madivaru (u) | a | 127 | 119 | 7 | 1 | 8 | 62.5 | 203 | 5.51 | 0.79 | 6.3 |
| | b | 281 | 263 | 11 | 7 | 18 | 94.0 | 299 | 3.91 | 2.49 | 6.41 |
| | c | 50 | 45 | 4 | 1 | 5 | 74.3 | 67.2 | 8.0 | 2.0 | 10.0 |
| Gaathafushi (u) | a | 61 | 52 | 6 | 0 | 6 | 73.1 | 83.5 | 9.84 | 0 | 9.84 |
| | b | 43 | 33 | 10 | 0 | 10 | 73.6 | 58.4 | 23.3 | 0 | 23.3 |
| | c | 24 | 20 | 4 | 0 | 4 | 78.3 | 30.7 | 16.7 | 0 | 16.7 |
| Velidhu (u) | a | 50 | 34 | 8 | 8 | 16 | 52 | 96.2 | 16.0 | 16.0 | 32.0 |
| | b | 14 | 7 | 7 | 0 | 7 | 30.6 | 546 | 50.0 | 0 | 50.0 |
| | c | 35 | 2 | 9 | 24 | 33 | 34.8 | 101 | 25.7 | 68.57 | 94.3 |
| Maayafushi (r) | a | 113 | 105 | 15 | 13 | 28 | 82.0 | 162 | 11.3 | 9.77 | 21.1 |
| | b | 157 | 138 | 11 | 8 | 19 | 77.6 | 202 | 7.01 | 5.10 | 12.1 |
| | c | 60 | 49 | 11 | 0 | 11 | 74.9 | 80.1 | 18.3 | 0 | 18.3 |
| Madoogali (r) | a | 27 | 11 | 11 | 5 | 16 | 57.3 | 47.1 | 40.7 | 18.52 | 59.3 |
| | b | 45 | 36 | 7 | 2 | 9 | 64.0 | 70.3 | 15.6 | 4.44 | 20.0 |
| | c | 33 | 26 | 7 | 0 | 7 | 67.5 | 48.9 | 21.2 | 0 | 21.2 |
| Kandholhudhoo (r) | a | 23 | 17 | 6 | 0 | 6 | 82.1 | 28.0 | 26.1 | 0 | 26.1 |
| | b | 70 | 55 | 11 | 4 | 15 | 79.0 | 88.6 | 15.7 | 5.71 | 21.4 |
| | c | 58 | 33 | 21 | 4 | 25 | 84.4 | 68.7 | 36.2 | 6.90 | 43.1 |

intercept point of these two parameters on the matrix (Fig. 2A, B) represents the *Amphistegina* Bleaching Index, which may fall in one of the nine squared fields describing an ecological status.

Water samples, which were collected into plastic bottles by SCUBA divers, were taken above the seabed from the same locations where coral rubble samples were collected. Immediately after collection of water samples, pH, temperature and conductivity were measured using a multiparameter meter Oriontm Star A325. Dissolved oxygen (DO) was measured with DO600 Waterproof ExStik[®] II Dissolved Oxygen Meter, which has an auto-calibration function. Full details of conductivity to salinity calculations, model sensors and calibrations are described in Pisapia et al. (2016). Water parameters were also measured at the sea surface to record potential difference between the surface and the sea floor (10 m depth). In some cases conditions, such as rough sea prevented the collection of water samples (e.g., Madivaru).

RESULTS

Live *Amphistegina* were recorded at all sites (Fig. 1, Table 1), though <20 specimens were found at 5 of the 34 sites (15%); when corrected for area sampled, <50 specimens/100 cm² were recorded at 10 sites (30%). At 23 sites (with ≥20 live specimens; 79% of total investigated sites), between 10–

41% of the *Amphistegina* specimens were partially bleached (Table 1). When bleached specimens are added to the totals, >6% of the specimens exhibited symbiont loss at all of the 29 sites and >10% at 25 of the 29 sites (Table 1). The percentage of live specimens exhibiting partial or complete bleaching exceeded 60% at only one site (Table 1).

The application of the ABI, based on the table matrix proposed by Ramirez (2008), and based on the percent bleached specimens (partially + completely bleached) as summarized in Figure 2A, B, revealed that most sites fell in fields BB (chronic photo-inhibitory stress, possibly other stressors) and BA (stress either chronic and mild or recent and moderate). Five samples all from sites near inhabited or resort islands fell within the CB field (acute photo-inhibitory stress or chronic photo-inhibitory stress with other stressors). Samples from one community island had very low density (AC or BC, environmental conditions unfavorable), while one higher density sample showed >40% exhibiting bleaching, likely indicating recent, acute photo-oxidative stress (Fig. 2B).

Mean values of temperature (°C), DO (mg L⁻¹), conductivity (mS/cm), salinity (‰) and pH are presented in Table 2. Mean temperatures ranged from 28.8–31.8°C, with the overall mean at 10 m depth of 30.3°C. During the cruise, a generalized warming occurred in the region and, as a result, the highest temperatures (up to 32.2°C, see Pisapia et al., 2016) were recorded at the resort island of Kandholhudhoo on

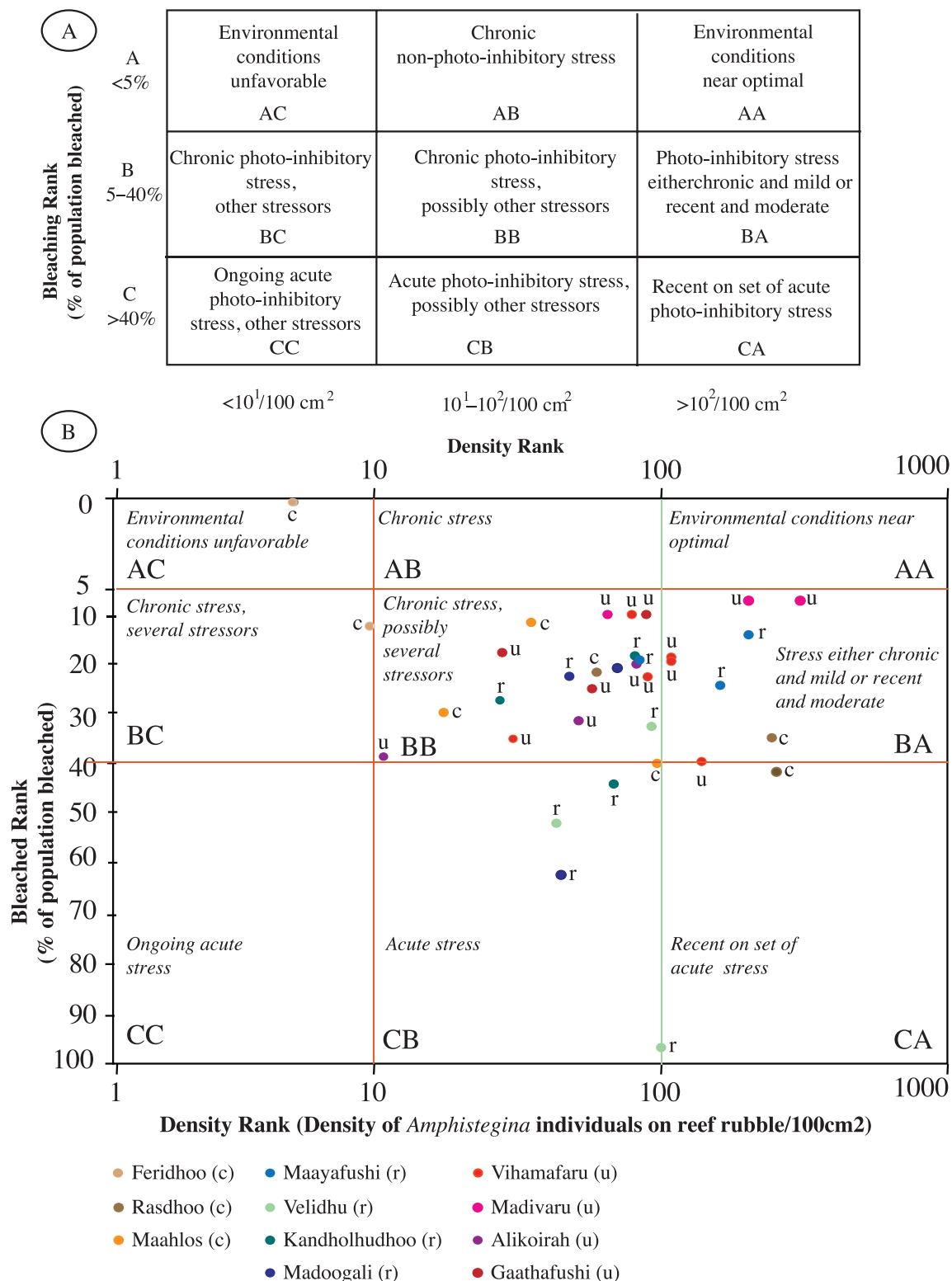


FIGURE 2. (A) Matrix modified from Ramirez (2008) used for the *Amphistegina* Photoc Index, based on the density of *Amphistegina* and the percent of *Amphistegina* specimens experiencing bleaching in a sample (re-drawn after Ramirez, 2008). High densities of live, healthy *Amphistegina* (>50/100 cm²) on rubble in reefal habitats indicates that ambient water quality supports this and other calcifying host-symbiont associations. Low densities, in which most samples indicate densities (<10/100 cm²) indicate that conditions do not support calcifying host-symbiont associations, with moderate densities (10–50/100 cm²) indicating marginal conditions. (B) The *Amphistegina* Bleaching Index (ABI) in the North Ari Atoll, based on the density of *Amphistegina* and the percent of *Amphistegina* specimens experiencing bleaching (modified after Ramirez, 2008). On the X-axis, the density rank (*Amphistegina* spp. per 100 cm² expressed in log₁₀); on the Y-axis is the bleached rank: % of total (partially bleached + completely bleached population). The sites are named with u (uninhabited islands), c (community islands) and r (resort islands) to distinguish the three categories. The full name of each site is presented in the legend.

TABLE 2. Mean values for water sample parameters measured at each site and group of sites. (Modified after Pisapia et al., 2016).

| Site | Depth (m) | pH | T (°C) | Cond. (ms/cm) | DO (mg.L ⁻¹) | Salinity (‰) |
|------------------|-----------|------|--------|---------------|--------------------------|--------------|
| Rasdhoo | 10 | 8.18 | 30.08 | 51.96 | 5.35 | 34.31 |
| Feridhoo | 10 | 8.04 | 30.30 | 51.61 | 5.94 | 33.92 |
| Maalhoss | 10 | 8.01 | 30.71 | 51.56 | 6.12 | 33.88 |
| Community Mean | 10 | 8.07 | 30.36 | 51.71 | 5.80 | 34.04 |
| Community | Surface | 8.14 | 30.59 | 51.72 | 7.99 | 34.11 |
| Velidhoo | 10 | 8.15 | 28.89 | 51.79 | 5.65 | 34.06 |
| Madoogali | 10 | ND | 30.02 | 51.81 | 4.80 | 34.07 |
| Mayafushi | 10 | 8.05 | 29.89 | 58.51 | 5.09 | 39.08 |
| Kandholhudhoo | 10 | 8.03 | 31.76 | 50.50 | 6.13 | 33.11 |
| Resort Mean | 10 | 8.08 | 30.39 | 53.15 | 5.42 | 35.08 |
| Resort | Surface | 8.16 | 30.59 | 58.17 | 8.27 | 35.09 |
| Vihamaafaru | 10 | 8.05 | 30.15 | 51.21 | 5.55 | 33.63 |
| Gaathafushi | 10 | 8.04 | 30.97 | 51.75 | 5.63 | 34.03 |
| Alikoirah | 10 | 8.04 | 30 | 50.52 | 5.24 | 33.14 |
| Uninhabited Mean | 10 | 8.04 | 30.37 | 51.16 | 5.47 | 33.60 |
| Uninhabited | Surface | 8.16 | 30.70 | 51.11 | 8.43 | 33.56 |

29 April 2015 (Fig. 3). Dissolved oxygen varied from 4.8–6.1 mg L⁻¹ at 10 m depth, and was ≥ 8 mg L⁻¹ in surface-water samples. The pH varied from 8.0 to 8.2. Salinity ranged from 33.0–35.1, with minimal variability across all sites, except for Maayafushi, where the anomalously high salinity values (up to 39) were probably due to instrumental problems.

DISCUSSION

Foraminifers are well-established indicators of water quality based on cellular responses (e.g., Murray, 2006; Prazeres et al., 2011, 2012) to morphological and community composition (Hallock, 2012; Raymond et al., 2012). Combining foraminiferal studies with ecological data and physical parameters may be helpful to describe environmental conditions that can induce bleaching. *Amphistegina* is a circumtropically-distributed genus of benthic foraminifers (e.g., Langer et al., 2013) that thrives in high water quality (e.g., low inorganic nutrients, low sedimentation and low turbidity). Talge & Hallock (2003) demonstrated that the cytological responses to bleaching of field-collected *Amphistegina* and specimens from laboratory cultures were statistically indistinguishable, indicating the potential for this genus to be

used as a tool to investigate the presence of photo-oxidative stresses that could lead to coral bleaching.

Temperature, DO and pH are highly dependent upon time of day of measurement, while both temperature and salinity can be influenced by tidal cycle and local conditions such as rainfall or evaporation in shallow reef flat in hot, dry weather (Yates et al., 2007; Wild et al., 2010). The range of pH (8.0–8.2) is typical of tropical waters during the warm, dry season (Table 2). The range of DO (4.8–8.4 mg L⁻¹) is typical of the diurnal cycle in well-oxygenated waters, where lower morning DO is reduced by the influence of nighttime respiration, and higher afternoon DO reflects the accumulated influence of photosynthesis over the course of the day (Yates et al., 2007; Wild et al., 2010). All measured water parameters were within the typical ranges in the Indian Ocean, tropical settings and/or coral reef environments (Ramamirtham, 1968; Wild et al., 2010; Zweng et al., 2013; Lauvset et al., 2015) and should not have stressed the *Amphistegina* populations. Additionally, Pisapia et al. (2016) demonstrated that the management regime (community, resort and uninhabited islands) does not remarkably influence the measured water parameters and that benthic foraminiferal assemblages are typical of tropical reefs in the North Ari Atoll, with minimal

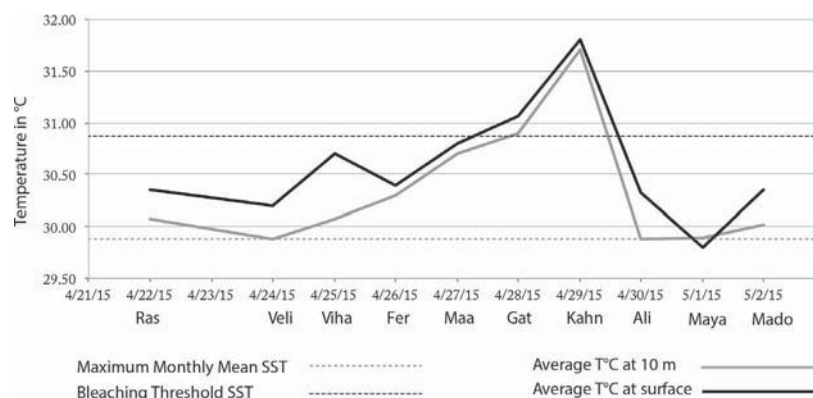


FIGURE 3. Average temperatures measured in selected islands from the North Ari Atoll showing the increasing temperature during the REGENERATE Cruise.

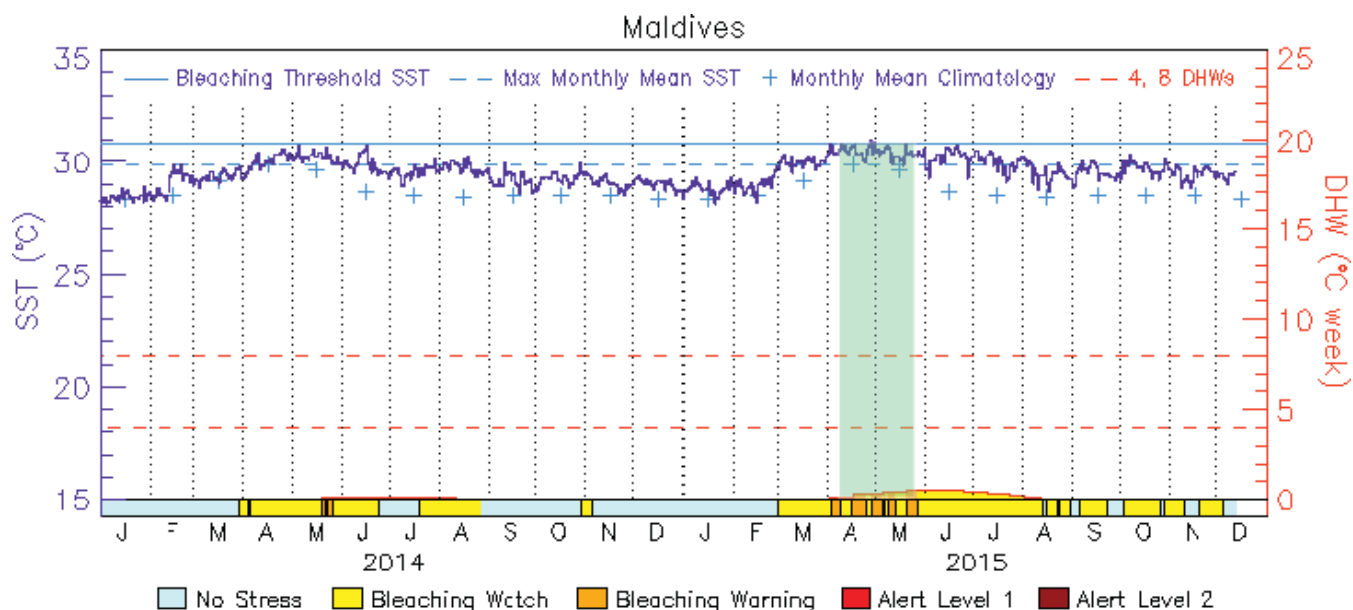


FIGURE 4. Summary of thermal conditions at the Maldives in 2014 and 2015. Modified after NOAA Coral Reef Watch (2015a). The period of the REGENERATE Cruise is marked with a green vertical bar. DHW = Degree Heating Week; SST = Sea Surface Temperature.

differences among sites. The only parameter assessed at the sampling sites that appears problematic is temperature (means range between 28.8–31.7°C), with only two of the means reported in Table 2 falling below 30°C, and several exceeding 30.5°C. Schmidt et al. (2011) have shown that temperature effects are species-specific, and that a temperature above 31°C has a negative effect on *Amphistegina*. Several authors have demonstrated that temperatures exceeding 30°C significantly decrease photosynthetic activity in the diatom symbionts of LBF (Sinutok et al., 2011; Uthicke et al., 2012; Schmidt et al., 2016a, b; Stühr et al., 2017).

Amphistegina spp. generally colonize hard and phytal substrates from shallow depths down to around 120 m, depending upon the penetration limits of visible radiation (Hallock, 1999; Hohenegger et al., 1999). Healthy *Amphistegina* are known to be negatively phototactic at light intensities only slightly above those supporting optimal growth rates (Lee & Anderson, 1991), therefore, they move to avoid photo-inhibitory stress. However, as noted in previous bleaching studies (Hallock et al., 1995), photo-oxidative damage in *Amphistegina* disrupts normal phototaxis, such that stressed specimens tend to seek light rather than retreat.

Amphistegina bleaches when exposed to photo-oxidative stress, whether the stress is primarily induced by light or by temperature. Hallock et al. (1995) demonstrated that onset of bleaching in *Amphistegina* consistently preceded maximum temperatures (see also Mendez-Ferrer et al., 2018). The response to acute photo-inhibition occurs within hours to days and to chronic stress over several days to a few weeks (Hallock et al., 1995, 2006a; Stühr et al., 2017). These responses can be detected cytologically before visible loss of symbionts becomes evident (Talge & Hallock, 1995, 2003). In contrast, the overall reaction of a coral ecosystem may occur weeks, even months, after initiation of the stress event.

In this study, the observed bleaching in *Amphistegina* in late April and early May 2015 occurred during the highest

temperatures and highest bleaching warning (Figs. 3, 4), as documented by NOAA Coral Reef Watch (2015a). Although neither solar irradiance nor photo-oxidative stress were directly assessed at the sites examined in this study, our field-work occurred soon after the sun was directly overhead at the ~4°N latitude sites sampled, at the end of the dry season, together indicating maximum solar irradiance reaching the seafloor at 10 m. April and May also are typically the hottest months (Fig. 4) which, combined with maximum solar irradiance, can result in the highest potential for photo-inhibitory stress. Thus, while this study did not definitely demonstrate that temperature was the predominant stressor at our study sites and did not explicitly test photo-oxidative stress, other potential sources of “stress” (salinity, pH, and DO, and management regime in the North Ari Atoll) assessed in this study all occurred within normal ranges for tropical waters and can be excluded. In particular, ABI values for resort, inhabited and community islands generally fall within the same fields of either chronic or recent onset of moderate stress (Fig. 2B) indicating that the management regime did not play a role in *Amphistegina* bleaching.

When a suite of samples are collected, *Amphistegina* densities in the absence of bleaching can reflect water quality (Hallock et al., 2003; Hallock, 2012). Thus, *Amphistegina* densities combined with bleaching prevalence together can indicate kinds, duration and intensity of stress (Hallock et al., 2006b). Specifically, low *Amphistegina* densities with low–intermediate bleaching percentages indicate unfavorable water quality, while low densities and high (acute) bleaching percentages in adult size-classes can indicate ongoing acute photo-oxidative stress. Intermediate–high densities with high bleaching percentages indicate favorable water quality and relatively recent acute photo-oxidative stress, while intermediate densities and intermediate bleaching percentages indicate chronic stressors, one of which is photo-inhibitory stress (Hallock et al., 2006b). The density data presented in

Figure 2, and the water quality data in Table 2, indicated that DO, pH and salinity conditions were suitable for *Amphistegina* and other calcifying symbioses at the sites sampled in North Ari Atoll. However, the incidences of partial to severe bleaching indicated either chronic or relatively recent onset of moderate stress. This observation corroborates the environmental assessment based on total benthic foraminiferal assemblages of Pisapia et al. (2016). This study used the Maldivian system as a novel example to test the ABI, and documented that ABI can be successfully used to assess relative degree of stresses including bleaching. Sampling methods for ABI are not destructive, only require collection of coral rubble, counts of living *Amphistegina* and degree of bleaching (absent, chronic, or acute), and therefore can easily be incorporated into a preexisting monitoring program (Hallock et al., 2006b) based on coral investigations.

Hoegh-Guldberg (1999) and Wilkinson (1999) linked sustained periods characterized by higher temperatures (a few degrees above the local average maximum) to coral mass bleaching. Strong et al. (1998) and Hoegh-Guldberg (1999) showed that only one degree above normal temperature lasting for one month may be responsible for bleaching in the majority of susceptible corals in an ecosystem. More recently, Frieler et al. (2013) concluded that limiting low-latitude warming to $<1.5^{\circ}\text{C}$ will be necessary to preserve coral reefs worldwide. The threshold for coral bleaching in the Maldives, given by NOAA as just below 31°C , was reached during April and May 2015, and lasted for at least 2 months (Fig. 4).

The major mass-bleaching events in the past two decades have occurred during ENSO years (Hoegh-Guldberg, 1999), and particularly the global bleaching events in 1998 (e.g., Hoegh-Guldberg, 1999; Wilkinson, 1999; Kelmo & Attrill, 2013) and 2010 (e.g., Miranda et al., 2013). Temperature anomalies ranged from $3\text{--}5^{\circ}\text{C}$ higher with respect to the normal annual average in 1998 (Wilkinson, 1999), but they were less extreme in 2010. A coral bleaching event occurred in June 2015 in the North Ari Atoll, triggered by El Niño-associated anomalous temperatures, affecting several coral taxa, in particular *Pocillopora* (CP, personal observation). Based on local observations, coral bleaching in the Maldives was variable but more severe than expected from the level of thermal stress (NOAA Coral Reef Watch, 2015b). The moderate photo-inhibitory stress, as shown by *Amphistegina* in April–May 2015, occurred when solar irradiance and water transparency were highest, indicating elevated photo-oxidative stresses predating the extreme thermal El Niño pulse heralding a mass bleaching event.

CONCLUSION

Bleaching in *Amphistegina* spp. was observed at 11 islands in the North Ari Atoll in the Maldives during the REGENERATE Cruise in April–May 2015. The *Amphistegina* Bleaching Index (ABI) proposed by Hallock et al. (2006b) indicated that the proportions of bleached specimens of *Amphistegina* in April–May 2015 at these sites were likely associated with photo-inhibitory stress induced by seawater temperatures exceeding 30°C during peak seasonal solar irradiance. The ABI indicated that environmental conditions in the North Ari Atoll were suitable for calcifying symbioses at most of the investigated sites, with chronic or relatively

recent onset of moderate stress at some sites (as indicated by observations of 5–40% specimens with bleaching), which likely reflected El Niño conditions. This study further demonstrated how these unicellular protists respond to stressors in reef environments and their potential to predict coral bleaching.

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