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Natural spatial variability of algal endosymbiont density in the coral *Acropora globiceps*: a small-scale approach along environmental gradients around Moorea (French Polynesia)

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This study provides a baseline describing natural small scale variability of Symbiodinium density in the sentinel coral Acropora globiceps during the summer, under non-bleaching conditions. Spatial scales investigated range from the colony scale (1–10 cm, i.e. among branches of the same colony) to the reef scale (1–10 km, i.e. among stations distributed over several locations and depths), at Moorea Island, French Polynesia. The coral–Symbiodinium symbiosis is a key process in scleractinian coral physiology, and Symbiodinium density provides an easy-to-measure and inexpensive biomarker of this symbiosis health. Spatial variability of three major environmental factors: light intensity, sedimentation and water motion was also assessed to evaluate their potential link with Symbiodinium density. Density of Symbiodinium did not significantly differ within colonies or among colonies within a station. However, a marked depth gradient was observed, showing increasing density with increasing depth and decreasing light intensity. These observations provide an interesting reference for forthcoming comparisons with disturbed conditions, such as bleaching events.

Keywords: *Symbiodinium* density, multi-scale variability, scleractinian corals, symbiosis, environmental gradients

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INTRODUCTION

Reef-building corals live in a symbiotic association with unicellular dinoflagellate algae, referred to as zooxanthellae (genus *Symbiodinium*). Translocated algal photosynthates satisfy most host energetic requirements and are essential for coral survival in oligotrophic tropical shallow waters (Muscattine & Porter, 1977). Algal symbionts supply the coral host with sugars, glycerol and amino acids, while algae benefit from host metabolic products, such as CO₂, phosphates and nitrogenous compounds (Hallock, 2001).

Symbiodinium density in coral colonies varies at several spatial and temporal scales, both under normal conditions (i.e. in the absence of perturbation) and after particular disturbances. Algal endosymbiont density can vary among coral species (Drew, 1972), as well as among colonies of the same species, at both local and regional scales (Fitt *et al.*, 2001). Light (D'Croz *et al.*, 2001; Bhagooli & Yakovleva, 2004), sedimentation and eutrophication (Brodie *et al.*, 2007; Sawall *et al.*, 2011), water motion (Finelli *et al.*, 2006), water temperature (Steen & Muscatine, 1987; Sunagawa *et al.*, 2008) and salinity (Hoegh-Guldberg & Smith, 1989; Sunagawa *et al.*, 2008) are known to influence the density of *Symbiodinium*. Despite these advances, the amount and causes of variability in coral algal endosymbionts density at a small spatial scale, i.e. from the colony scale (1–10 cm) to the reef scale (1–10 km), remain poorly documented in natural populations. The symbiotic relationship between

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Symbiodinium and its coral host is vulnerable and highly sensitive to environmental or anthropogenic disturbances, and may be disrupted (Bhagooli & Yakovleva, 2004; Weis, 2008). The disruption of this symbiosis is commonly referred to as coral bleaching, which is broadly defined as the drastic loss of endosymbiotic dinoflagellates or their associated pigments from the coral host cells (Douglas, 2003). Mass-bleaching events, which occur over large spatial scale, are typically associated with higher than average seawater temperature periods (Goreau & Hayes, 1994; Baker *et al.*, 2008), often in conjunction with increased light (Lesser *et al.*, 1990). These events can cause mass mortality within coral populations and subsequent cascading effects on coral-associated fauna (McClanahan *et al.*, 2009; Leal *et al.*, 2012), and may induce a long-term shift in the composition of reef assemblages (Adjeroud *et al.*, 2009). Surviving coral colonies often show decreased growth and fecundity, reduction in competitive abilities and increased susceptibility to diseases (McClanahan *et al.*, 2009). In recent decades, mass bleaching events have raised increasing concern, especially in the present situation of climate change (Baker *et al.*, 2008). In this context, studies have been set up to estimate coral reef health and document the consequences and extent of catastrophic disturbances such as mass bleaching events. Most of these programmes only document the abundance of coral colonies, and do not take into account physiological processes underlying coral health, such as the coral–*Symbiodinium* relationship (Fitt *et al.*, 2001). However, estimating coral–*Symbiodinium* symbiosis health through measures of parameters such as *Symbiodinium* density has been demonstrated to be relevant in studies investigating bleaching events (see, for example, Fagoonee *et al.*, 1999; Stimson *et al.*, 2002; Shenkar *et al.*, 2006; Li *et al.*, 2008). In this context, documenting *Symbiodinium* density variation under natural, non-bleaching conditions is critical to provide a baseline allowing comparisons when a bleaching event occurs. This is especially true in the present context of climate change, in reef systems under the influence of recurrent mass bleaching events, such as islands of the Central Pacific (Salvat, 1992; Adjeroud *et al.*, 2005, 2009; Penin *et al.*, 2007, 2013).

In this context, the present study aims at documenting intra-colony and small scale natural spatial variation in *Symbiodinium* density in a sentinel coral species under non-bleaching conditions during the summer season (warm period). *Symbiodinium* density was chosen because it is an inexpensive and easy-to-measure variable that is a good proxy for the health of the coral–*Symbiodinium* relationship (Moothien-Pillay *et al.*, 2005). The method used can be implemented in many locations with very basic laboratory equipment (Bürker type haemocytometer and dissecting microscope).

The present study documents intra-colony variation (colony scale: 1–10 cm) as well as small spatial scale variation in the field thanks to a hierarchical sampling design encompassing the station scale (1–10 m), the location scale (50–100 m), and the reef scale (4–7 km). Additional measurements of light intensity, sedimentation rate, and water motion allowed the spatial patterns of variation of these key environmental factors to be compared with *Symbiodinium* density, thus providing a better understanding of the implications of these factors for the coral–*Symbiodinium* relationship in the field.

MATERIALS AND METHODS

Sampling strategy

The present study focused on the coral *Acropora globiceps* (Dana, 1846), a major reef-building species in Moorea. *Acropora globiceps* is a ubiquitous species in the Society Archipelago, abundant both in the lagoon and on the whole depth range of the outer slope, and is easy to identify in the field. It is widespread in the Indo-Pacific, from the central Indian Ocean (Andaman Sea) to south central Pacific (Pitcairn) via the Great Barrier Reef, Micronesia and Polynesia (Wallace, 1999). It is also highly sensitive to changes of environmental conditions, and particularly to temperature variations, like most species of this genus (Marshall & Baird, 2000; Penin *et al.*, 2007, 2013; Kayal *et al.*, 2011). As a consequence, *A. globiceps* can be considered as a sentinel species and an adequate candidate for surveys documenting coral health in the Society Archipelago.

Moorea Island (17°30'S, 149°50'W, Society Archipelago, French Polynesia) exhibits a narrow coral reef belt surrounding the island, which compresses the spatial organization along highly marked environmental gradients (Adjeroud, 1997); therefore, it is a unique system to study spatial variability of *Symbiodinium* density and the role environmental factors may play in causing these patterns.

First, colony scale variability of *Symbiodinium* density was studied at three different depths (6, 12 and 18 m) at one site (Vaipahu). At each of these three depths, eight colonies were randomly chosen. For each colony, the extremity of four branches (2 cm long apex), two internal, and two external, were collected for comparison of *Symbiodinium* density (Oliver, 1984).

A hierarchical sampling design (Figure 1), which includes various depths and locations was used to determine small spatial scale variation of *Symbiodinium* density: (1) at the station scale (1–10 m), among colonies within a sampling station; (2) at the location scale (50–100 m), among stations implemented at different depths (6, 12 and 18 m) within a location; and (3) at the reef scale (4–7 km), among three locations within the outer reef slope of Moorea Island (Figure 1: Haapiti on the west coast, Tiahura and Vaipahu on the north coast; see Penin *et al.* (2007) for habitat description). Since no significant differences were observed at the colony scale, small spatial scale variability was assessed through sampling three branches of each of eight colonies randomly chosen within a 100 m² area at the nine sampling stations.

Because *Symbiodinium* density is known to vary seasonally (Fagoonee *et al.*, 1999; Moothien-Pillay *et al.*, 2005), coral samples were collected in March 2007, which is the warmest month of the year in Moorea (CRIOBE temperature data), in order to provide a baseline corresponding to warm period, i.e. when mass bleaching events are most likely to happen (Adjeroud *et al.*, 2009). Indeed, about one month after the sampling, the first signs of bleaching were observed around Moorea (mid-April; Penin *et al.*, 2013).

Sample analysis

Coral samples were initially preserved at –20°C. Tissues of the frozen fragments were then separated from the coral skeleton with a high-pressure water jet (Water-PickTM;

Fig. 1. Map of Moorea indicating the position of the nine sampling stations encompassing three locations (Haapiti, Tiahura and Vaipahu) and three depths (6, 12 and 18 m) on the outer reef slope. Variation of *Symbiodinium* density has been characterized at the colony scale as well as at three different hierarchical small spatial scales represented by different line patterns. Distances among stations within a location are not to scale.

Pitt *et al.*, 2000) using 0.22 μm -filtered seawater (50 ml per sample) and allowed to settle. The slurry was then ground in a glass tissue homogenizer and fixed with 4% formalin for further counts and observations (Lasker, 2003). Density was determined from counts of three replicate aliquots, using a haemocytometer (Bürker type), under an optical microscope. The *Symbiodinium* cell counts were normalized to total coral surface using a paraffin method adapted from Chancerelle (2000), based on the weight difference between

the clean and dry skeleton and the same skeleton coated with paraffin (i.e. sealing fragments of coral skeletons with a varnish and single dipping in paraffin wax at 65°C).

Other methods exist to document coral-*Symbiodinium* symbiosis performances (see for example Frade *et al.*, 2008a, b). However, they imply using expensive equipment and laboratory facilities (such as aquarium systems, pulse-amplitude modulation fluorometers, etc.), that are not always available, especially in remote locations like

Fig. 2. Colony-scale variation of *Symbiodinium* densities: mean *Symbiodinium* density ($\text{zoox} \cdot \text{cm}^{-2}$) in external vs internal branches for each of the eight colonies (1-8) sampled at each of the three depths (6, 12 and 18 m) at Vaipahu site. Error bars represent standard deviations.

Table 1. Non-parametric statistical analysis of spatial variation of *Symbiodinium* density at the colony, station, location and reef scales.**Colony scale**

Mann–Whitney (MW) tests, internal vs external branches.

 α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0125

Depth	U	P	sig. with α_1	sig. with α_2
All depths	1200.00	0.4059	NS	NS
6 m	111.00	0.1657	NS	NS
12 m	108.00	0.0875	NS	NS
18 m	111.00	0.5217	NS	NS

Station scale

Kruskal–Wallis (KW) tests among colonies within stations.

 α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0056

Station	H	P	sig. with α_1	sig. with α_2
Haapiti 6 m	6.813	0.4486	NS	NS
Haapiti 12 m	3.000	0.8850	NS	NS
Haapiti 18 m	15.293	0.0324	*	NS
Tiahura 6 m	7.640	0.3654	NS	NS
Tiahura 12 m	1.840	0.9682	NS	NS
Tiahura 18 m	10.080	0.1841	NS	NS
Vaipahu 6 m	10.240	0.1754	NS	NS
Vaipahu 12 m	10.893	0.1433	NS	NS
Vaipahu 18 m	3.173	0.8685	NS	NS

Location scaleKW tests among stations within sites, and MW pairwise *post-hoc* tests (6 vs 12 m, 6 vs 18 m, and 12 vs 18 m). α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0042**KW tests**

Location	H	P	sig. with α_1	sig. with α_2
Haapiti	47.39	<0.0001	*	*
Tiahura	61.941	<0.0001	*	*
Vaipahu	59.909	<0.0001	*	*

MW tests

Station	U	P	sig. with α_1	sig. with α_2
Haapiti 6 m vs Haapiti 12 m	0.00	<0.0001	*	*
Haapiti 6 m vs Haapiti 18 m	0.00	<0.0001	*	*
Haapiti 12 m vs Haapiti 18 m	272.00	<0.0001	*	*
Tiahura 6 m vs Tiahura 12 m	0.00	<0.0001	*	*
Tiahura 6 m vs Tiahura 18 m	0.00	<0.0001	*	*
Tiahura 12 m vs Tiahura 18 m	11.00	<0.0001	*	*
Vaipahu 6 m vs Vaipahu 12 m	0.00	<0.0001	*	*
Vaipahu 6 m vs Vaipahu 18 m	0.00	<0.0001	*	*
Vaipahu 12 m vs Vaipahu 18 m	31.00	<0.0001	*	*

Reef scaleKW tests among locations and MW pairwise *post-hoc* tests (Haapiti vs Tiahura, Haapiti vs Vaipahu, and Tiahura vs Vaipahu). α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0125**KW test**

	H	P	sig. with α_1	sig. with α_2
Moorea	0.371	0.8305	NS	NS

MW tests

Station	U	P	sig. with α_1	sig. with α_2
Haapiti vs Tiahura	2506.00	0.7311	NS	NS
Haapiti vs Vaipahu	2448.00	0.5651	NS	NS
Tiahura vs Vaipahu	2509.00	0.7402	NS	NS

the French Polynesian islands. *Symbiodinium* counting method used in the present study presents the advantage of being easy to implement with basic laboratory equipment.

Environmental factors

To identify major factors potentially associated with spatial variations of *Symbiodinium* density, light intensity (relative photosynthetic photon flux, rPPF, in $\mu\text{mol.m}^{-2}.\text{s}^{-1}$), sedimentation (total sedimentation rate, SR, in $\text{mg.cm}^{-2}.\text{d}^{-1}$), and water motion (diffusion factor, DF) were measured at each station. Variability in light intensity was assessed through a relative photosynthetic photon flux (rPPF), which is calculated as the ratio between underwater and surface photosynthetic photon flux (PPF, $\mu\text{mol.m}^{-2}.\text{s}^{-1}$), within the range of the photosynthetically active radiations (400–700 nm). Measures were made using a MQ-200 quantum meter (Apogee Instruments Inc., Logan UT, USA) at zenith and on cloud-free days. For each replicate, underwater and surface PPF were measured five times within 60 s at each of five random replicate plots. Three replicates were performed at each station, on three different days. Variability in water motion was characterized through comparison of diffusion factor (DF), calculated as the ratio between weight loss of clod cards deployed in the field for 24 h and weight loss of identical cards kept in a motionless seawater tank (Thompson & Glenn, 1994). At each station, five replicate racks, each encompassing four clod cards, were used on each of five randomly chosen days. Variability in sedimentation was quantified through a comparison of dry sediment weight deposited per cm^2 and per day. At each station, five sediment collectors were deployed for ten days in three replicate periods, following Stewart *et al.* (2006). Temperature was not measured, because it does not significantly vary within the studied depth range at these sites during the warm season (Penin *et al.*, 2007).

Statistical analysis

Due to lack of normality and homoscedasticity of the distributions of *Symbiodinium* densities, even after appropriate

transformations, parametric statistics like ANOVA could not be used. As a consequence, non-parametric statistical analyses were used. Intra-colony comparisons (between internal and external branches) were performed using Mann–Whitney rank tests (MW). For comparisons among stations, locations and depths, Kruskal–Wallis rank tests (KW) were conducted, completed by MW rank tests for *post-hoc* pairwise comparisons. Spatial variability of light intensity, water motion, and sedimentation rate were explored through the use of KW rank tests and complementary MW rank tests for pairwise comparisons. Non-parametric Spearman correlations were used to detect significant relationships between variability of *Symbiodinium* density and variability of light intensity, sedimentation, and water motion among the nine sampling stations. Results are presented with two values of α , the first one being the classical 0.05, and the second one being the α obtained after standard Bonferroni corrections, which is a method aiming at adjusting the α risk to the number of tests run, thus limiting the risk of Type I errors (i.e. rejecting H_0 when H_0 is true). Results were virtually identical with the two methods, but we choose to present both due to controversy raised by the use of Bonferroni corrections (Cabin & Mitchell, 2000; Moran, 2003).

RESULTS

Symbiodinium density ranged from 0.77 to $2.32 \times 10^6 \text{ cm}^{-2}$. No difference was observed between internal and external branches at the Vaipahu site, whatever the depth being considered (Figure 2; Table 1). Similarly, *Symbiodinium* density did not significantly vary at the station scale (i.e. among colonies within a sampling station, Table 1). In contrast, significantly higher densities were observed at deep stations than at shallow stations at all three locations (Figure 3; Table 1). Marked gradients were also observed among the nine sampling stations for the measured environmental variables. The rPPF decreased with increasing depth, but no significant variation was observed among locations (Figure 4; Table 2). Total dry sediment weight did not vary among depths, but was significantly lower at Haapiti than at Vaipahu or Tiahura (Figure 4; Table 2). Diffusion factor decreased with

Fig. 3. Multi-scale variation of *Symbiodinium* densities: mean *Symbiodinium* density (zoox.cm^{-2}) for each of the eight colonies sampled at each of the nine study stations around Moorea. Error bars represent standard deviations.

Fig. 4. Spatial variation of: (A) light intensity (relative photosynthetic photon flux rPPF); (B) sedimentation (dry sediment weight, $\text{mg} \cdot \text{cm}^{-2} \cdot \text{d}^{-1}$); (C) water motion (diffusion factor) over the nine stations. Error bars represent standard deviations.

depth, and significant differences were detected among locations, Haapiti presenting the highest values and Vaipahu the lowest (Figure 4; Table 2).

Spatial variability in *Symbiodinium* density was strongly and negatively correlated with light intensity, but not with sedimentation rates or water motion (Figure 5; Table 3).

DISCUSSION

Symbiodinium density observed in *Acropora globiceps* tissues around Moorea was of the same order of magnitude as

values previously measured in *A. palmata* and *A. cervicornis* in the Caribbean (Fitt *et al.*, 2000), in *A. formosa* in the Indian Ocean (Fagoonée *et al.*, 1999), in different *Acropora* species in the South China Sea (Li *et al.*, 2008), and also in *A. millepora* at the Palm Island Group, Great Barrier Reef, Australia (Moothien-Pillay *et al.*, 2005). This suggests that the range of *Symbiodinium* density is relatively consistent within the *Acropora* genus, even for highly divergent host species and symbiont clades, and from different biogeographic regions or environmental conditions.

At the colony scale, results showed homogeneity of *Symbiodinium* densities between inner and outer branches

Table 2. Kruskal–Wallis tests among stations ($N = 9$ stations), among locations ($N = 3$ locations) and among depths ($N = 3$ depths) on light (photosynthetic photon flux), sedimentation (dry sediment weight), and water motion (diffusion factor). α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0167.

Light	H	P	sig. with α_1	sig. with α_2
Station	556.996	<0.0001	*	*
Location	2.540	0.2808	NS	NS
Depth	552.520	<0.0001	*	*
Sedimentation	H	P	sig. with α_1	sig. with α_2
Station	42.670	<0.0001	*	*
Location	18.662	<0.0001	*	*
Depth	2.902	0.2343	NS	NS
Water motion	H	P	sig. with α_1	sig. with α_2
Station	569.013	<0.0001	*	*
Location	319.882	<0.0001	*	*
Depth	238.675	<0.0001	*	*

in *A. globiceps* at the Vaipahu site, regardless of the depth considered. This outcome seems in contradiction with results of previous surveys on other *Acropora* species (Oliver, 1984; Moothien-Pillay *et al.*, 2005) and suggests that intra-colony variation in *Symbiodinium* density in reef-building corals could be species-specific. At Moorea, this absence of intra-colony differences in *Symbiodinium* density could also be due to high light intensity ($>200 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) and water motion (>3.5 ; Figure 4) observed at all study sites and/or to the presence of only small differences in these parameters between internal and external branches, especially when considering the upper part of the branches, which usually contain less *Symbiodinium* (Allemand *et al.*, 2011). These hypotheses could be addressed through intra-colony measurements of light intensity and water motion on different species of the *Acropora* genus.

At the station scale, no differences were observed among colonies in the density of *Symbiodinium*. This homogeneity within a particular habitat indicates the preponderance of extrinsic vs intrinsic factors, and suggests environmental factors are probably homogeneous enough at this scale not to induce significant variability in *Symbiodinium* density. At the location scale, a marked and consistent increase in *Symbiodinium* density with increasing depth was observed

at all three locations. At the reef scale, significant differences in *Symbiodinium* density have been observed among the nine stations, but not among the three locations (Figure 3). This shows that variability in *Symbiodinium* density is mostly driven by depth and associated parameters such as light, rather than by location. This depth pattern is probably related to the strong negative correlation observed between light intensity and *Symbiodinium* density, and underlines the importance played by light in the coral–algal symbiosis (Falkowski *et al.*, 1984). A similar depth/light pattern in *Symbiodinium* density was demonstrated in other cnidarians, such as other scleractinian corals (Drew, 1972; Dustan, 1979) or the sea anemone *Aiptasia tagetes* (Steele, 1976). Reduced light intensity is known to induce an increase of *Symbiodinium* density and photosynthetic pigments concentration under experimental conditions (Titlyanov *et al.*, 2001) or in the field, in relation with depth (Li *et al.*, 2008) or cloud cover (Titlyanov *et al.*, 2001; Sunagawa *et al.*, 2008). Titlyanov *et al.* (1980) have also established a relationship between the increase of *Symbiodinium* density and the decrease of light. These patterns are linked with acclimatization to low light, which involves maximization of the light harvesting capacity by increasing photosynthetic pigment concentration in *Symbiodinium*, and *Symbiodinium* population density in coral branches. Another mechanism for corals to acclimatize to low light may be to change their *Symbiodinium* clades (Rowan & Knowlton, 1995; Toller *et al.*, 2001; Bongaerts *et al.*, 2010), in a similar way to that sometimes observed with acclimatization to high temperature (Stat *et al.*, 2006). The different clades present variable volume and circumference (Wilkerson *et al.*, 1988), and deeper corals generally harbour smaller *Symbiodinium* (Wilkerson *et al.*, 1988). Moreover, there is a relation between the size and density of symbionts and the host tissue volume (space availability for symbionts; Jones & Yellowlees, 1997). In the present study, we did not detect any visible difference in *Symbiodinium* size, and we can thus assume that variability in size of *Symbiodinium*, in order to counterbalance higher density, is probably limited. However, it would be of particular interest to examine this hypothesis in further studies aiming to precisely quantify and qualify the size and the clade of *Symbiodinium* extracted from *Acropora globiceps* sampled at different depth.

The observed higher *Symbiodinium* density at deep stations can also be one of the causes of spatial variability in corals response to high temperature observed during the

Fig. 5. Relationships between variation of *Symbiodinium* density (zoox.cm^{-2}) and environmental factors among the nine stations: (A) light intensity (relative photosynthetic photon flux); (B) sedimentation (dry sediment weight, $\text{mg.cm}^{-2}.\text{d}^{-1}$); (C) water motion (diffusion factor). ρ is the Spearman's rank correlation coefficient.

Table 3. Spearman non-parametric correlations between *Symbiodinium* density and environmental factors (light, sedimentation, and water motion). α_1 (without Bonferroni correction) = 0.0500; α_2 (with Bonferroni correction) = 0.0167.

Factor	ρ	P	sig. with α_1	sig. with α_2
Light	−0.900	0.0109	*	*
Sedimentation	0.350	0.3222	NS	NS
Water motion	−0.533	0.1314	NS	NS

bleaching event that occurred at Moorea a few weeks after this study. During this bleaching event, corals at deeper stations displayed a higher bleaching response than the shallower ones (Penin *et al.*, 2013). Coral bleaching is clearly linked to photodamages faced by *Symbiodinium* under thermal stress (Venn *et al.*, 2008). These damages cause overproduction of reactive oxygen species (ROS) leading to coral bleaching via a complex cellular cascade (Weis, 2008). As a consequence, corals from deeper stations, with high *Symbiodinium* density and high concentration of photosynthetic pigments might suffer from higher oxidative stress during temperature anomalies than corals at shallower stations, characterized by lower *Symbiodinium* and pigment densities (Stat *et al.*, 2006).

Results of this study demonstrate that *Symbiodinium* density in *Acropora globiceps* is strongly influenced by light intensity, as it is the case for photophysiological and symbiotic mechanisms in reef-building coral species (Venn *et al.*, 2008; Mass *et al.*, 2010). Homogeneity in *Symbiodinium* density at the colony (i.e. between branches of the same colony) and station scales (i.e. between colonies of the same habitat) allows considering *Symbiodinium* density in *A. globiceps* as a potential biomarker of coral health in monitoring surveys, since *Symbiodinium* density seems typical of a particular habitat. In the present study, *Symbiodinium* density was measured in non-disturbed conditions (i.e. in the absence of major perturbations), just before the season when bleaching events generally occur (Penin *et al.*, 2007, 2013), and at various depths and locations. Therefore, it provides a valuable baseline that could be used in the future as a reference, to be compared with measures realized in disturbed conditions, such as during a bleaching event. In this perspective, *Symbiodinium* density can represent an inexpensive and easy to implement biomarker of coral–*Symbiodinium* symbiosis health, and complement other tools used in studies investigating the effects of bleaching events on coral reef health.

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