Variable thermal and biochemical stress responses of tissue balls from *Lithophyllon repanda*, *Pocillopora damicornis* and *Acropora muricata*

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Abstract This study examined the effects of thermal stress (28 and 31°C) and biochemical stress (sponge *Haliclona madrepora* and ascidian *Didemnum molle* crude methanolic extracts) on density of coral tissue balls (TBs) and on their symbiont photosystem II functioning. Coral TBs were obtained from scleractinian corals harbouring symbionts *Cladocopium* [*Lithophyllon repanda*, *Pocillopora damicornis* and *Acropora muricata*] and symbionts *Durisdinium* [*Acropora muricata*]. Thermal stress experiments at 28 and 31°C tended to have negative effect on TB density from *A. muricata* (harbouring *Cladocopium* or *Durisdinium*) and *L. repanda* (harbouring *Cladocopium*) but not from *P. damicornis* (harbouring *Cladocopium*). Effective quantum yield, Φ_{PSII} , decreased at temperature 31°C in *A. muricata* (*Cladocopium*) but remained stable in *A. muricata* (*Durisdinium*). Combined thermal (31°C) and biochemical (50 $\mu\text{g ml}^{-1}$ *D. molle* extract) stressors had a relatively more pro-

nounced effect on TB density, but did not affect Φ_{PSII} in the three species, irrespective of symbiont genus. Thermal stress and 200 $\mu\text{g ml}^{-1}$ *D. molle* affected Φ_{PSII} in *A. muricata* (*Cladocopium*) and *L. repanda* (*Cladocopium*) as compared to *P. damicornis* (*Cladocopium*). Thermal stress and 50 $\mu\text{g ml}^{-1}$ *H. madrepora* affected TB density in three coral species but caused a drop in Φ_{PSII} only in *A. muricata* (*Cladocopium*). Thermal stress and 200 $\mu\text{g ml}^{-1}$ *H. madrepora* affected both TB density and Φ_{PSII} in the three tested coral species. These results suggest a variable susceptibility among corals, with *A. muricata* (*Cladocopium*) being the most susceptible and *P. damicornis* (*Cladocopium*) the least susceptible to studied thermal and biochemical stressors.

Keywords PSII, zooxanthellae clade, scleractinian corals, stressors, tissue balls

Introduction

Coral reefs support a high productivity and enormous diversity of marine organisms. As primary reef ecosystem engineers, corals provide the trophic and structural foundation of the coral reef ecosystem (Wild et al. 2011). Coral reefs are under threat worldwide due to a number of natural and anthropogenic pressures. The dysfunction and collapse of both symbiosis (between the coral host and *in hospite* unicellular algae *zooxanthellae*) and calcification in corals is central to the severe global decline of coral reefs (Weis and Allemand 2009). This is due to environmental stressors imposed by climate change such as elevated sea temperatures. The collapse of symbiosis in turn leads to coral bleaching – a phenomenon characterized by the loss of photosynthetic pigments from symbiont or loss of symbionts from coral host tissues (Glynn 1993; Jones et al. 1998), leading to paling, whitening and subsequent mortality of coral colony in the absence of recovery. Wild et al. (2011) proposed that thermal stress-induced bleaching and subsequent coral mortality, along with ocean acidification, can further lead to long-term shifts in benthic community structure, changes in topographic reef complexity and modification of reef functioning.

Elevated sea temperature is considered to be a primary factor that cause mass coral bleaching (Brown and Suharsono 1990; Brown 1997; Bhagooli and Hidaka 2004; Lesser and Farrell 2004; Dove et al. 2006; Hoegh-Guldberg 2011) following photoinhibition of symbiont photosynthetic machinery. During photoinhibition, photosystem II (PSII) reaction centres in symbiotic dinoflagellate cells undergo damage (Warner et al. 1999) due to generation of reactive oxygen species (ROS) (Wiebke et al. 2012). Repair of both PSII (Takahashi et al. 2004) and the Calvin/Benson cycle (Bhagooli 2013) of the photosynthetic machinery of the coral symbionts have also been implicated in bleaching mechanisms. Loss of photosynthetic activity is measured as maximum quantum yield of PSII (F_v/F_m) in dark-adapted samples and as effective quantum yield of PSII (Φ_{PSII}) in light-adapted samples. However, the role of the host cannot be ignored (Bhagooli and Hidaka 2003; Bhagooli et al. 2008; Baird et al. 2009).

Several studies have reported the effect of different environmental stressors on corals leading to coral bleaching in several parts of the world (Glynn 1993; Brown 1997; Coles and Brown 2003; Lesser and Farrell 2004). In Mauritian waters coral bleaching was not severe during the 1998 worldwide mass bleaching/mortality event (Turner et al. 2000). During the recent years Mauritian reefs have not been spared by bleaching events: In 2004, there was a non-severe bleaching followed by a major bleaching event in 2009 whereby coral cover declined from >50% in 2000 to ~1% cover at some sites in 2010 and with a significant increase in macro-algal cover around Mauritius Island (AFRC 2010). Bhagooli and Sheppard (2012), using sea surface temperature-based models, indicated that reefs around Mauritius Island may have ‘extinction date’ of 2070.

Bleaching has been reported to be variable among coral species, and within and among reefs (Marshall and Baird 2000; Loya et al. 2001; Bhagooli and Yakovleva 2004). In the Mauritian waters, variation in bleaching has been reported among coral species, within a coastal site and among reefs as well. Bhagooli and Taleb-Hossenkhan (2012), during a warming event, reported variable thermal regimes reciprocally corresponding to bleaching patterns in *Acropora muricata* occurring at near-coast, lagoonal and reef zones at Flic en Flac and Belle Mare. These observations can be explained by the fact that *A. muricata* colonies harbour clade A-like *Symbiodinium* (Louis et al. 2016a), which might confer enhanced acclimatization capacity. Acclimatization capacity can be in terms of photo-physiological (Louis et al. 2016b), antioxidant and Hsp70 (Louis et al. 2020) features related to the prevailing thermal environment progressively increasing at the studied zones during summer. Variable bleaching pattern observations among eight corals revealed that *Pocillopora damicornis*, *Pocillopora eydouxi*, *Galaxea fascicularis* and *Fungia* sp. did not bleach (Mattan-Moorgawa et al. 2012). Tabular corals *Acropora cytherea* and *A. hyacinthus* exhibited extensive bleaching/mortality and branching *A. muricata* suffered only partial colony mortality (Mattan-Moorgawa et al. 2012). Field observations of post-bleaching recovery of colony colour and photosystem II functioning of symbionts indicated a fast recovery in solitary corals followed by branching ones and least among tabular

corals at Belle Mare (Mattan-Moorgawa et al. 2018). Use of tissue balls (Mattan-Moorgawa et al. 2014) and isolated zooxanthellae (Ghoora et al. 2018) have also experimentally indicated variable responses. These studies suggest that Mauritian corals exhibit differential bleaching responses.

According to Pochon and Gates (2010) dinoflagellate genus, *Symbiodinium* is divided into nine divergent lineages referred to as clades A–I, and several subclade types. Scleractinian corals predominantly associate with clade C zooxanthellae and to a lesser extent with clades A, B, D, F and G (Stat et al. 2009). In a study by Silverstein et al. (2012) many scleractinian coral species were shown to be capable of associating with multiple zooxanthellae clades (A–D). Such symbiotic flexibility may provide one mechanism by which corals can respond to environmental change, for example, increasing sea surface temperatures. Also, this type of endosymbiont shuffling is beneficial to the host under conditions of environmental stress (Pochon and Gates 2010). Members of zooxanthellae clade D, particularly ITS-2 types D1 and D1a, can be relatively thermotolerant (Silverstein et al. 2012). Coral colonies associated with members of this clade have exhibited increasing bleaching resistance (Glynn et al. 2001; Berkelmans and Van Oppen 2006; Jones 2008; Baker et al. 2004; LaJeunesse et al. 2009). Stat and Gates (2011) suggested that clade D are mostly opportunistic endosymbionts and they outcompete and replace optimal symbionts in health-compromised corals. Corals harbouring endosymbiotic communities dominated by clade D symbionts have nevertheless certain drawbacks such that they grow more slowly than their conspecifics harbouring clade C symbionts. This acts as a major fitness trade-off and have implications for the long-term growth and survival of coral reefs (Stat and Gates 2011). LaJeunesse et al. (2018) proposed new systematics in the family Symbiodiniaceae: clade C, D and A have been renamed as genera *Cladocopium*, *Durusdinium* and *Symbiodinium*. Chauka and Macdonald (2019) have reviewed the Symbiodiniaceae studies in the Western Indian Ocean region.

In this study, coral tissue balls (TBs) have been used as a miniaturized model to study the effects of various environmental stressors and other factors on their viability

and density, their differential susceptibility to thermal stress and biochemical stressors (Nesa and Hidaka 2008, 2009; Lecoointe et al. 2013; Mattan-Moorgawa et al. 2014; Gardner et al. 2015; Mattan-Moorgawa et al. 2018). Biochemical stressors may arise from competition with sessile and sedentary reef organisms, for example, seaweeds (Brandt et al. 2019), sponges (Wang et al. 2012; Brown et al. 2018) and ascidians (Roth et al. 2018). Mattan-Moorgawa et al. (2014) studied the effects of extracts of sponges and one ascidian on the formation and density of tissue balls from hard corals, and reported *P. damicornis* to be most robust and *A. muricata* to be most susceptible to the stressors (thermal and sponge/ascidian crude extracts) to which they were exposed. Responses of coral tissue ball harbouring different zooxanthellae genera to thermal and biochemical stressors have not been investigated before. Here, we aimed to investigate the response of TBs from three scleractinian corals harbouring similar or different symbiont genera (*Cladocopium* or *Durusdinium*) to thermal and biochemical stressors. The response was measured in terms of TB density and zooxanthellae PSII functioning following exposure to the stressors.

Specific objectives of this experimental study were to: (i) investigate effects of stressors, thermal and biochemical, on the density of TBs from three scleractinian corals harbouring different zooxanthellae *Cladocopium* or *Durusdinium* genera; and (ii) determine PSII functioning of TBs from three coral species harbouring different zooxanthellae genera following exposure to thermal and biochemical stressors.

Materials and methods

Field collection of samples

Specimens of hard coral, *Acropora muricata* (Linnaeus, 1758), *Lithophyllon repanda* (Dana, 1846) and *Pocillopora damicornis* (Linnaeus, 1758) were collected from Flic en Flac, on the west coast of Mauritius Island (Fig. 1). Five (5) colonies of each species were selected and tagged, and fifteen (15) fragments/ nubbins were taken from each colony sampled. Coral samples were kept in 0.45 µm-filtered seawater under dark conditions prior to laboratory

Fig. 1 Mauritius located in the Western Indian Ocean on the east coast of Madagascar; **B**, Map of Mauritius showing Flic en Flac site on the west coast and Ile d'Ambre site on the north-east coast where samples were collected; **C**, Flic en Flac site where corals were sampled; **D**, Ile D'Ambre site where sponges and ascidians were collected. [Source: Google Earth Pro 2018 (**A** & **B**); MauritiusAttractions.com (**C** & **D**)]

processing for preparation of coral tissue balls, which took place on the day of collection to avoid tissue degradation.

Specimens of sponges and ascidians (three per species) were collected at Ile D'Ambre islet located on north-east of Mauritius Island. One sponge species, *Haliclona madrepora*, and one ascidian species, *Didemnum molle*, were selected for study. The samples were stored in seawater in zip-lock bags and transported under dark conditions in cooler boxes to prevent any potential degradation of bioactive compounds in the sponge and ascidian samples. Once in laboratory, samples were wrapped in aluminium foil and stored at -20°C , before extraction of crude extracts following a modified extraction protocol of Li Kam Wah et al. (2006).

Preparation of coral tissue balls

Coral TBs from collected coral samples were prepared

using the protocol of Nesa and Hidaka (2008). Surface area of coral samples was first estimated according to Marsh (1970). Fresh coral samples were carefully blotted lightly with paper towels and then carefully wrapped in aluminium foil which was trimmed to avoid any overlap. The aluminium foil was then removed and weighed. Surface area was determined using the following relationship:

$$\text{Surface area of coral (cm}^2\text{)} = \frac{\text{weight of Al foil (g)}}{\text{density of foil (g cm}^{-2}\text{)}}$$

Coral samples were then gently rinsed with fresh seawater. A WaterPik WP-100 dental water flosser was used to remove coral tissue from the skeleton of coral nubbin samples. Coral blastate obtained from the blasting was, firstly, filtered through a $155\text{ }\mu\text{m}$ nylon mesh to remove mucus produced during the isolation procedure and, secondly, was homogenized manually and filtered again through a $40\text{ }\mu\text{m}$ nylon mesh. The filtrate was centrifuged at 1000 rpm for 5 minutes, re-suspended in fresh seawater

and centrifuged again at 1000 rpm for 5 minutes, after which the supernatant was discarded. The suspended pellet obtained was dispersed in a test-tube using a vortex mixer. This suspension containing dissociated coral cell aggregates and zooxanthellae was then used immediately for stress experiments.

Preparation of crude extracts of sponge and ascidian

Crude extracts of sponge *H. madrepora* (HS) and ascidian *D. molle* (DM) were prepared following a modified protocol of Li Kam Wah et al. (2006). Frozen sponge and ascidian samples were cut into 1 cm³ pieces, stored at -20°C for 48 hours, and then freeze-dried for additional 24 hours. After freeze-drying, samples were lyophilized and sponge or ascidian tissue were macerated in a 1:1 methanol: dichloromethane mixture. After 72 hours, the suspension was filtered using a coarse filter paper and the filtrate obtained was flash-evaporated to obtain crude extracts that were free of solvent. Crude sponge and ascidian extracts were prepared in two different concentrations each, 50 µg ml⁻¹ and 200 µg ml⁻¹. These were stored in glass vials at -20°C to prevent degradation of its bioactive molecules.

Stress experiments on coral tissue balls

Suspensions containing coral tissue cells and zooxanthellae were then used for stress experiments at different temperatures: 25 (control), 28 and 31°C. For each colony sampled for *A. muricata*, *L. repanda* and *P. damicornis* (n=5 per species), 2 ml of suspension were placed in separate 50 ml glass beakers and incubated at 25, 28 and 31°C, respectively. Samples were examined under an inverted microscope (Olympus CK40) to assess density of live and rotating coral TBs (Mattan-Moorgawa et al. 2014) and recorded at start of experiment (time 0) and at time intervals 60 and 120 minutes for each treatment. Tissue ball density varied from 0 to 2 at time 0 for each treatment, and the data were presented for time intervals 60 and 120 minutes only. This is in contrast to the method used by Nesa and Hidaka (2008) whereby they kept the suspension of the dissociated coral cell aggregates and microalgae overnight to allow the formation of the TBs prior to starting of the stress experiments. Number(s) of live TBs were counted at time intervals, 60 and 120

minutes, and was expressed as density (number of coral TBs per cm² of coral surface). Suspensions containing coral tissues and zooxanthellae were exposed to sponge and ascidian extracts at two different concentrations, 50 and 200 µg ml⁻¹, at time intervals 60 and 120 minutes, and at three different temperatures, 25 (control), 28 and 31°C. For each sample of coral species, 1.5 ml of suspension was placed in a 50 ml glass beaker and exposed to 1 ml volume of extracts as indicated above. Then, density of coral TBs was counted at 60 and 120 minutes. For the controls, no extract was used. It was instead replaced by 1 ml of filtered seawater.

Zooxanthellae genera identification

Coral nubbins, 1–2 cm per species, were used for extraction of total genomic DNA by the method of Rowan and Powers (1991) with slight modifications. Primers specific to zooxanthellae, namely ss5Z (5'-GCAGTTA TARTTTATTGATGGTYRCTGCTAC-3') and ss3Z (5'-AGCACTGCGTCACTCCGAATAATTCACCGG-3'), were then used for amplification of subunit 18S rRNA gene from DNA originally extracted from the holobiont (Rowan and Powers, 1991). Following amplification, restriction enzymes *HhaI* and *TaqI* were used for the digestion of amplicons for assessment of clades based on the Restriction Fragment Length Polymorphism (RFLP) profiles generated.

Chlorophyll a fluorescence measurements

A diving Pulse-Amplitude-Modulated Fluorometer (D-PAM, Heinz Walz GmbH) was used to measure chlorophyll *a* fluorescence (PSII functioning) of zooxanthellae symbionts in coral TBs formed. This was done by pooling together only the coral TBs for each stress experiment and measuring effective quantum yield (EQY) in light-adapted samples. EQY of PSII (ΦPSII) was determined according to the following expression by Genty et al. (1989):

$$\Phi\text{PSII} = (F_m' - F_t) / F_m' = \Delta F / F_m'$$

F_m' is the maximum fluorescence yield in illuminated sample with all PSII centres closed

F_t is the fluorescence yield immediately before applying a saturating pulse

Statistical analyses

Mean number of live coral TBs/ per species/ per treatment were recorded and expressed in terms of mean number per unit area (cm²) of coral surface. Non-parametric tests, Kruskal-Wallis multiple comparison test and Post-Hoc were used for significance testing for effects of coral species, zooxanthella genera, temperature stress, biochemical stress and exposure.

Result

This study examined the photo-physiological responses of coral TBs to thermal and biochemical stressors, and differential responses between source corals harbouring similar or different zooxanthellae genera (*Cladocopium* or *Durusdinium*). Thermal and biochemical stress (sponge and ascidian crude extracts) had significant negative effect

on density of coral TBs (Table 1). *Acropora muricata* (genus *Cladocopium*) were more affected than *A. muricata* (genus *Durusdinium*). Though genus *Cladocopium* was present in three tested coral species, variable responses to thermal and/or biochemical stressors were observed. HS extract affected density of coral TBs (which represent the holobiont) and PSII functioning (of symbiont) (Table 2). *Lithophyllon repanda* was most affected and *Pocillopora damicornis* was least affected among studied corals. This effect increased with higher temperatures. DM ascidian extract prevented the formation of TBs in the suspensions, but it did not affect symbiont PSII functioning except at higher temperatures (31°C).

Zooxanthellae genera identification

RFLP profiles of 18S rRNA gene amplicons were analysed. Genus *Cladocopium* was identified in *L. repanda* and *P. damicornis* samples (Fig. 2). *A. muricata*

Table 1 Summary of statistical analyses (Kruskal-Wallis) on tissue ball density among the four studied corals (*A. muricata* – genus *Cladocopium*, *A. muricata* – genus *Durusdinium*, *L. repanda* – genus *Cladocopium* and *P. damicornis* – genus *Cladocopium*) at three different temperatures (25, 28, 31°C), with sponge extract *H. madrepora* at concentrations 0, 50 & 120 µg/ml, at time intervals 60 and 120 minutes. *P<0.05; **P<0.01;***P<0.001; NS: no significant difference (n=5 per treatment).

Coral species	Temp/ °C	Time interval 60 minutes			Time interval 120 minutes		
		<i>H. madrepora</i> 0 µg/ml	<i>H. madrepora</i> 50 µg/ml	<i>H. madrepora</i> 200 µg/ml	<i>H. madrepora</i> 0 µg/ml	<i>H. madrepora</i> 50 µg/ml	<i>H. madrepora</i> 200 µg/ml
<i>A. muricata</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	NS	NS	NS	***
	25 – 31	NS	***	***	**	***	NS
	28 – 31	NS	NS	NS	NS	***	***
<i>A. muricata</i> (genus <i>Durusdinium</i>)	25 – 28	NS	NS	NS	NS	NS	**
	25 – 31	NS	NS	*	NS	***	**
	28 – 31	NS	NS	NS	NS	***	NS
<i>L. repanda</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	NS	NS	NS	NS
	25 – 31	NS	*	*	NS	NS	NS
	28 – 31	NS	NS	*	NS	NS	NS
<i>P. damicornis</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	NS	NS	NS	NS
	25 – 31	NS	NS	NS	NS	***	**
	28 – 31	NS	NS	NS	NS	*	NS

Table 2 Summary of statistical analyses (Kruskal-Wallis) on effective quantum yield among the four studied corals – Symbiodiniaceae clade (*A. muricata* - *Cladocopium*, *A. muricata* - *Durusdinium*, *L. repanda* - *Cladocopium* and *P. damicornis* - *Cladocopium*) at three different temperatures (25, 28, 31°C) and with sponge extract *H. madrepora* at concentrations 0, 50 & 120 µg/ml at time intervals 60 minutes and 120 minutes. *P<0.05; **P<0.01; ***P<0.001; NS: no significant difference (n = 5 per treatment).

Coral species	Temp/ °C	Time interval 60 minutes			Time interval 120 minutes		
		<i>H. madrepora</i> 0 µg/ml	<i>H. madrepora</i> 50 µg/ml	<i>H. madrepora</i> 200 µg/ml	<i>H. madrepora</i> 0 µg/ml	<i>H. madrepora</i> 50 µg/ml	<i>H. madrepora</i> 200 µg/ml
<i>A. muricata</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	*	NS	**	*
	25 – 31	NS	*	***	**	***	***
	28 – 31	NS	*	*	NS	*	*
<i>A. muricata</i> (genus <i>Durusdinium</i>)	25 – 28	NS	NS	NS	NS	NS	NS
	25 – 31	NS	NS	*	**	NS	**
	28 – 31	NS	NS	NS	NS	NS	NS
<i>L. repanda</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	*	NS	NS	*
	25 – 31	NS	NS	**	NS	NS	**
	28 – 31	NS	NS	NS	NS	NS	NS
<i>P. damicornis</i> (genus <i>Cladocopium</i>)	25 – 28	NS	NS	NS	NS	NS	*
	25 – 31	NS	NS	NS	NS	NS	**
	28 – 31	NS	NS	NS	NS	NS	NS

Fig. 2 RFLP profiles of zooxanthellae clade following digestion with Hha 1 and Taq 1: **A.** *Cladocopium* (formerly known as clade C) in *L. repanda* (LR), *P. damicornis* (PD) and *A. muricata* (AM); **B.** *Durusdinium* (formerly known as clade D) in *A. muricata* (AM).

colonies in this study hosted either genus *Cladocopium* or genus *Durusdinium* (Fig. 2).

Stress experiments on coral tissue balls

Results indicated that in the presence of thermal stress (28 and 31°C), mean density of TBs in *A. muricata* (genus *Cladocopium* or *Durusdinium*) was significantly lower as compared to *L. repanda* (genus *Cladocopium*) and *P. damicornis* (genus *Cladocopium*) (Fig. 3). The decrease in TBs density with higher temperatures (28 and 31°C) at longer time interval (120 minutes), was not significantly different in all corals, to the exception of *A. muricata* (genus *Cladocopium*). *A. muricata* (genus *Cladocopium*) was more affected than *A. muricata* (genus *Durusdinium*). Higher concentration of sponge *H. madrepora* negatively affected TBs density, with highest effect in *L. repanda*.

Fig. 3 Mean density of coral TBs per unit area of coral recorded for three species (*A. muricata C*, *L. repanda*, *P. damicornis* - genus *Cladocopium*, *A. muricata D* - genus *Durudsinium*) exposed to three temperatures (25, 28 and 31°C), at two time intervals (60 and 120 minutes) for: **A.** In the absence of extracts (Control), **B.** extracts of *H. madrepora* (50 µg ml⁻¹), and **C.** extracts at *H. madrepora* (200 µg ml⁻¹); n=5. *** represent significant differences from the Control (25°C). Summary of statistical analysis is given in Table 1.

Fig. 4 (A-F): Photosystem II functioning of symbiont of coral TBs from three studied coral species [*A. muricata* C, *L. repanda*, *P. damicornis* – genus *Cladocopium*, *A. muricata* D – genus *Durudinium*] exposed to three different temperatures [25 (control), 28 and 31°C], at two time intervals (A, C, E - 60 and B, D, F - 120 minutes) and extracts of *H. madrepora* (A, B - 0 µg ml⁻¹, C, D - 50 µg ml⁻¹ and E, F - 200 µg ml⁻¹). *** represent significant differences from the Control (25°C). Summary of statistical analysis is given in Table 2.

Fig. 5 (A–D): Photosystem II functioning of symbiont of coral TBs from three coral species [*A. muricata* C, *L. repanda*, *P. damicornis* – genus *Cladocopium*, *A. muricata* D – genus *Durusdinium*] exposed to three different temperatures [25 (control), 28 and 31°C], at two time intervals (A, C - 60 and B, D - 120 minutes) and extract *D. molle* (A, B - 50 and C, D - 200 $\mu\text{g ml}^{-1}$).

Effective Quantum Yield

Effective quantum yield, ΦPSII , was measured in coral TBs following stress experiments (Fig. 4). Under control conditions in the absence of thermal and biochemical stress, ΦPSII was normal and ranged between 0.5–0.6 in TBs from all three corals species. In the presence of HS extract at higher temperature (31°C), PSII functioning is affected (<0.2) in *A. muricata*, *L. repanda* and *P. damicornis*, with *A. muricata* (genus *Cladocopium*) more affected than *A. muricata* (genus *Durusdinium*). DM did not affect PSII functioning of suspension from three corals in the absence of TBs formation, irrespective of zooxanthellae clades harboured (genus *Cladocopium* or genus *Durusdinium*) (Fig. 5).

Discussion

The results of this study indicated a negative effect of thermal stress on density and effective quantum yield of coral TBs, mostly at high temperature (31°C). A higher susceptibility of TBs was noted from coral *Acropora muricata* (harbouring genus *Cladocopium*) as compared to coral *A. muricata* (harbouring genus *Durusdinium*). A lower susceptibility was observed in coral *Pocillopora damicornis* (harbouring genus *Cladocopium*). However, there was a significant difference ($p < 0.05$) between *A. muricata* (genus *Cladocopium*) and *P. damicornis* (genus *Cladocopium*), and also between *A. muricata* (genus *Cladocopium*) and *Lithophyllon repanda* (genus *Cladoco-*

pium) ($p < 0.05$). With thermal stress only, at 28°C, mean density of coral TBs was lower in both *A. muricata* (genus *Cladocopium*) and *A. muricata* (genus *Durusdinium*) as compared to *L. repanda* (genus *Cladocopium*) and *P. damicornis* (genus *Cladocopium*). At higher temperature 31°C, there was a pronounced effect of thermal stress on TB density between *Cladocopium*-harbouring *A. muricata* and *Durusdinium*-harbouring *A. muricata*, with *Cladocopium*-harbouring *A. muricata* being more affected.

The difference in TBs density with respect to thermal stress (31°C) may be explained by the higher resistance of genus *Durusdinium* to thermal stress as evidenced by other studies (Sylverstein et al. 2012). For example, genus *Durusdinium* isolated from *P. damicornis* in Guan showed evidence of photoprotection at 32°C, while *Cladocopium* isolated from same host showed photoinhibition (Rowan 2004). This explains the higher resistance of corals hosting genus *Durusdinium* (Baker et al. 2004). Berkelmans and Van Oppen (2006) have evidenced that a shift in dominance to genus *Durusdinium* in *A. millepora* has resulted in an acquired tolerance of 1–1.5°C. However, genus *Durusdinium* is able to outcompete other symbiont types only under stressed conditions, and may not be an advantage under non-stressful conditions (Baker et al. 2013). In this study, the thermal tolerance of *Durusdinium* harbouring-*A. muricata* was more evident at longer exposure time and in the presence of biochemical (sponge extract) and thermal stressors combined.

Although genus *Cladocopium* was present in all three tested coral species, *A. muricata*, *L. repanda* and *P. damicornis*, a variable response was observed in terms of TB density in the three species harbouring similar zooxanthellae genus. This observation may be attributed to the effects of zooxanthellae genus subclades which may attribute different susceptibilities to stress. Similar observations were made in a previous study which demonstrated a higher susceptibility of *A. muricata* compared to other hard corals (Mattan-Moorgawa et al. 2012) *in situ*. Density of TB was null in the presence of DM extract in all conditions, however, PSII was recorded. The DM extract is suspected to exhibit cytotoxic effects on the dissociated coral cells and microalgae, thus causing them to form lumps and preventing the formation of rotating tissue balls. Ascidians have been reported to contain bioactive

alkaloids which enable them to outcompete corals and deter their predators (Rodriguez-Martinez et al. 2012; Koplovitz et al. 2015; Gewing et al. 2017). However, the contribution of the different zooxanthellae species as per LaJeunesse et al. (2018) classification needs further investigation to be able to thoroughly investigate the role of the zooxanthellae and that of the host in the studied coral species.

PSII functioning ranged between 0.5–0.6 in all coral TBs from all source coral species in the absence of thermal and biochemical stresses. Here also there was a significant difference ($p < 0.05$) between *A. muricata* (genus *Cladocopium*) and *L. repanda* (genus *Cladocopium*), and between *A. muricata* (genus *Durusdinium*) and *L. repanda* (genus *Cladocopium*), but not between the Acroporids harbouring two different *Symbiodinium* clades except in the presence of biochemical stressor and longer exposure time to thermal stressor. The biochemical stress comes from the bioactive compounds present in the sponge and ascidian extracts, which may interfere with the PSII functioning in the TBs. Similar to ascidians, sponges also produce bioactive compounds which help them deter predators and compete for food and space with corals and other reef organisms, for example, *T. hoshinota* which is a coral-killing sponge (Wang et al. 2012). In the presence of HS extract (200 µg ml⁻¹) at higher temperature stress (31°C), PSII functioning was affected in all three corals with *P. damicornis* being most affected and *A. muricata* (genus *Durusdinium*) least affected. A reverse trend was observed in PSII functioning among all coral species in the presence of DM extract (200 µg ml⁻¹) at 31°C, with *A. muricata* (genus *Cladocopium*) most affected and *P. damicornis* least affected. The results of this study suggest a differential response among the three corals to thermal stress and biochemical stressors from extracts of sponge and ascidians.

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Compliance

Sampling procedures used in this study comply with national and international legal requirements and local regulations. Permit for sample collection was obtained from the then Ministry of Agro Industry and Fisheries (now Ministry of Blue Economy, Fisheries, Marine Resources and Shipping) under permit number F20/2V.6.

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