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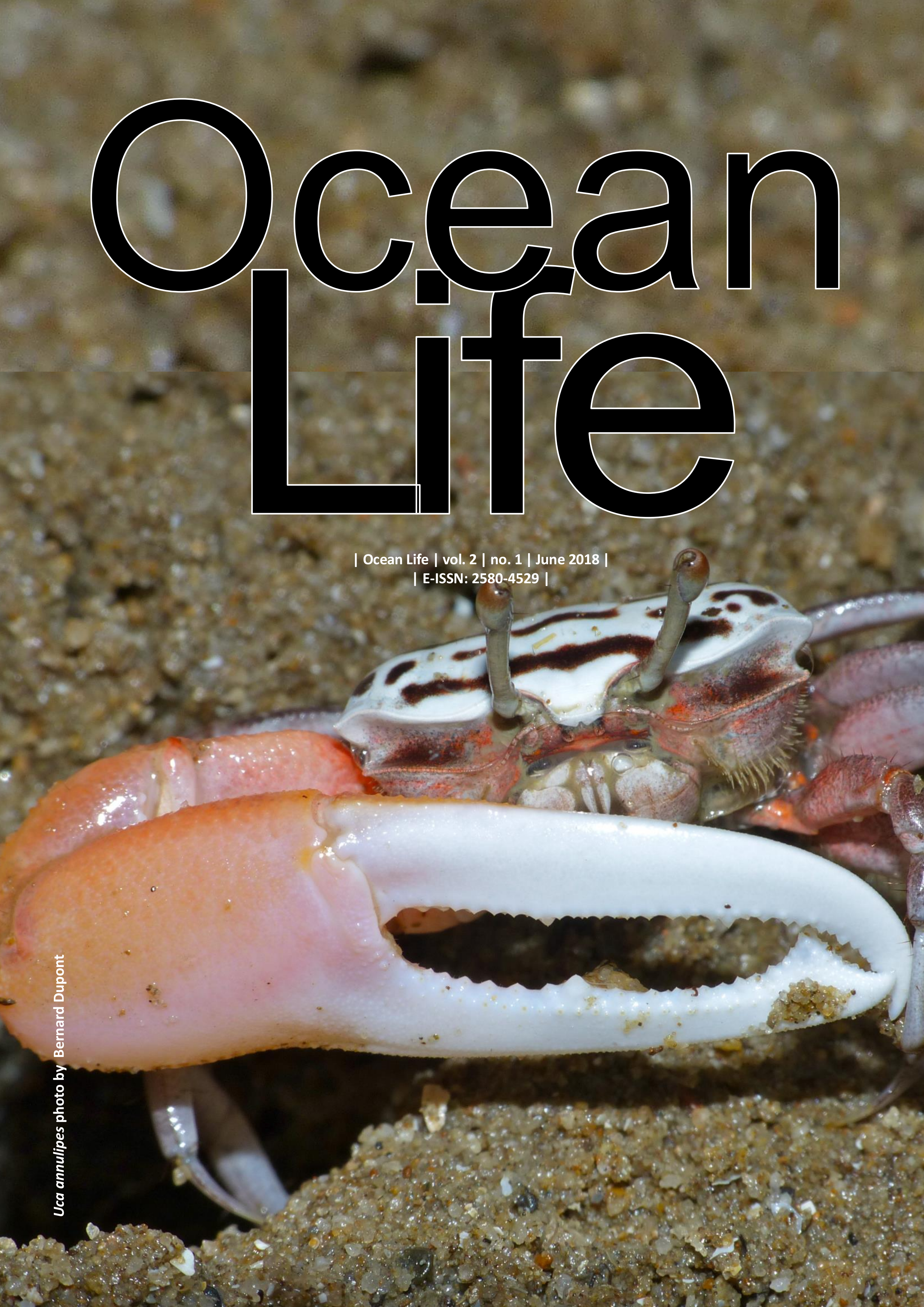

Uca annulipes photo by Bernard Dupont

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Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Variable PSII functioning and bleaching conditions of tropical scleractinian corals pre-and post-bleaching event

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Abstract. *Mattan-Moorgawa S, Rughooputh SDDV, Bhagooli R. 2018. Variable PSII functioning and bleaching conditions of tropical scleractinian corals pre-and post-bleaching event. Ocean Life 1: 1-10.* This study compared pre-bleaching and post-bleaching conditions of eight reef-building corals, *Acropora cytherea, Acropora hyacynthus, Acropora muricata, Acropora* sp.*, Pocillopora damicornis, Pocillopora eydouxi, Galaxea fascicularis,* and *Fungia* sp., in terms of visual coloration (non-bleached (NB), pale (P), partially bleached (PB) and bleached (B)) and chlorophyll fluorescence yield at photosystem II (PSII)). A total of twenty colonies from twelve stations along four transects were surveyed at Belle-Mare, Mauritius, from October 2008 to October 2009 and compared to the CoralWatch Coral Health Chart. PSII functioning, measured as Fv/Fm, was recorded in coral samples using a pulse-amplitude-modulated (PAM) fluorometer. Physico-chemical parameters (sea surface temperature, dissolved oxygen, salinity, and pH) were recorded in situ. An increase in SST up to 31.4ºC in February 2009 triggered the site's bleaching event observed in May 2009. Acroporids showed the first sign of bleaching and paling from January 2009, when the mean SST was at 30ºC. Branching coral (*P. eydouxi*) and solitary coral (*Fungia* sp.) exhibited only 15% of their colonies and paling by April 2009. *A. cytherea*, *A. hyacynthus*, and *A. muricata* showed varying bleaching conditions [Pale (P), Partially-bleached (PB), and Bleached (B)] at the onset of the bleaching event while Acropora sp. showed only a paling of its colonies. Post-bleaching data indicated a differential recovery in visual coloration and PSII functioning among the corals. *P. eydouxi* and *Fungia* sp. showed no bleaching conditions throughout the study. P. damicornis and G. fascicularis indicated a quick coloration recovery from P to NB after the bleaching event. However, their maximum quantum yield at PSII did not show significant changes in P and NB samples. *A. muricata* recovered faster than *A. hyacynthus* and *A. cytherea* in terms of PSII functioning. A differential recovery was observed post-bleaching event among the eight coral species in terms of recovery of color and PSII functioning. The recovery order was as follows: massive-like/ solitary corals > branching and semi-bulbous corals > tabular corals.

Keywords: Bleaching, climate change, PSII functioning, reef-building corals, PAM

INTRODUCTION

Coral bleaching is a stress response in reef-building corals exposed to acute short-term or long-term environmental stress from both natural and anthropogenic sources. Muscatine et al. (1981) reported that in the symbiotic relationship between the coral host and its symbiont, the host could obtain up to 100% of its daily carbon requirements from photosynthesis in its endosymbiont. The coral bleaching process takes place due to the breakdown of the photosynthetic machinery (Photosystem II or PSII) of Chlorophyll *a* (Chl *a*) of the endosymbiotic dinoflagellate (Genus *Symbiodinium*). Breakdown of PSII and subsequent degradation can lead to loss of pigments and/or loss of zooxanthellae, leading to subsequent paling or whitening of corals. However, the cellular processes and/or mechanisms for expulsion of zooxanthellae during bleaching are still unclear (Baker et al. 2008).

The extent of damage at the level of PSII in zooxanthellae symbionts has been attributed to: (i) interactions between temperature and light (Iglesias-Prieto. 1997; Fitt and Warner, 1995; Lesser et al., 1996; Warner et al., 1996; Jones et al. 1998; Brown et al. 2000; Fitt et al. 2001; Bhagooli and Hidaka 2006); (ii) production of reactive oxygen species (ROS) (Baird et al. 2009); lipid composition of the symbiont thylakoid membranes that affect its structural integrity at higher temperatures (Tchernov et al. 2004); and increased levels of nitric acid synthase (Trapido-Rosenthal et al. 2005).

Intra-specific and intra-specific differential responses of scleractinian corals have been reported in several studies (Loya et al. 2001; Brown et al. 2002; Bhagooli and Hidaka 2003; Visram and Douglas 2007; Sampayo et al. 2008; Louis et al. 2016) and have been attributed to both dinoflagellate symbiont and animal host. In symbionts, physiologically distinct lines (or clades) of *Symbiodinium* spp. may confer differential thermal thresholds on a coral host. The coral host may also contribute to the differential response (Baird et al. 2009; Bhagooli et al. 2008; Baird et al. 2010) in the coral by adopting different ways to reduce

UV and light flux to its symbionts, such as the production of fluorescent pigments, mycosporine-like amino acids, antioxidant systems, and stress enzymes.

Few *ex-situ* (Hoegh-Guldberg and Jones 1999; Warner et al. 1999; Grottoli et al. 2006) and *in-situ* studies (Warner et al. 1999; Bhagooli and Hidaka 2003, 2004; Yakovleva and Hidaka 2004) have reported the mechanisms of recovery from bleaching in scleractinian corals. Jones and Yellowlees (1997) reported that algal densities in corals remain remarkably constant as a result of carefully regulated control mechanisms such as pre-mitotic control of zooxanthellae; growth inhibitory factors or limitation of algal nutrient supply; post-mitotic control by digestion of healthy or senescent zooxanthellae; or expulsion of excess or senescent zooxanthellae. Bleached corals recover their algal population by division of remaining zooxanthellae (Jones and Yellowlees 1997) or shifts in symbiont communities to opportunistic and resilient members of Clade D (Thornhill et al. 2006; LaJeunesse et al. 2009) or by symbiotic flexibility which provide corals with a mechanism to respond to environmental change (Sylverstein et al. 2012). Space limitation, that is, space availability and symbiont size, determines algal densities in corals and the constancy of algal density between species, growth forms, and over depth and geographic range.

Rodrigues et al. (2008) studied the changes in chlorophyll fluorescence over long-term bleaching and recovery in two Hawaiian coral species, *Porites compressa*, and *Montipora capitata*, under controlled *ex-situ* conditions. The study reported that zooxanthellae of *P. compressa* were more resilient to bleaching and exhibited faster recovery due to the following factors: host feeding strategies during recovery from bleaching; zooxanthellae clade type (Type C15 in *P. compressa*); and Chl *a* recovery by zooxanthellae symbionts.

Van Woesik et al. (2011) revisited the winners and losers of coral bleaching over 14 years. The authors concluded that short-term winners were the thermally-tolerant encrusting and massive coral morphologies (*Porites* and faviids) and *Acropora* colonies (smaller than <5 cm in diameter). Long-term winners were revealed as (i) thermally tolerant, locally persistent colonies, (ii) remnant survivors that rapidly regrew, and (iii) regionally persistent colonies that were recruited. McCowan et al. (2012) hypothesized that in species with polyps that are physiologically independent (e.g., massive colonies), only polyps directly affected by both heat and light respond as predicted by the photoinhibition model of coral bleaching of Jones et al. (1998). As a result, bleaching within the colony is patchy, and rates of whole colony mortality are low, which is a typical response of most massive species. Moreover, highly integrated taxa cannot contain the damage, and rates of whole colony mortality are therefore high (e.g., *Acropora*) (McCowan et al. 2012).

The present study investigated the recovery of eight species of reef-building corals in Mauritius following a major single bleaching event recorded in May 2009 at the lagoon of Belle Mare, Mauritius (Mattan-Moorgawa et al. 2012). The study aimed the following: (i) to investigate the bleaching susceptibilities before and after the recovery post-May 2009 bleaching event; (ii) to assess recovery of coloration in bleached, partially-bleached, pale, and non-bleached colonies of eight test coral species; (iii) to investigate recovery of photosynthetic functioning of zooxanthellae in same coral colonies in terms of maximum quantum yield, Fv/Fm, at photosystem II (PSII) using a Pulse-Amplitude-Modulated fluorometer, and; (iv) to investigate the effect of physical parameters on bleaching and recovery patterns of the coral colonies.

MATERIALS AND METHODS

Fieldwork

Four transects of approximately 800m running from coast to reef were surveyed in the lagoon of Belle Mare, each comprising 3 stations at near-shore, mid-lagoon, and back-reef habitats. A total of 12 stations with 20 colonies of eight reef-building coral species were surveyed to assess bleaching and subsequent recovery over time. The twenty coral colonies were first tagged in October 2008 for bleaching monitoring. After the single bleaching event recorded in May 2009, the colonies exhibiting different bleaching conditions [non-bleached (NB), pale (P), partially bleached (PB), and bleached (B)] were re-tagged for follow-up on the recovery from May to October 2009. In addition, coral colonies were tagged with fluorescent tapes for easy identification during the study, and the tapes were removed after the completion of the study.

Sample collection and preparation

Colonies of eight reef-building corals, *Acropora cytherea*, *Acropora hyacynthus*, *Acropora muricata*, *Acropora* sp., *Pocillopora damicornis*, *Pocillopora eydouxi*, *Galaxea fascicularis*, and *Fungia* sp., of varying conditions (partially bleached (PB), pale (P), bleached (B) and non-bleached (NB) were collected from twenty tagged colonies at twelve stations at the study site. Non-bleached (NB) condition indicates that the whole colony looks healthy and normal color; the Pale (P) condition indicates the overall paling of the colony; Partially-bleached (PB) condition indicates a colony that exhibits less than 30% bleaching/ whitening; Bleached (B) condition indicates colony which exhibits >90% bleaching/ whitening. Coral tips of 2-3 cm were collected and kept in 250 ml sampling bottles in seawater. Coral samples were brought to the laboratory and dark-adapted to allow the photosynthetic endosymbionts to relax all their PSII reaction centers before measurement of maximum quantum yield, $F_v/F_{m.}$

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a teaching PAM fluorometer. Initial fluorescence (Fo) was measured by applying pulses of weak red light (< 1 µmol quanta m^{-2} s^{-1}) and a saturating pulse (3,000 µmol quanta m^{-2} s^{-1}, 0.8 s duration) was applied to determine maximal fluorescence (F_m) when all PSII centers were closed. The ratio of change in fluorescence (F_v/F_m) caused by the saturating pulse to the maximal fluorescence (F_m) in a dark-adapted sample is correlated to the maximum quantum yield of PSII. It thus represents the PSII functioning (Genty et al. 1989).

In-situ measurements of physical parameters

Temperature (OAKTON pH/mv/^{0}C meter pH300 series) and other physical parameters, namely dissolved oxygen (Hach Sension 6), salinity, and pH (OAKTON waterproof pH300 series), were measured *in situ* and recorded every month from May 2009 to October 2009.

Statistical analysis

Mean values and standard deviations were calculated for the maximum quantum yield for the four different conditions (PB, P, B, and NB) in the eight species of coral.

RESULTS AND DISCUSSION

Physical parameters

Recorded physical parameters from October 2008 to October 2009 included average temperature, dissolved oxygen, salinity, and pH values (Figure 1). Mean salinity varied over the study period, with the highest mean salinity recorded in August 2009 at 35.25 ‰ and the lowest at 33.25 ‰ recorded in April 2009. Mean dissolved oxygen fluctuated over the study period, with the lowest levels (7 mgL^{-1}) recorded in October 2009 and peak levels (20.38 mgL^{-1}) in May 2009. Mean pH levels varied slightly over the study period at an average of 7, with an abnormally lower pH recorded at 8.45 in August 2009. Mean seawater temperature at sampling stations indicated a seasonal trend, with the lowest temperatures recorded at 23.9ºC in the winter of September 2009 and the highest at 31.4ºC in February 2009. There was a gradual increase in sea surface temperatures from October 2008 (26.6ºC) to February 2009 (31.4ºC), which decreased gradually to 27.5ºC in May 2009. The lowest mean sea surface temperatures were recorded in October 2009 at 24.1ºC

Percentage bleaching occurrence in sampled colonies pre-bleaching event

All surveyed coral colonies appeared non-bleached and healthy over the first three summer months from October 2008 to December 2008. In January 2009, the first cases of paling (P) and bleaching (B) were recorded in *A. muricata* (15%), *Acropora* sp. (25%), *A. cytherea* (15%), and *A. hyacynthus* (20%) (Figure 2).

All (100%) colonies of *Fungia* sp. and *P. eydouxi* were non-bleached (NB) throughout the study period. Surveyed colonies of *P. damicornis* and *G. fascicularis* showed 100% NB condition from October 2008 until March 2009. In April 2009, both *P. damicornis* and *G. fascicularis* showed paling (P) for 15% of the colonies. In May 2009, the percentage of colonies showing paling increased to 25% in P. damicornis and 30% in *G. fascicularis*.

A. cytherea colonies showed an increasing occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (60%). For example, in February 2009, *A. cytherea* showed all four visual conditions at 35% NB, 35% P, 15% PB, and 15% B, while in May 2009, it was 25% NB, 15% PB, and 60% B in May 2009 (Figure 2).

A. hyacynthus colonies also showed an increased occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (65%). For example, in February 2009, *A. hyacynthus* showed all bleaching conditions at 60% NB, 15% P, and 25% B; at 45% NB, 15% PB, and 40% B in March 2009; at 40%NB, 15%PB and 45%B in April 2009; and at 20%NB, 15%NB and 65% B in May 2009 (Figure 2).

A. muricata colonies also showed an increased occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (35%). For example, in February 2009, *A. muricata* showed all four visual conditions at 25% NB, 50% P, and 25% PB, compared to 25% NB, 15% P, 25% PB and 35%B in May 2009 (Figure 2).

Acropora sp. colonies, on the contrary, showed the occurrence of only two conditions, NB and P, from January 2009 to May 2009, with no record of PB and P conditions. Here, P conditions increased from 25% in January 2009 to 50% in May 2009 (Figure 2).

Post-bleaching conditions of corals/ recovery

The bleaching state/recovery conditions were investigated in surveyed coral colonies post-bleaching from May 2009 until October 2009. Figure 3 compares the bleaching condition in the eight studied species. Results indicated that all NB coral colonies of the eight species remained non-bleached (NB = 1) and in a healthy state up to October 2009.

All pale (P) colonies recorded in May 2009 in the four species, namely *A. hyacynthus*, *A. muricata*, *Acropora sp.*, *P. damicornis*, and *G. fascicularis*, recovered to the NB condition in June 2009, continuing this trend until October 2009, to the exception of P colonies of *A. cytherea* which remained in the same condition (P = 2) for May-June 2009 and only recovered (NB = 1) as from July 2009.

Moreover, all partially bleached (PB = 3) colonies recorded in May 2009 for *A. muricata* recovered to non-bleached condition (NB = 1) from June 2009 onwards, indicating a rapid recovery. However, results also indicate that partially bleached (PB) colonies recorded for *A. cytherea* in May 2009 took some time to recover, attaining recovery (NB = 1) only in August 2009.

All bleached (B) colonies recorded in May 2009 for *A. cytherea*, *A. hyacynthus*, and *A. muricata* showed no improvement in June 2009 in its bleaching condition, except for *A. muricata*, which indicated a quick recovery from bleached (B = 4) to pale condition (P = 2) from May 2009 to June 2009, respectively. All B colonies of *A. muricata* gained complete recovery in July 2009. Bleached colonies recorded for *A. cytherea* and *A. hyacynthus* during the May 2009 bleaching event showed an initial recovery to PB condition only after two months, i.e., in July 2009. Complete recovery in these two species to non-bleached (NB = 1) conditions occurred in August 2009.

Chlorophyll fluorescence ratio, Fv/Fm

Figure 4 indicated the chlorophyll fluorescence ratio of *inhospite* zooxanthellae cells from coral samples collected from tagged coral colonies showing different visual conditions, namely non-bleached (NB), pale (P), partially bleached (PB), and bleached (B), which was carried out over 6 months following the May 2009 bleaching event.

Results indicated that PSII functioning, Fv/Fm, was normal at an average of 0.6 in all NB colonies in the eight studied corals. For all tagged pale (P) colonies, recorded Fv/Fm values were as high as 0.6 in *P. damicornis* and *G. fascicularis* and as low as 0.4 in pale (P) colonies of *A. hyacynthus*, *A. muricata*, and *Acropora* sp. over the post-bleaching months from May 2009 to October 2009. Pale colonies of *P. damicornis* and *G. fascicularis* showed no significant change in PSII functioning over the post-bleaching months (May 2009 to October 2009). However, P colonies of *A. hyacynthus*, *A. muricata* and *Acropora* sp. showed an overall recovery of PSII functioning from May 2009 (Fv/Fm = 0.4) to June 2009 (Fv/Fm = 0.6), July 2009 (Fv/Fm < 0.6) and August 2009 (Fv/Fm = 0.6). That indicated a slow recovery from late summer 2009 (May) to early summer 2009 (October). However, among the three Acroporids, *A. muricata* showed a faster recovery of its PSII functioning than *A. hyacynthus* and *Acropora* sp.

For all tagged partially-bleached (PB) colonies for *A. cytherea* and *A. muricata*, recorded Fv/Fm values were as high as 0.6 and as low as 0.45 during the post-bleaching from May 2009 to October 2009. In fact, in May 2009 bleaching month, PSII functioning was lower in *A. cytherea* (Fv/Fm = 0.45) and higher in *A. muricata* (Fv/Fm = 0.56). From June 2009 to October 2009 indicated a faster recovery of PSII functioning in *A. muricata* from 0.56 in May 2009 to 0.6 in early June 2009. In contrast, PSII functioning indicated a slower recovery in *A. cytherea* from 0.45 in May 2009 to 0.6 in October 2009. In May 2009, only three corals exhibited the bleached (B) condition, namely *A. cytherea*, *A. hyacynthus*, and *A. muricata*. PSII functioning recorded in May 2009 in bleached (B) colonies was as low as 0.14 in A. cytherea and A. hyacynthus and 0.19 in *A. muricata.* A gradual recovery of PSII functioning was observed in all three species post bleaching from May 2009 (Fv/Fm = 0.14-0.19) to October 2009 (Fv/Fm = 0.6). However, results indicated that *A. muricata* recovered fastest in July 2009, with the highest chlorophyll fluorescence ratio of 0.57 compared to the other two Acroporids.

Discussion

This study indicated a differential recovery among eight reef-building corals in terms of recovery of color and recovery of PSII post-May 2009 bleaching event.

Physical parameters

It is observed that an increase in sea surface temperatures up to a maximum of 31.4ºC in February 2009 triggered the bleaching event in May 2009. However, other physical parameters such as DO, salinity, and pH had insignificant variations over the study period and did not affect the bleaching and/or recovery in the studied corals.

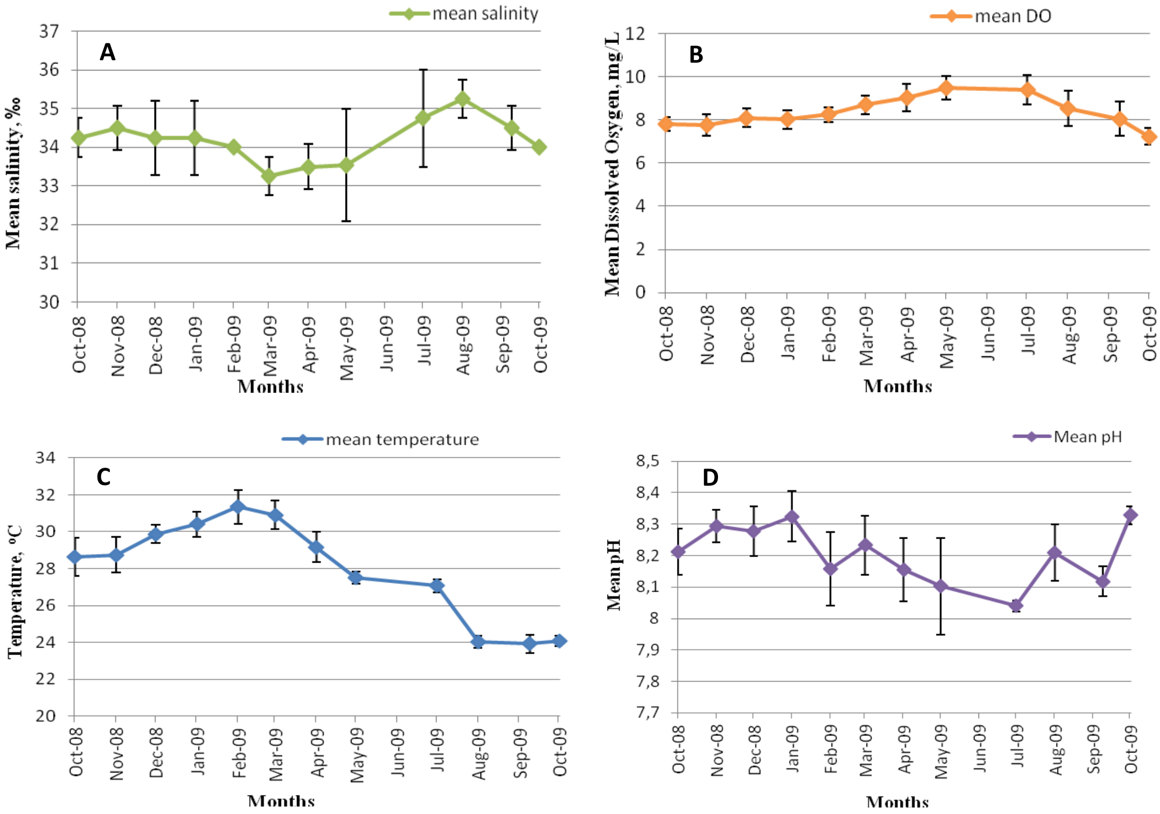

Figure 1. Physical parameters measured in situ during Oct 2008-Oct 2009: A. salinity (ppt); B. dissolved oxygen (mgL-1); C: seawater temperature (°C); D. pH. Data represent mean±SD (n=12 for each month)

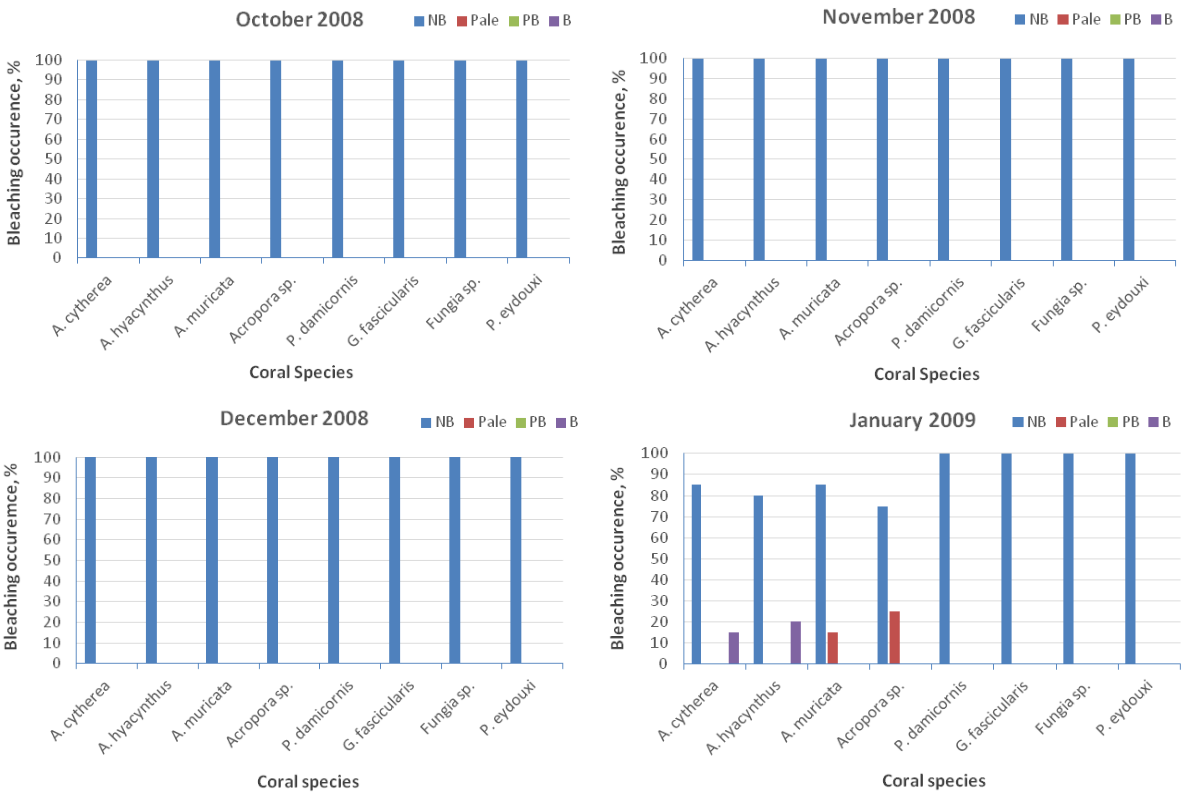

Figure 2A. Percentage bleaching occurrence (% out of 20 colonies) in the eight studied coral species over a one-year study from October 2008 to January 2009

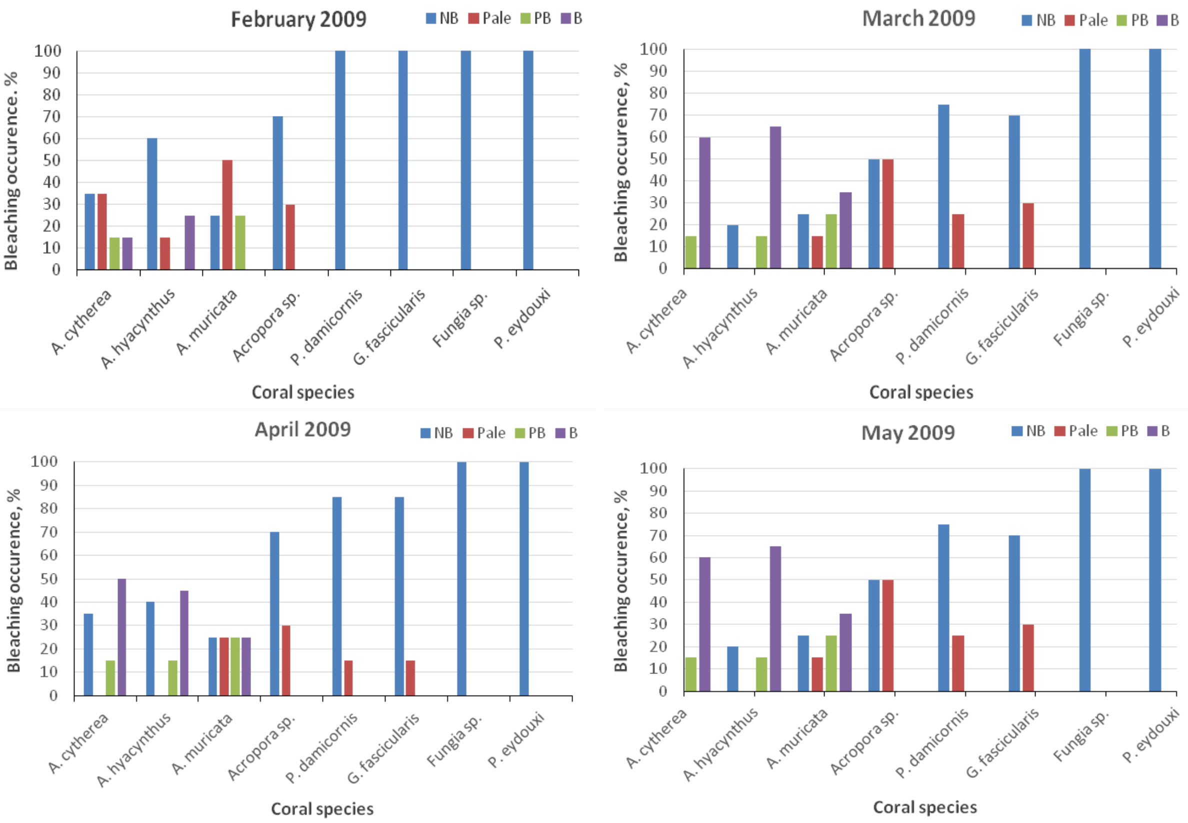

Figure 2B. Percentage bleaching occurrence (% out of 20 colonies) in the eight studied coral species over a one-year study from February 2009 to May 2009

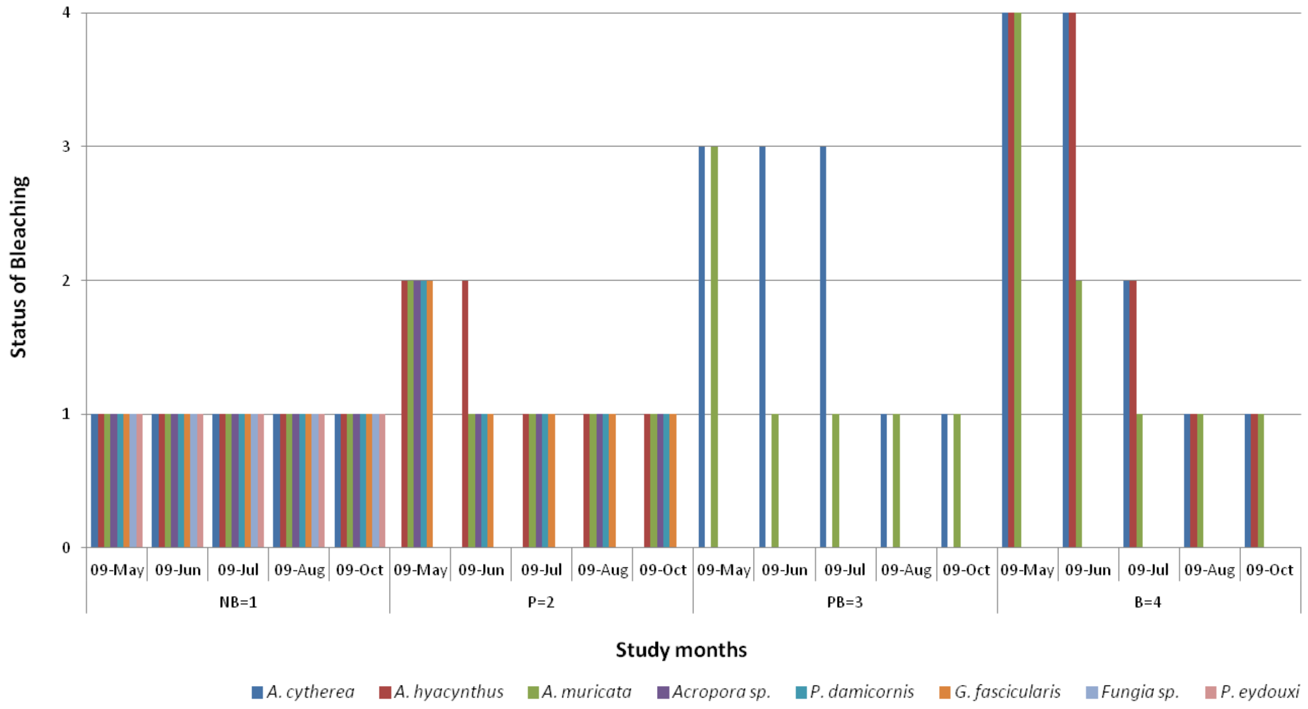

Figure 3. Percentage bleaching occurrence (% out of 20 colonies) in the eight studied coral species over a one-year study period from October 2008 to May 2009

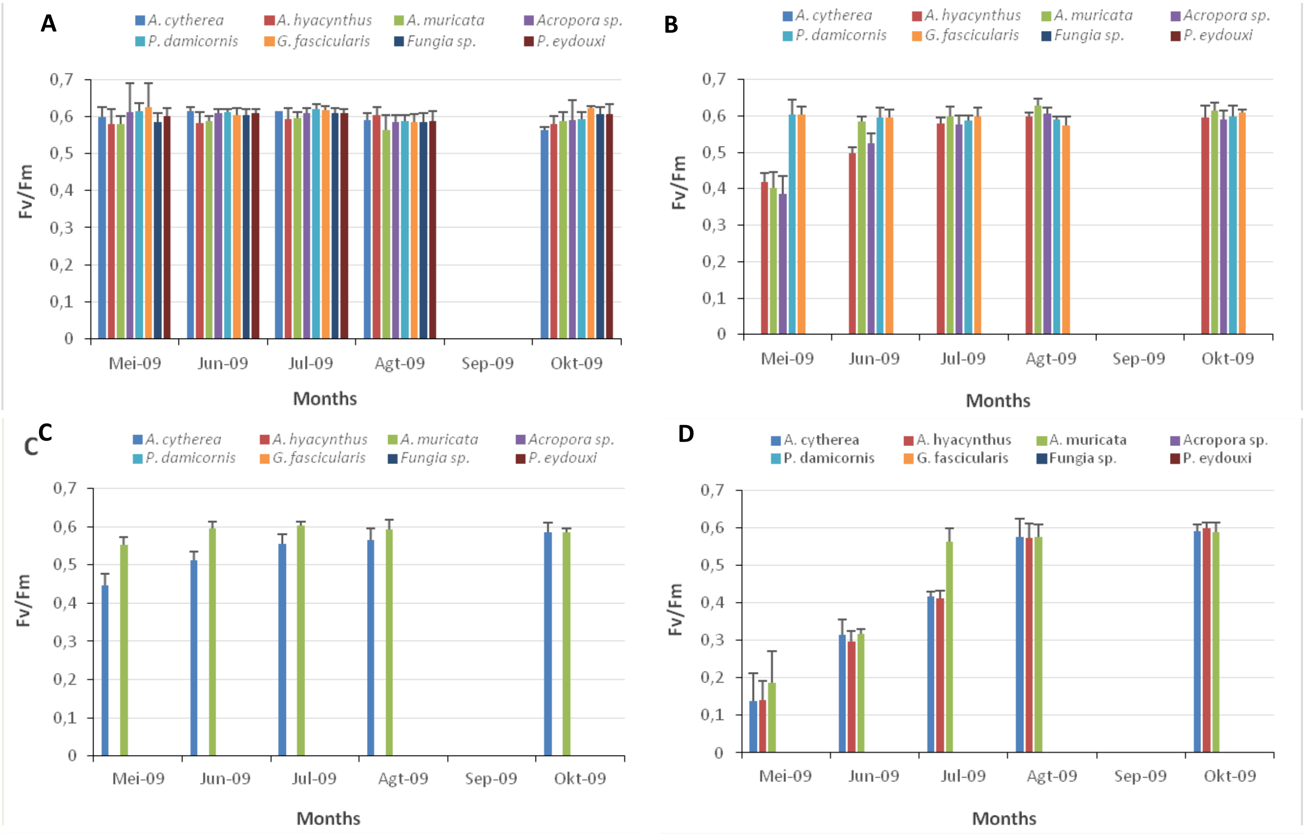

Figure 4. Post-bleaching recovery and PSII functioning (Fv/Fm) among the four different conditions of the surveyed eight coral species: A. non-bleached (NB); B. pale (P); C. partially-bleached (PB); C. bleached (B)

Percentage bleaching occurrence in sampled coral species pre-bleaching event

Paling and bleaching signs were observed in the *Acroporids* from January 2009 when mean sea surface temperatures rose above 30 ºC, which may have been an onset of the bleaching event down the line in May 2009. This result also suggests a pre-disposition or susceptibility of the *Acroporids* to elevated sea surface temperatures.). Past studies have highlighted the importance of coral growth and morphology as a contributory factor in the susceptibility and mortality of scleractinian corals to bleaching (Brown and Suharsono 1990; McClanahan 2000; Nakamura and van Woesik 2001; Baird and Marshall 2002; Riegl 2002; Jones 2008; McCowan et al. 2012). Families of corals mostly characterized by branching growth forms (e.g., Acroporidae and Pocilloporidae) are most susceptible to bleaching and experience the highest rates of mortality once bleached (Baird and Marshall 2002; Jones 2008). Conversely, families of corals that typically have massive morphologies (e.g., Faviidae, Mussidae, and Poritidae) appear fairly resistant to increasing temperature and are among the last to bleach.

Consequently, they more frequently experience partial rather than whole colony mortality (Brown and Suharsono 1990; McClanahan 2000; Baird and Marshall 2002; Riegl 2002). McCowan et al. (2012) reported that overall patterns of bleaching susceptibility were significantly different among coral growth forms, whereby branching, columnar and tabular corals have greater susceptibility and mortality than massive, submassive, encrusting, and free-living corals. Nakamura and van Woesik (2001) reported that flatter and smaller corals have a greater capacity to remove potentially deleterious superoxides and other oxygen radicals compared to more erect and branching forms. That may explain observations of the present study whereby Acroporids (tabular and branching) tend to be thermally most susceptible and show different bleaching conditions (paling, partial bleaching, and complete bleaching) at the onset of a bleaching event. Massive coral (*P. eydouxi*) and solitary coral (*Fungia sp.*) were the most robust of the eight studied corals, with only 15% of their colonies paling in April 2009 before the bleaching event.

Interestingly, the results of this study also indicate a differential susceptibility among Acroporids themselves, with *A. cytherea*, *A. hyacynthus*, and *A. muricata* showing varying bleaching conditions (P, PB, and B) at the onset of the bleaching event and *Acropora* sp. showing relatively lower susceptibility/ higher robustness in terms of bleaching conditions, observed only as paling of coral colonies. The following factors have explained this differential susceptibility among Acroporids in other studies: variations in morphology (Loya et al. 2001; McCowan et al. 2012); inherent differences in growth rates (Baird and Marshall 2002); thermal tolerances of photo-endosymbionts in terms of damage at PSII (Bhagooli and Hidaka 2003; Berkelmans and van Oppen 2006; Bhagooli 2009; Oliver and Palumbi 2011) and/or inhibition of Calvin-Benson cycle (Jones et al. 1998; Bhagooli 2013); tissue thickness and/or marked differences in colony size and age (Loya et al. 2001); differential susceptibility and adaptive mechanisms of a coral host (Baird et al. 2009); and combined physiology of coral symbiont and coral host forming the holobiont (Sampayo et al. 2008). Bhagooli (2012) also reported that zooxanthellae density of > 0.5, ~ 0.2, ~ 0.1 and < 0.02 x 10^6 cells cm^{-2} represented bleaching severity of 0, 50, 75 and > 90 %, respectively. Horizontal branches of *A. muricata* were more susceptible to bleaching than vertical ones indicating solar bleaching (Bhagooli 2012). Stemming from the above, the differential susceptibility among the Acroporids observed in this study could be explained by variations in morphology and thermal tolerances of endosymbiotic dinoflagellates.

Post bleaching recovery and *PSII functioning among studied corals*

Bleaching state/ recovery conditions were investigated in surveyed coral colonies post-bleaching event in May 2009 until October 2009. During that period, all NB colonies exhibited normal color and normal chlorophyll fluorescence ratio (Fv/Fm = 0.6). Results also indicated a quick recovery in June 2009 of 100% of P colonies of *A. hyacynthus*, *A. muricata*, *A. sp.*, *P. damicornis*, and *G. fascicularis* to NB condition, except for *A. cytherea*, which took slightly longer to recover. Moreover, all PB colonies recorded in May 2009 for *A. muricata* recovered to NB from June 2009 onwards, indicating a rapid recovery, except PB colonies of *A. cytherea* recorded in May 2009, which underwent a slower recovery to NB in August 2009. Similarly, 100% of B colonies of *A. muricata* recorded in May 2009 achieved quicker recovery to NB in July 2009, compared to B colonies for *A. cytherea*, *A. hyacynthus*, and *A. muricata*, which showed no complete recovery until August 2009.

The above observations suggest that tabular corals, *A. cytherea*, were more affected by bleaching events and thus took longer to recover. When this observation is tallied by PSII functioning data (Figure 4), it is deduced that recovery of visual condition from P to NB also involved a recovery of PSII functioning. This observation strongly suggests that recovery of visual appearance/color of coral may be linked to a recovery of PSII functioning in zooxanthellae. Rodrigues et al. (2008) demonstrated that M. capitata bleached six days earlier than P. compressa and that PSII repair recovered 6.5 months earlier than in *M. capitata*. The authors suggested that the zooxanthellae of *P. compressa* were more resilient to bleaching stress. In this study, it is to be noted that P colonies of *P. damicornis* and *G. fascicularis*. However, pale appearance showed no remarkable change or improvement in PSII functioning, suggesting that paling may have involved loss of pigments rather than damage to the PSII functioning of Chl *a* of zooxanthellae symbionts in these two species of scleractinian corals. This observation may be explained by the fact that flatter and smaller corals have a greater capacity to remove potentially deleterious superoxides and other oxygen radicals compared to more erect and branching forms (Nakamura and van Woesik 2001). Bhagooli and Yakovleva (2004) also demonstrated bleaching susceptibility and mortality in massive coral *Platygyra ryukyuensis* (no mortality) and *Seriatopora* *caliendrum* (100% mortality) after exposure to thermal stress.

When comparing bleaching conditions/ visual status/ coloration of PB and B corals with their PSII functioning data, it is noted that recovery from B to PB, PB to P, and P to NB conditions normally involved an improvement of PSII functioning of photosynthetic apparatus of coral symbionts. In PB and B samples, *A. muricata* normally exhibited higher Fv/Fm values compared to the other two Acroporids, *A. cytherea*, and *A. hyacynthus*, showing a faster recovery of functioning of Chl *a* in its zooxanthellar symbionts in June and July 2009. All P, PB, and B samples in this study gained coloration by October 2009, simultaneously showing an improvement in PSII functioning.

The results indicated that recovery of zooxanthellae Chl *a* fluorescence, i.e., PSII functioning (to Fv/Fm = 0.6), varied among the studied corals within days and/or months, with some recovering faster (*A. muricata* and *A. sp.*) and others recovering much slower (*A. cytherea* and *A. hyacynthus*). Although showing a loss of coloration (visual paling) during the bleaching event and subsequent gain of coloration (visual non-bleaching) post-bleaching event, *P. damicornis* and *G. fascicularis* showed no remarkable changes in fluorescence quantum yield during and post-bleaching event. Higher bleaching susceptibility in *A. cytherea* and *A. hyacynthus* and their slower recovery in terms of PSII functioning was also observed. Jones and Yellowlees (1997) have reported how a bleached colony of a staghorn coral, *Acropora formosa* (Dana 1846), now *Acropora muricata*, recovered its algal symbionts after a major bleaching event and the processes involved in its algal regulation and control. In their study, the authors suggested that algal cell size appeared invariant of seasonal change, zooxanthellae density, chl *a* concentration, and division frequency. If the algal size can be determined by host or external light conditions or nutrient supply, then it may ultimately determine the whole nature of the association.

Differential post-bleaching recovery among studied species may suggest chronic photoinhibition, also indicating that photodamage may have affected the structures and functions of PSII in these species ((Jones and Hoegh-Guldberg 2001). Mechanisms of recovery can be explained by the following: elevated Chl *a* per zooxanthellae (Rodrigues et al. 2008); an increase in mitotic index and therefore number of zooxanthellae cells (Bhagooli 2012a); zooxanthellar density in terms of zooxanthellae reproduction and zooxanthellae release rates from bleached hosts (Jones and Yellowlees 1997); space availability and size of algal symbionts (Jones and Yellowlees 1997); repair of donor side of PSII (Rodrigues et al. 2008); reduced metabolic rates (decreased net photosynthesis and coral plus zooxanthellae respiration) (Rodrigues and Grottoli 2007); zooxanthellae clade types (Baker et al. 2004; Mc Clanahan et al. 2005); morphological variability on surface skeleton of host as a means for photoprotection (Bhagooli 2012b); and host's strategies during recovery, for example, stored energy reserves and photosynthetically acquired carbon and/or heterotrophically acquired carbon by host feeding when photosynthesis is not available by the symbionts (Rodrigues et al. 2008); nutrient limitations for zooxanthellae growth (Jones and Yellowlees 1997). Also, the activity at one or more other levels within the chloroplast (i.e., electron transport, photosystem I, ATP synthase, or carbon fixation) may differ between zooxanthellae types and coral species and account for visible differences in bleaching and recovery (Smith et al. 2006; Tchernov et al. 2004). The resilience of the zooxanthellae symbiont and/or resilience of the coral host is also important in determining the rate of recovery (Rodrigues et al. 2008). Anthony et al. (2009) reported that survival following bleaching was also strongly influenced by remaining lipid reserves, heterotrophy rates, and photopigment (or symbiont) recovery rates. However, these factors were not measured within this study and provide an opportunity for further investigation into the effect of zooxanthellae size, density, clades, and gene expression in coral animal host and *Symbiodinium* (Louis et al. 2017). The observations made in this study might also be compared to a non-bleaching year.

In conclusion, the study's results highlight a differential recovery among eight species of reef-building corals in terms of recovery of color (visual condition) and recovery of the PSII functioning of zooxanthellae endosymbionts post-May 2009 bleaching event. The recovery order was as follows: Massive-like/ solitary corals > Branching and semi-bulbous corals > tabular corals. The rate of recovery was fastest in *P. damicornis* and *G. fascicularis*, followed by *Acropora sp.*, and lastly by *A. cytherea* and *A. hyacynthus*. Normally, a recovery in bleaching conditions, i.e., gain in coloration from B to PB, PB to P, and P to NB, also indicated a recovery of PSII functioning n coral except for *P. damicornis* and *G. fascicularis*.

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Short-term effects of heavy metal and temperature stresses on the photo-physiology of *Symbiodinium* isolated from the coral *Fungia repanda*

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Abstract. *Ghoora MD, Pilly SS, Chumun PK, Jawaheer S, Bhagooli R. 2018. Short-term effects of heavy metal and temperature stresses on the photo-physiology of Symbiodinium isolated from the coral* Fungia repanda. *Ocean Life 2: 11-20.* This study aimed to investigate the effects of the heavy metals copper, zinc, and lead, on the photo-physiology of the symbiotic dinoflagellate *Symbiodinium* isolated from the coral *Fungia repanda.* Freshly isolated *Symbiodinium* that belonged to clade C were exposed to different concentrations of the three heavy metals for 3-hour and 18-hour treatments at 28°C and 32°C. The Pulse Amplitude Modulated (PAM) fluorometry technique was used to determine the maximum quantum yield (F_v/F_m), relative maximum electron transport rate ($rETR_{max}$), and maximum nonphotochemical quenching (NPQ_{max}) of the photosystem II (PSII). An increase in nonphotochemical quenching and a decrease in photosynthetic capacity was noted for copper at 50 µg/L for both temperatures. The F_v/F_m was not significantly affected by the Zn treatments. However, at 28 °C, isolates treated with 100 µg/L Zn for 18 hours showed an increase in nonphotochemical quenching and a decrease in photosynthetic capacity. Pb had the most profound effect on all of the isolates. The F_v/F_m significantly decreased, and an increase in NPQ_{max} was noted. The decrease of $rETR_{max}$ and increase in NPQ_{max} for the heavy metal bioassays under 32 °C were more significant than at 28 °C. This study suggests that Cu (≥50 µg/L), Zn (≥ 100 µg/L), and Pb decrease the photosynthetic capacity of the *Symbiodinium* isolates from *F. repanda,* especially more so with increasing temperatures.

Keywords: *Fungia,* heavy metal, photosynthetic parameters, Pulse Amplitude Modulated fluorometry, *Symbiodinium*, thermal stress

Abbreviations: PAM: Pulse Amplitude Modulated; F_v/F_m: maximum quantum yield; $rETR_{max}$: relative maximum electron transport rate; NPQ_{max}: maximum nonphotochemical quenching; PSII: photosystem II; rpm: revolutions per minute; RLCs: rapid light curves; F_o: initial fluorescence; F_m: maximum fluorescence

INTRODUCTION

Although rising seawater temperature, one of the major indicators of global climate change (National Climatic Data Centre 2011), might exert damaging effects on the marine biota in the long term (from decades to centuries), chemical contaminants such as heavy metal pollution may pose more immediate threats to the coastal residents (Hu et al. 2017). The release of heavy metals into the marine environment mainly results from atmospheric and river inputs, direct discharges, industrial dumping, and sewage sludge, which are among the important contributors to metal pollution (Valavanidis and Vlachogianni 2010). At low concentrations, heavy metals are essential to the metabolism of the organisms, but at higher levels, they may lead to toxicity (Phillips 1995; Sunda and Huntsman 1998; Pinto et al. 2003). For example, heavy metals reduce photosynthesis by affecting the light-harvesting complex, oxygen evolution complex, cytochrome complex, plastoquinone, plastocyanin, ferredoxin, and $NADP^+$ (Baumann et al. 2009).

The marine environment undergoes rapid fluctuations in seawater temperature, which may change the conditions necessary for optimum metabolism (Oukarroum et al., 2012). Field and laboratory studies on corals and their symbiotic associations have established a causal link between temperature stress and bleaching events (Lesser 1996) in symbiotic corals that build reefs. Exposure to sub-lethal temperatures (Iglesias-Prieto et al. 1992) leads to photoinhibition of photosynthetic processes in marine organisms. The elevated temperature has been found to cause damage to photosystem II (Warner et al. 1999) and recovery of the D1 protein (Takahashi et al. 2009), which forms part of the water-splitting complex in photosystem II. Moreover, the Calvin-Benson cycle is compromised under high-temperature exposures (Jones et al. 1998; Bhagooli and Yakovleva 2004; Bhagooli and Hidaka 2006), and the site of damage has been speculated to be the enzyme RuBisCO (Lesser 1996; Lilley et al. 2010). Temperature increase in aquatic systems has also been found to enhance the toxicity of some metals on algae (Cairns et al. 1975; Heugens et al. 2001) by increasing the rate of diffusion or active transport.

Environmental stresses threaten the fragile coral reef ecosystems, which are hosts to a highly diverse group of dinoflagellate symbionts of the genus *Symbiodinium* (Baker

2003). These symbionts are responsible for the existence of the coral reefs as we know them (Stanley and Swart 1995) and contribute substantially to coral reef productivity. This study focused on the scleractinian coral *Fungia*, a genus tolerant to environmental stresses (Mattan-Moorgawa et al., 2011). Many studies used the chlorophyll *a* fluorescence technique estimated by the pulse-amplitude-modulated (PAM) fluorometer to assess the photo-physiology of corals and/or their associated symbionts under heavy metal stress (Bielmyer et al. 2010; Gorbunov and Falkowski 2011) or temperature stress (Bhagooli and Hidaka 2002, 2006; Bhagooli and Yakovleva 2004) as individual stress factors. A few studies even looked at the interactive effects of heavy metal and temperature on photosynthetic physiology (Baumann et al. 2009; Oukarroum et al. 2012), but Baumann et al. (2009) worked with macroalgae over 14 days, and Oukarroum et al. (2012) worked with cultured microalgae over 24 hours. However, to the best of our knowledge, there is a shortage of information on the short-term effects of heavy metals assessed individually and in combination with temperature stress on the photosynthetic physiology of freshly-isolated symbionts of a thermally resistant coral-*Fungia repanda*. The main objective of the present study is thus to expose freshly isolated *Symbiodinium* of *F. repanda* to increasing concentrations of heavy metals, namely Cu, Zn, and Pb, for 3-hour and 18-hour treatments under two temperatures-28°C and 32°C to assess the photosynthetic physiology of the organism in response to the stress conditions using PAM fluorometry to determine the three chlorophyll a fluorescence parameters-the maximum quantum yield (F_v/F_m) of PSII, the maximum relative electron transport rate ($rETR_{max}$) and the maximum nonphotochemical quenching (NPQ_{max}).

MATERIALS AND METHODS

Specimen collection and symbiont isolation

Medium-sized scleractinian coral individuals of *Fungia repanda* (diameter ~10 cm) were collected at a depth of ~ 2 m at Trou aux Biches, one of the world-renowned beaches located on the northern coast of the island of Mauritius (20.0350 °S, 57.5450 °E) (Figure 1). The reefs of Trou aux Biches harbor a diversity of coral species, including *Acropora*, *Alveopora*, *Echinopora*, *Favia*, *Favites*, *Fungia*, *Galaxea*, *Pavona*, *Pocillopora*, *Porites*, amongst various other genera (AIMS 2017).

The coral was allowed to recover from handling in a plastic container filled with seawater for 1 hour at ambient temperature (25.0 ± 1.0 °C) before further processing. *Symbiodinium* cells were obtained from *F. repanda* by blasting the coral with filtered seawater (FSW) (0.47 µm) using an oral hygiene device (Water Pik). The blasted tissue was then homogenized using a tissue grinder at 9500 rpm. The homogenate was filtered twice, first with a coarse (180 µm) and then a fine (35 µm) filter mesh, and subsequently centrifuged at 2000 x *g* for five minutes. The pellets were resuspended with FSW before second centrifugation at 1800 x *g* for ten minutes to obtain clean *Symbiodinium* pellets. A cell count was performed using the Neubauer Hemocytometer Chamber, and 1 ml of the isolated symbionts suspension was adsorbed onto 0.22 µm Millipore filters (Ø-13 mm) using a syringe apparatus. Cell densities above 10^5 cells cm^{-2} were used for experimental trials as these densities ensure reliable PAM measurements (Bhagooli and Hidaka 2004a).

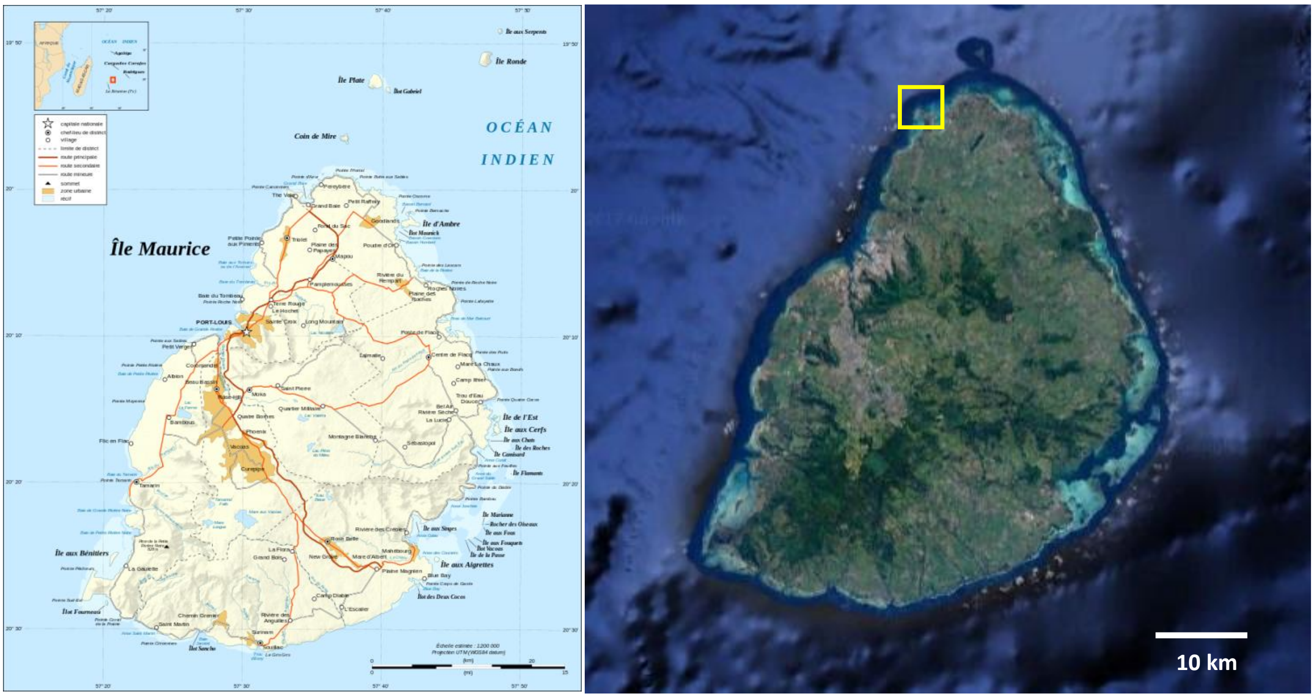

Figure 1. Mauritius and its aerial view showing the sampling site, Trou aux Biches

Experimental protocol

In the laboratory, *Symbiodinium* cells were harvested on millipore filters and separately exposed to four different concentrations of the heavy metals Cu, Zn, and Pb. For each heavy metal assay, the *Symbiodinium* was cultured under two temperature regimes, 28°C and 32°C, and two exposure periods, 3-h and 18-h. Each test was carried out in triplicate. These two temperatures were chosen to represent two conditions leading to non-bleaching and bleaching responses, respectively, in corals reported from Mauritian waters (Bhagooli and Taleb-Hossenkhan 2012; Mattan-moorgawa et al. 2012) and the Great Barrier Reef (Jones et al. 1998). Heavy metal test concentrations were prepared by dilution of standard solutions of Cu, Zn, and Pb (1000 ppm) with seawater filtered through a 0.2 µm membrane filter (Schleicher and Schuell Nitrocellulose Membrane Filters) to produce the following concentrations-Cu: 0, 10, 30 and 50 µg/L; Zn: 0, 25, 50 and 100 µg/L; Pb: 0, 10, 30 and 50 µg/L. The concentrations were chosen based on the range of heavy metals reported in coral reef areas (e.g., Ali et al., 2011) and set toxic thresholds (ANZECC, 1992). Five milliliters of each heavy metal solution was added to McCartney bottles, followed by the subsequent addition of 1 Millipore filter with adsorbed symbionts per vial. The McCartney bottles were then immersed in two water baths, one set at 28°C and the other at 32°C. The treatments were illuminated by a light source of 200 µmol m^{-2} s^{-1} measured by a light meter (Hagner Digital Luxmeter, EC1-Y) during the 3-h and 18-h stress.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a Pulse Amplitude Modulated (PAM) fluorometer (DIVING-PAM, Heinz Waltz GmbH, Germany). The initial fluorescence (F_o) was determined by applying a weak pulse-modulated measuring light (< 0.1 µmol quanta m^{-2} s^{-1}) when the PSII reaction centers were open. The maximum fluorescence (F_m) was determined after applying a saturating pulse (> 6000 µmol quanta m^{-2} s^{-1}) when the reaction centers were closed. The ratio of the change in fluorescence to maximum fluorescence ($[F_m\text{-}F_o]$ / F_m) gives the dark-adapted photosynthetic parameter F_v/F_m, which is a good proxy of the maximum quantum yield of PSII (Genty et al. 1989). Samples were dark-adapted for 15 minutes before measurement. After the dark-adapted measurement, the samples were light adapted for 5 minutes, followed by a 30 s dark period, and initial and maximum fluorescence (F_o' and F_m' respectively) were determined again. The product of the ratio of change in fluorescence to maximum fluorescence of light-adapted samples ($[F_m'\text{-}F_o']/ F_m'$) (also known as the effective quantum yield) and the photosynthetically active radiation (PAR) gives the parameter relative Electron Transport Rate (*rETR*). The nonphotochemical quenching (*NPQ*) parameter, which regulates the dissipation of excess energy in the form of heat, is derived from the ratio of change in maximum fluorescence from the dark-adapted to the light-adapted stage to the maximum fluorescence of the illuminated sample ($NPQ = [F_m\text{-}F_m']/ F_m'$) (Bilger and Björkmann 1990).

The *rETR* and the *NPQ* were derived from the rapid light curves (RLCs) obtained after light adapting the samples. The RLCs determine the symbionts' physiological flexibility to adapt their photosynthetic apparatus to rapidly changing light intensities. Rapid irradiances occurred at an interval of every 10 s and gave fluorescence measurements fitted as an exponential decay curve. The $rETR_{max}$ was obtained by fitting *rETR* curves in the Sigma Plot software using the Platt et al. (1980) equation. NPQ_{max} represents the highest nonphotochemical quenching value.

Symbiodinium isolation, DNA extraction, and clade identification

Coral tissues were removed using a Waterpik and filtered seawater (FS, 0.45µm). The blastate was centrifuged for 10 min at 4,000 rpm, washed with filtered seawater, and centrifuged again to pellet the *Symbiodinium* cells. The pellet was suspended in 1ml of FS. Following centrifugation for 5 min at 4,000 rpm, the pellet was resuspended with 1% sodium dodecyl sulfate (SDS) and DNA isolation buffer (0.4 M NaCl; 50 mM EDTA, pH 8), vortexed, and treated for 1-2 hrs at 65˚C and stored at room temperature for later analyses. DNA extraction was carried out using a slightly modified method by Rowan and Powers (1991). First, proteinase-K was added to the *Symbiodinium* suspension and incubated for 2-3 hrs at 55˚C. Next, 64 µl of 5M NaCl was added, followed by 60 µl of 10% cetyltrimethylammonium bromide (CTAB), and was topped up to 600µl with sterile distilled water. The lysate was then heated for 30min at 65˚C, followed by the addition of 600µl of chloroform. The lysate was subject to chloroform extraction once and phenol extraction twice. First, 900µl of cold ethanol was added, followed by 45µl 3M Sodium Acetate (NaOAc). The DNA was precipitation at-20˚C overnight, and excess chloroform was washed with 70% ethanol. The DNA was then air dried and resuspended in 50µl TE buffer. Polymerase chain reaction (PCR) was done using *Symbiodinium*-specific primers ss3z and ss5z that anneal to the 18S-rDNA region of the *Symbiodinium* DNA. Restriction digest was performed by incubating the PCR product for 2 hours with Taq I enzyme. The banding pattern of the RFLP was then visualized in agarose gel.

Statistical analyses

Chlorophyll fluorescence data were arcsine transformed before statistical analyses. Multivariate analysis of variance (ANOVA) was carried out using the statistical software STATISTICA version 10.0 to compare the effects of the heavy metals (Cu, Zn, and Pb) and their respective concentrations, temperatures (28°C and 32°C), and exposure times (3-h and 18-h) per se and in combination on the photosynthetic parameters, F_v/F_m, $rETR_{max}$ and NPQ_{max}. The Post Hoc Tukey HSD test determined differences between groups.

RESULTS AND DISCUSSION

Genotyping results showed that *F. repanda* harbored the *Symbiodinium* Clade C (Figure 2). An increase in temperature, heavy metal concentrations, and exposure time reduced the maximum quantum yield (F_v/F_m) of the symbionts significantly ($P < 0.05$). The maximum nonphotochemical quenching (NPQ_{max}) was increased significantly ($P < 0.001$) by temperature, heavy metal concentration, and exposure time. However, no marked difference ($P > 0.05$) was noted across the three heavy metals. The $rETR_{max}$ remained invariant ($P > 0.05$) under temperature stress but was significantly reduced by the heavy metals and their concentrations and exposure time. Interaction of stress factors, evaluated by the multivariate ANOVA analyses, had variable effects on the photosynthetic parameters, as shown in Table 1.

Effects of Cu

Figure 3 shows the variation of the three fluorescence-based parameters, F_v/F_m, $rETR_{max}$, and NPQ_{max}, with Cu concentrations over three and eighteen-hour treatments. Zooxanthellae isolated from *F. repanda* had initial F_v/F_m values of 0.593 ± 0.011. At 28 °C, no change in F_v/F_m was noted ($P > 0.05$), the light-adapted parameters were affected significantly; 50 µg/L copper reduced the $rETR_{max}$ significantly for all isolates ($P < 0.001$ for 3 h; $P < 0.01$ for 18 h) and increased the NPQ_{max} for the 18 h treatment ($P < 0.01$). The combined effects of copper and high-temperature stresses (32°C) considerably reduced the photosynthetic outputs of PSII. Although 10µg/L copper did not affect the photosynthetic parameters measured, a significant reduction in $rETR_{max}$ ($P < 0.001$) during the 18 h treatment was evident with 30 µg/L Cu. The highest copper concentration used (50 µg/L) caused marked decreased in F_v/F_m ($P < 0.05$ for 3 h; $P < 0.01$ for 18 h) and $rETR_{max}$ ($P < 0.01$ for 18 h; no change for 3 h) and significant increase in NPQ_{max} ($P < 0.001$ for both 3h and 18 h).

Effects of Zn

Zn exposure on isolated *Symbiodinium* (Figure 4) showed no significant change in F_v/F_m for all treatments. However, the $rETR_{max}$ was significantly reduced at 28°C when the symbionts were treated with 100 µg/L zinc for 18 h ($P < 0.05$). An associated increase in NPQ_{max} ($P < 0.01$) was noted for the same treatment. At 32°C, zinc concentrations of 50µg/L and 100µg/L caused significant increase in NPQ_{max} ($P < 0.001$ for 50 µg/L; $P < 0.01$ for 100 µg/L) but not the other measured photosynthetic parameters.

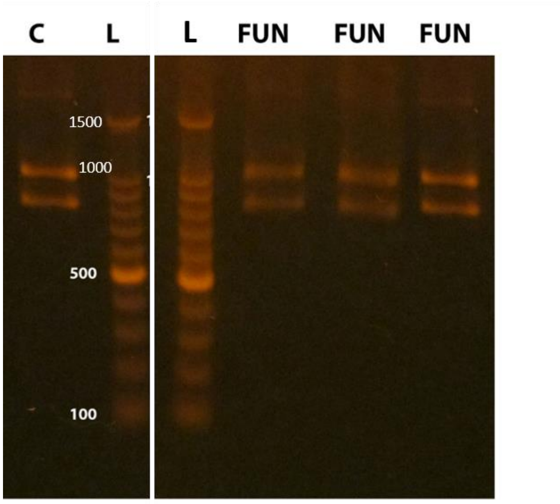

Figure 2. Restriction fragment length polymorphism (RFLP) of *Symbiodinium* Clade harbored by *F. repanda.* C-clade C; L-DNA ladder; FUN-*F. repanda*

Table 1. Summary of multivariate ANOVA analyses testing the effect of temperature (28°C and 32°C), heavy metals (Cu, Zn, and Pb), and their respective concentrations (Cu and Pb: 0, 10, 30, and 50 µg/L; Zn: 0, 25, 50 and 100 µg/L) and exposure time (3h and 18h) individually and in combination (s), on F_v/F_m, NPQ_{max} *and* $rETR_{max}$). Significant differences are indicated in red. [Abbreviations: Temp: temperature; HM: heavy metals; Conc: concentration; DF: degree of freedom; MS: mean square; F: variance ratio; P: probability value] (n = 3)

Source of variation	F_v/F_m				NPQ_{max}				$rETR_{max}$			
	DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Temp	1	0.001	4	0.037	1	0.018	29.66	0.000	1	0.004	3.70	0.057
HM	2	0.001	9	0.000	2	0.001	1.08	0.341	2	0.004	4.35	0.015
Conc	3	0.005	35	0.000	3	0.038	63.76	0.000	3	0.027	25.88	0.000
Time	2	0.005	36	0.000	2	0.039	65.51	0.000	2	0.223	217.36	0.000
Temp*HM	2	0.000	0	0.890	2	0.003	5.59	0.005	2	0.008	7.33	0.001
Temp*Conc	3	0.000	1	0.512	3	0.001	1.47	0.226	3	0.001	1.20	0.312
HM*Conc	6	0.000	1	0.536	6	0.000	0.68	0.663	6	0.002	1.68	0.131
Temp*Time	2	0.000	2	0.120	2	0.005	7.93	0.001	2	0.007	6.88	0.001
HM*Time	4	0.000	2	0.047	4	0.002	3.93	0.005	4	0.008	7.46	0.000
Conc*Time	6	0.000	2	0.172	6	0.011	18.98	0.000	6	0.009	8.69	0.000
Temp*HM*Conc	6	0.000	0	0.933	6	0.000	0.71	0.640	6	0.005	5.36	0.000
Temp*HM*Time	4	0.000	0	0.899	4	0.002	2.65	0.036	4	0.008	7.67	0.000
Temp*Conc*Time	6	0.000	0	0.950	6	0.001	2.16	0.050	6	0.002	1.92	0.081
HM*Conc*Time	12	0.000	0	0.993	12	0.001	2.45	0.006	12	0.003	2.55	0.004
Temp*HM*Conc*Time	12	0.000	0	0.998	12	0.001	1.39	0.179	12	0.004	3.55	0.000

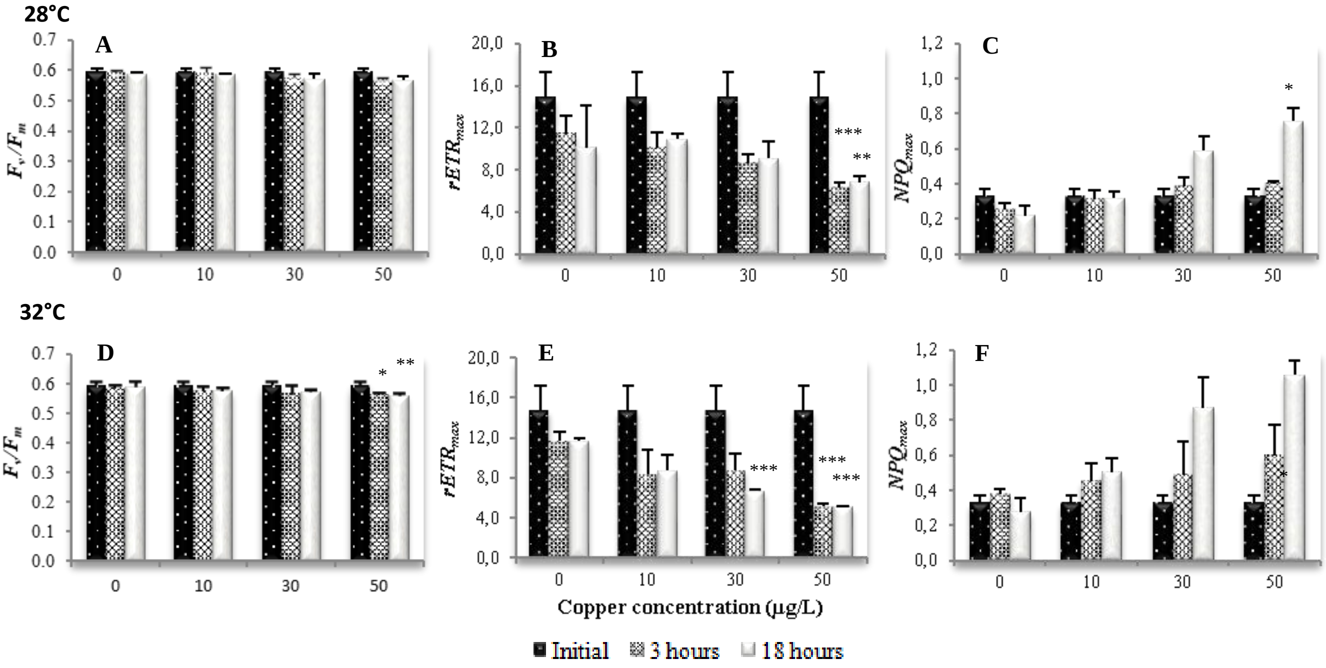

Figure 3. The maximum quantum yield of PSII (F_v/F_m), maximum relative electron transport rate (rETR$_{max}$), and nonphotochemical quenching (NPQ$_{max}$) of zooxanthellae isolated from F. repanda under Cu treatments at 28°C (A, B, and C) and 32° (D, E, and F). Note: * represents $P < 0.05$, ** represents $P < 0.01$ and *** represents $P < 0.001$ between the treatment and initial. Data are represented as mean ± standard deviation (n = 3).

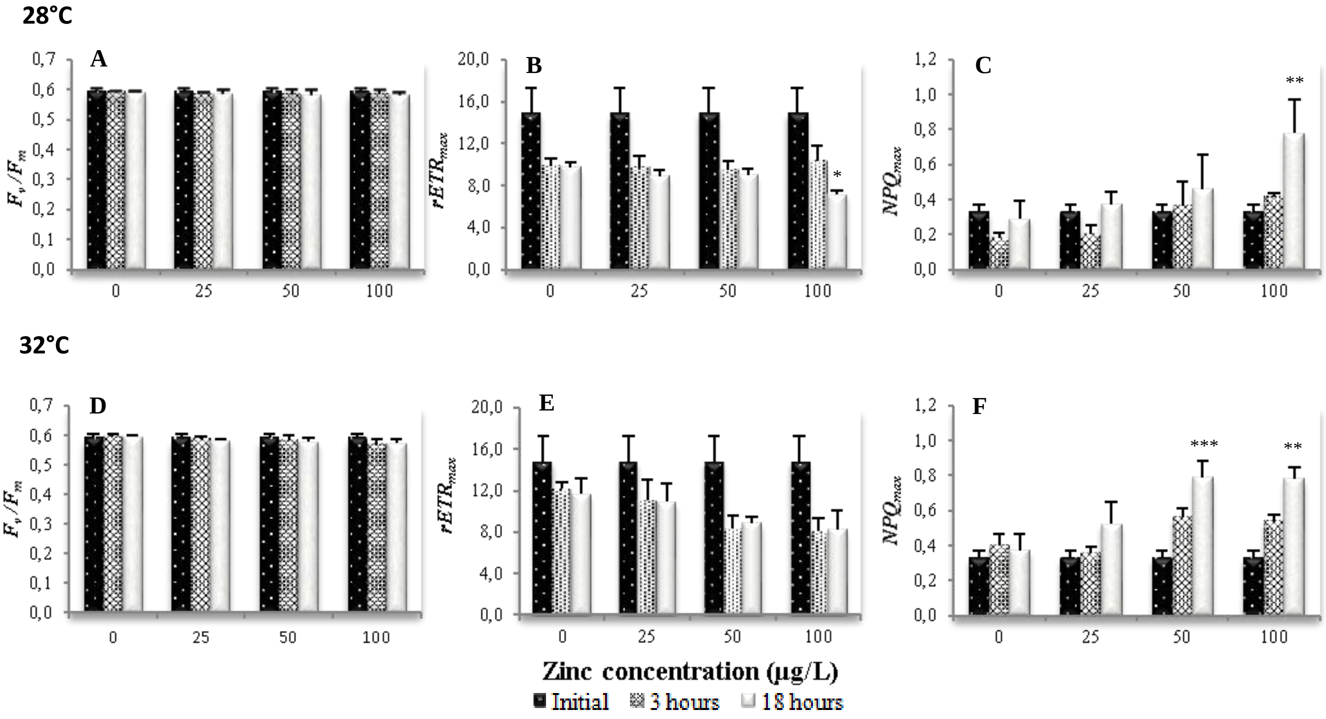

Figure 4. The maximum quantum yield of PSII (*F_v/F_m*), maximum relative electron transport rate (*rETR$_{max}$*), and nonphotochemical quenching (*NPQ$_{max}$*) of zooxanthellae isolated from *F. repanda* under Zn treatments at 28°C (A, B and C) and 32° (D, E, and F). Note: * represents $P < 0.05$, ** represents $P < 0.01$ and *** represents $P < 0.001$ between the treatment and initial. Data are represented as mean ± standard deviation (n = 3).

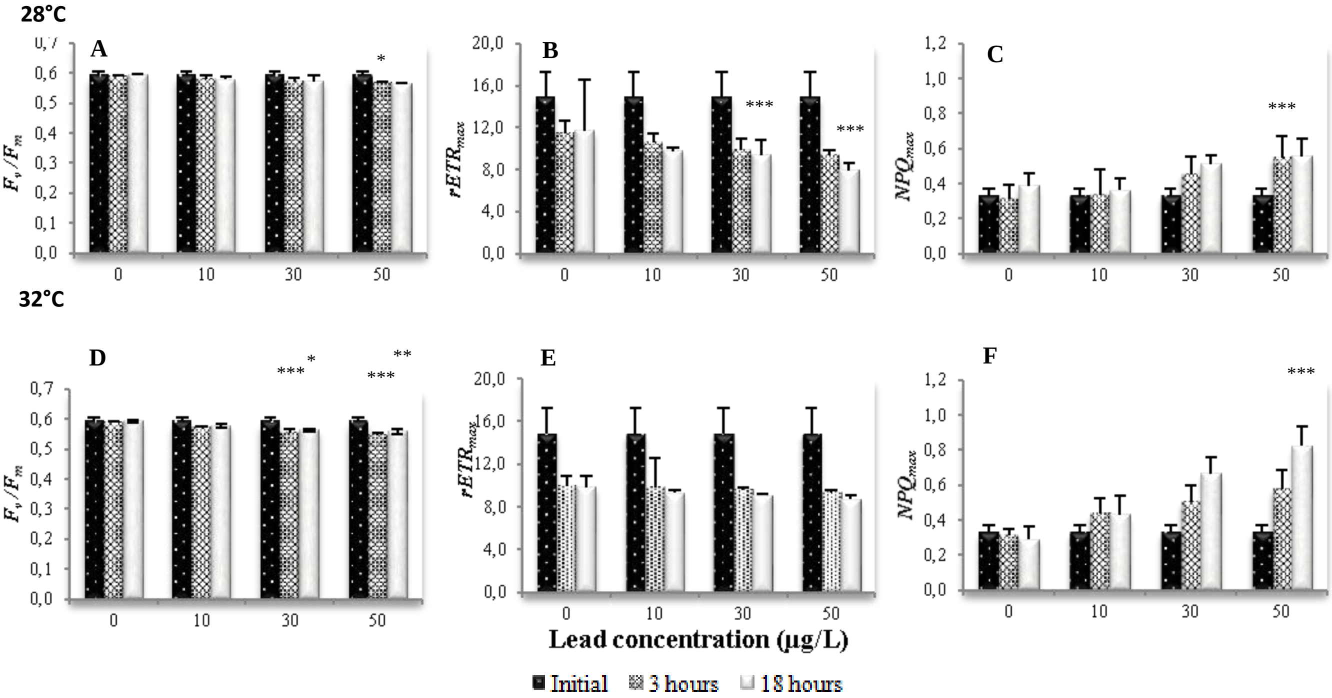

Figure 5. The maximum quantum yield of PSII (F_v/F_m), maximum relative electron transport rate ($rETR_{max}$), and nonphotochemical quenching (NPQ_{max}) of zooxanthellae isolated from *F. repanda* under Pb treatments at 28°C (A, B and C) and 32° (D, E, and F). Note: * represents $P < 0.05$, ** represents $P < 0.01$ and *** represents $P < 0.001$ between the treatment and initial. Data are represented as mean ± standard deviation (n = 3)

Effects of Pb

Pb reduced the photosynthetic capacity of the isolated *Symbiodinium* by affecting all the photosynthetic parameters monitored (**Figure 5**). A pronounced decrease was noted in F_v/F_m over both temperature treatments (28 °C and 32 °C). While the F_v/F_m value of the 50µg/L lead treatment over 3 h was significantly reduced ($P < 0.05$) at 28°C, marked reduction was noted as from 30µg/L for the same parameter at 32 °C; the symbionts exposed for 3 h and 18 h under 30 µg/L and 50 µg/L lead treatments at 32°C had significantly lower F_v/F_m values (30 µg/L: $P < 0.001$ for 3 h, $P < 0.05$ for 18 h; 50 µg/L: $P < 0.001$ for 3 h, $P < 0.01$ for 18 h). The $rETR_{max}$ was significantly reduced after 18 h at lead concentrations of 30µg/L and 50µg/L for the 28 °C treatment ($P < 0.001$) but not for 32 °C. However, for treatments at both temperatures (28 °C and 32 °C) showed a significant increase in NPQ_{max} ($P < 0.001$ for 3 h at 28 °C, $P < 0.001$ for 18 h at 32 °C).

Discussion

A Cu concentration of 50 µg/L did not result in any significant changes in F_v/F_m; however, a decrease in $rETR_{max}$ accompanied by an increase in NPQ_{max} was recorded within 18hrs of exposure. That suggests that 50 µg/L Cu decreased the photosynthetic capacity of the *Symbiodinium*, and the excess energy was effectively dissipated. That is consistent with Yruela et al. (1992), who noted that Cu inhibits electron transfer at the pheophytin Q_A-Fe domain of the PSII reaction center. Han et al. (2008) noted no significant change in photosynthetic yield at Cu concentrations of 25-50 µg/L; a higher Cu concentration of 250 µg/L significantly reduced the Fv/Fm. A large body of research has shown that Cu is toxic to the photophysiology of marine organisms and causes damage to several target sites along the photosynthetic pathway (Parales-Vela 2007; Han et al. 2008; Bielmyer et al. 2010; Connan and Stengel 2011; Oukarroum et al. 2012). As toxicity is generally considered dose-dependent, a high Cu concentration is expected to cause a decline in the photosynthetic yield.

Moreover, toxicity is linked to the sensitivity of the test organisms since Cu concentration as low as 4 µg/L has been found to reduce the quantum yield in algal symbionts of *Pocillopora damicornis*, as reported by Bielmyer et al. (2010). Kuzminov et al. (2013) investigated Cu toxicity over several days in a cultured *Symbidinium* (CCMP 2467) isolated from the coral *Stylophora pistillata*. They reported no significant change in F_v/F_m and a slight but not significant increase in the maximum rate of photosynthetic electron transport (P*max*) up to 2 days of exposure to 50µM Cu at 25ºC. However, after 3 days of exposure, a significant decline was observed in both F_v/F_m and P*max*. They also reported that the time of electron transport between photosystems ($\tau_{PSII-PSI}$) increased significantly within 12hrs of treatment. It is noteworthy that in higher plants such as *Arabidopsis thaliana*, a significant increase in *ETR* after exposure to Cu concentrations of 50-100 µg/L was observed (Martínez-Peñalver et al. 2012), suggesting that Cu, which is a micronutrient may have been limiting in the multicellular organism.

A significant reduction in the $rETR_{max}$ was recorded within 18hrs of exposure to Zn, which is in line with other investigations on the effect of Zn on photosynthesis carried out by many authors (Davies and Sleep 1979; Tripathy and Mohanty 1980; El-Sheekh 1993). Experiment using the O_2-evolution method has shown that Zn inhibits the photosynthetic electron transport through PSII (Tripathy and Mohanty 1980), and this corresponds with the results of the present study where Zn has been shown to exert its effects at the oxidizing (H_20-splitting) side of PSII, possibly inhibiting the manganese complex (Miller and Cox 1983; Van Assche and Clijsters 1986). Baker et al. (1982) proposed a second site for Zn^{2+} action in the electron transfer chain between the PSII and the PSI, and this has been attributed to plastoquinone (Mohanty et al. 1989). That is in line with the observed decrease in $rETR_{max}$. Though the F_v/F_m did not vary significantly, it has been proposed that F_v/F_m is not sensitive to Zn; hence, it may not be a good indicator of Zn stress (Joshi and Mohanty 2004). Baumann et al. (2009) reported a significant reduction in macroalgae yield when exposed to a Zn concentration of 10 µg/L after 4 days. In the latter study, Zn was reported to irreversibly bind to the test macroalgal species, causing the death of the organisms, which was confirmed by F_v/F_m values of zero. Kuzminov et al. (2013) reported Zn toxicity in a cultured *Symbidinium*. They found no significant change in F_v/F_m but a significant increase in the maximum rate of photosynthetic electron transport (P*max*) up to 2 days of exposure to 100µM Zn at 25°C. After 3 days of exposure significant decline in both F_v/F_m and P*max* was observed.

Studies have reported that Pb stress can cause inhibition of photosynthesis at the level of the light-harvesting complexes of PSI and PSII (Miles et al. 1972) and the photosynthetic reduction cycle (Stiborova et al. 1986). Moreover, PSII is more sensitive to Pb than PSI. Pb inhibition site is located at the donor side of PSII, between the oxygen-evolving complex and the reaction center of PSII (Joshi and Mohanty 2004), following a decrease in $rETR_{max}$ with increasing concentration of Pb. While in the present study, the Fv/Fm of Symbiodinium was severely reduced when exposed to a Pb concentration of 50 µg/L, Baumann et al. (2009) reported Pb as one of the least toxic among 5 metals, including Cu and Zn. In the latter study Pb caused no reduction in fluorescence yield of 7 species at 10 µg/L, possibly because the macroalgae were tolerant to moderately high Pb concentration (Strömgren 1980; Lamai et al. 2005). However, Hussain et al. (2006) found a drastic reduction in yield parameters when mash plants were exposed to 20-40 mg/L Pb. Reduction in photosynthesis in algae by Pb has also been reported by Woolery and Lewin (1976). Extensive inhibition of photosynthetic electron transport was observed when isolated chloroplasts were exposed to 2.4 mM Pb for a few minutes (Miles et al. 1972), which corresponds to the effects of Pb in our study. As a result, the system significantly increased its nonphotochemical quenching to harness excitation energy safely. Kuzminov et al. (2013) documented Pb toxicity in a cultured *Symbidinium*. They reported no significant change in F_v/F_m up to 3 days of exposure to 50µM Pb at 25°C but a slight decrease in the maximum rate of photosynthetic electron transport (P*max*) up to 2 days of exposure. After 4 days of exposure, a significant decline in both F_v/F_m and P*max* was observed. However, $\tau_{PSII\text{-}PSI}$ was the first parameter to be affected.

Under stress conditions such as combined heavy metal and thermal stress, the higher capacity for nonphotochemical quenching helps to protect the photosynthetic organism. In the present study, all heavy metal treatments carried out at elevated temperatures (32°C) recorded significantly high NPQ_{max}. A proposed photoprotection mechanism involves the inter-conversion between the two pigments, diatoxanthin and diadinoxanthin (Ting and Owens 1993). Ruban et al. (2004) demonstrated that *NPQ* is tightly correlated to the presence of diatoxanthin and that the triggering key factor was the proton gradient across the thylakoid membranes. The alternative sources of protons, such as the PS I cyclic electron transfer and/or chlororespiration, are likely important in generating the proton gradient sufficient to trigger *NPQ*. Excess energy dissipation in the form of heat prevents the formation of reactive oxygen species, which can induce lipid peroxidation and destruction of membrane structure and function. Both excess essential and non-essential metals and elevated temperature are known to affect algal and coral, among other coastal species, physiology, metabolism, and growth (El-Sarraf and Taha 1995; Bertrand and Poirier 2005; Mitchelmore et al. 2007; Baumann et al. 2009; Bielmyer et al. 2010; Main et al. 2010; Connan and Stengel 2011; Kuzminov et al. 2013). It is noteworthy that along with heavy metal stresses, the combined effects of temperature pose a greater threat to marine life forms (Cairns et al. 1978; Sokolova and Lannig 2008; Oukarroum et al. 2012). Oukarroum et al. (2012) noted that heavy metal toxicity on photosynthetic performance is temperature-dependent, consistent with the present study. However, research carried out by Cairns et al. (1978) on four algal species revealed differential effects of heavy metal toxicity to temperature, most probably due to different culturing methods of the algae, representing distinctly different habitats. Mauritian waters are not spared from metal contamination (Daby 2006) and elevated thermal anomalies (Mattan-Moorgawa et al. 2012; Bhagooli and Taleb-Hossenkhan 2012; Bhagooli and Sheppard 2012).

Time of stress under heavy metals significantly influenced the photosynthetic parameters in this study. Algal cells can accumulate heavy metals when exposed to high concentrations, which can interfere with photosynthesis. However, specific responses of a given heavy metal on photosynthesis vary among species; thus, broad generalization cannot be made about the combined effects of heavy metal exposure and time. The severity of the stress response depends on the exposure time and the concentration of heavy metals. Mitchelmore et al. (2007) showed that the coral *Pocillopora damicornis* could accumulate Cu 3-fold, 30-fold at 5 and 50 μgl^{-1}, respectively, after 4d of exposure, with the *in hospite*

Symbiodinium accumulating 1.5-fold of Cu in 5μgl^{-1} treatment higher than that in control. Bielmyer et al. (2010) investigated the effect of Cu on the coral *A. cervicornis* for 5 weeks. They observed that Cu exposure and accumulation might affect the symbiont by reducing the CO_2 available for photosynthesis. Kuzminov et al. (2013) also reported time-dependent exposure toxicity of essential and non-essential metals along with differential photophysiological responses to the metals of cultured *Symbiodinium*. However, sensitivity to heavy metals may vary with the organism's physiology; hence, it is important to understand the mechanisms of action of these heavy metals to evaluate the effects of heavy metal stress better. It is also noteworthy that the accumulation of heavy metals for an effective concentration resulting in significant photophysiological changes may be time-dependent. Thus, short-term exposures in hours may need to be extended to days of exposures to thoroughly evaluate the impacts of heavy metals on Symbiodinium.

The rise in the surface sea water temperature is expected to cause mass bleaching events leading to the 'extinction' of some coral reefs in Mauritius, and the 'extinction dates' have been suggested to occur between the years 2025-2070 based on the bleaching/mortality thermal threshold (Bhagooli and Sheppard 2012). This situation is further aggravated in the presence of heavy metal contaminants. Sokolova and Lannig (2008) reported synergistic effects of temperature and heavy metal stress. Elevated temperature is known to increase the rate of uptake and accumulation of heavy metals (Cairns et al. 1975; McLusky et al. 1986; Hutchings et al. 1996; Heugens et al. 2002). *Symbiodinium* exposed to thermal stress reduced the dark-acclimated maximum quantum yield of PSII compared to the non-stressed ones (Hoegh-Guldberg 2005). In the present study, Cu and Pb treatments at 32°C significantly reduced the F_v/F_m, suggesting damage at the level of photosynthetic functioning in *Symbiodinium*. This phenomenon is mainly attributed to photoinhibition of the PSII (Warner et al. 1999). Within the PSII, numerous components are known to be susceptible to damage by elevated temperatures. These include the oxygen-evolving complex (Havaux 1993), the reaction center (Heckathorn et al. 1998), as well as the connectivity between the light-harvesting complex and the reaction center of PSII (Schreiber and Armond 1978). Warner et al. (1999) and Lesser and Farrell (2004) have shown that the main site of photoinhibition damage at the PSII is the D1 proteins, the loss of which is correlated with reductions in F_v/F_m. Bhagooli and Hidaka (2003) suggested that enzymes involved in the synthesis or resynthesis of the D1 protein could be affected by heat stress. Bhagooli (2013) proposed that inhibition of the Calvin-Benson cycle under elevated temperature may suppress the recovery of PSII. That enforces the suggestion that thermal stress exacerbates the pathway of cellular damage that occurs due to heavy metal stress, as observed in the present study.

Scleractinian corals have been reported to harbor different genetic types of *Symbiodinium*, several clades (A, B, C, D, E, F, G, H, I) (Pochon and Gates 2010). Due to global ocean warming, corals tend to change their *Symbiodinium* communities (Rowan et al. 1997; Baker 2003), with clade D as a thermally tolerant type (Rowan 2004). Members within different *Symbiodinium* clades can be further subdivided into internal transcribed spacer 2 (ITS2) types exhibiting differential thermal stress photophysiological responses (Bhagooli and Hidaka 2004b; Bhagooli 2009; Bhagooli 2010). Bielmyer et al. (2010) reported variable copper accumulation and susceptibility among three coral species harboring Symbiodinium clade types, A3, C1, and D1a. The coral species harboring *Symbiodinium* D1a exhibited the highest metal tolerance. Kuzminov et al. (2013) demonstrated differential metal toxicity in culture *Symbiodinium* of clade A1. In the present study, *F. repanda*, which has been reported to be one of the resistant coral species to bleaching events both locally (Mattan-Moorgawa et al. 2012; Bhagooli and Kaullysing 2018) and worldwide (Marshall and Baird 2000; Loya et al. 2001), was found to host *Symbiodinium* clade C. Recently, LaJeunesse et al. (2018) detailed the existing sub-cladal types (e.g., ITS2 types) and provided new names for them as distinct species. For instance, they have renamed clade C *Symbiodinium* as *Cladocopium* species. Thus, the differences in responses of *Symbiodinium* isolates to metal exposure between the present study and the other reports may be partly attributed to a difference in *Symbiodinium* clade types or sub-types.

Further studies on the sub-clade types, for example, ITS2 types of *Symbiodinium* in *F. repanda* may provide for detailed comparison with other related studies, and sub-cladal variability may imply that the present results for responses to heavy metals may not be generalized for all members of clade C. In addition, some *Symbiodinium* types, such as clade A occurring in some abundant but bleaching susceptible coral species, namely the branching *Acropora muricata*, occurring near the coast with more fluctuating temperatures, may also have some potential to acclimatize to high-temperature regimes and may thus resist bleaching events (Louis et al. 2016). However, higher levels of essential and non-essential metals may also occur in the immediate coastal areas. Consequently, when the sea temperature rises gradually instead of yielding into acclimatization processes that may reduce bleaching incidences, the *Symbiodinium* photophysiology may be negatively affected, thus making the corals more vulnerable to thermal events in the coastal waters.

In conclusion, Cu (≥50 μg/L), Zn (≥ 100 μg/L), and Pb (≥30 μg/L) decreased the photosynthetic capacity of the *Symbiodinium* isolates from the coral *F. repanda* with more pronounced effects at a higher temperature. The present study showed that a higher temperature enhanced the harmful effect of heavy metals, leading to a marked decline of the photo-physiology of symbionts of the thermally resistant coral *Fungia repanda* even under short exposure time (< 24-h). These findings suggest that coral species that may be thermally robust and either resistant or resilient to thermal anomaly events may be photo-physiologically vulnerable to global warming-induced mass coral bleaching/mortality events by local coastal heavy-metal contamination. It is important to note that differences in response to both essential and non-essential metals may be specific to the local *Symbiodinium* clades and duration of metal exposure. This work provides an impetus for further investigation to determine the effects of heavy metals in the face of global warming.

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Short Communication: Seawater Mollusca (bivalve) diversity at Dullah Laut Beach, Tual City, Southeast Moluccas, Indonesia

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Abstract. *Roshitafandi DA, Sartika HW, Dewi AK, Nashrurrokhman M, Ratman N, Trijoko. 2018. Short Communication: Seawater Mollusca (bivalve) diversity at Dullah Laut Beach, Tual City, Southeast Moluccas, Indonesia. Ocean Life 2: 21-26.* Indonesia is a country that has a high level of biodiversity supported by diverse types of beaches. Dullah Laut Village is located on one of the small islands in Southeast Moluccas. Dullah Laut Beach is a natural white sandy beach with algae, seagrass, and rocky substrate. Bivalve is part of the Mollusca found mostly on the Dullah Laut Beach. The purpose of this research is to know the diversity of the Bivalve class in the intertidal zone of Dullah Laut Beach, Southeast Molucca, related to the environmental parameters. This research was conducted in July and August 2017. The environmental parameters recorded at the time of the study were 27°C for temperature, 0,01 mg/L for nitrite, and 0,5 mg/L for Ammonia. The method used in this study is the purposive sampling method. After data collection was completed, the next steps were documentation, preservation, and identification conducted in the Faculty of Biology, Universitas Gadjah Mada, Indonesia. Based on this research, we found 4 genera of the class Bivalve in Dullah Laut Beach: *Atactodea, Macrocallista, Mactra,* and *Hippopus.*

Keywords: Diversity, Dullah Laut, Mollusca, Southeast Moluccas

INTRODUCTION

Indonesia is a country with high levels of biodiversity supported by its location in the tropics (Supriatna 2008). In addition to mega diversity countries, Indonesia is a maritime country with abundant marine wealth. Various types of beaches in Indonesia support various kinds of marine life. Marine commodities such as fish, crustaceans, mollusks, echinoderms, and seaweed can be cultivated today. One of the most live animals includes well-exploited mollusks such as meat oysters, pearl oysters, green shells, blood clams, abalone, and Kima (Sudradjat 2015). One of the abundant marine biotas in Indonesia is Mollusca. Mollusks are suitable to live in sandy beach environments and coral fragments (Romimohtarto and Suhardjono 1999). Mollusks are covered with an exoskeleton. Eastern Indonesia is one part of Indonesia with various types of beaches that are still natural. The beach consists of various substrates such as algae, seagrass, sand, and rocks suitable for the Mollusca habitat. Dullah Laut Village is a small island in Southeast Moluccas with various substrate beaches. The island is located at the coordinate point -5.533990 LS / 748941 BT and is obscured by other small islands. These sandy and rocky beach characteristics are very suitable for habitat for Molluscs, especially bivalves. Following the word's origin (bi = two, valve = kaleal kalkareus), bivalves are a type of Mollusca with a shell (Holley 2015). The sedentary live bivalves are filter-feeders.

The sleek bushes secrete strong threads that attach them to the substrate of rocks, docks, ships, other animal shells, and so on. Yet there are also bivalves such as clams that can live with interesting self-enters to the sand or mud using their muscle legs as an anchor. Then there are also bivalves, such as mussels, that move fast along the seafloor by flapping their shells (Campbell et al. 2008). This study will analyze bivalves taken based on different substrate types. In addition, with the existence of bivalves still awake there, data collection is needed to assess the potential in the village of Dullah Laut. The state of the village beach Dullah an unspoiled sea, and becoming an archipelago becomes a compelling reason for proper research. That results in Mollusca being a good environmental indicator. This study was conducted to determine the diversity of bivalves in the coastal village of Dullah Laut. The expected outcome of this research could be a database for conservation and science concerning Mollusca for the wider community.

MATERIALS AND METHODS

Study area

The study was located at Dullah Laut Beach, Duroa Island, Tual City, Southeast Moluccas, Indonesia (coordinate -5.533990LS/132.748941BT) (Figure 1). Sampling was conducted in the intertidal zone of Dullah Laut Beach. These sites have varied types of substrate. Substrate types were classified as sandy substrate, seagrass substrate, and rocky substrate (low tide near subtidal zone).

Procedures

The collection of mollusks was done by the purposive sampling method in various substrates within the intertidal zone. If the substrate was sandy, the collection of mollusks was done by digging. Sampling was conducted four times in two months, on 13, 21 June, and 14, 26 July at 04.45 pm until 6.00 pm WIT. All bivalve specimens found alive were collected and placed into a plastic container. Residents assisted in the collection of mollusk samples mollusk. Samples were preserved by washing, boiling, then cleaning the contents. The mollusk samples were documented on mm block paper and brought to the Faculty of Biology, Universitas Gadjah Mada, Indonesia, for identification. Preserved mollusk samples were identified with the help of FAO, Worms, EOL, and other literature. A thermometer tested the environmental parameters like water temperature four times in two months. Test of Nitrite and Ammonia was done by LIPI (Lembaga Ilmu Penelitian Indonesia) using KIT ammonia and nitrate.

RESULTS AND DISCUSSION

Results

The list of species is presented in Table 1, as well as Figures 2-5. The mollusk samples (bivalve) belonged to genus *Atactodea*, *Hippopus*, *Macrocallista*, *Mactra*, and *Tridana*.

The substrate of Dullah Laut Beach in the intertidal zone was sandy, seagrass, and then rocky. Atactodea was found on the sandy substrate by digging. *Macrocallista* and *Mactra* were found on a substrate that was overgrown with seagrass. *Hippopus* was found on sandy bottoms of rock substrate.

The water sample from Dullah Laut Beach was brought to LIPI to test the nitrate and ammonia content using KIT nitrate and Ammonia. The nitrite content results are 0.01 mg/L, and Ammonia is 0.5 mg/L. Therefore, the value of nitrite contained when compared with water quality standards from government decisions (KMLH No 51 2004) is not more than the standard value, which means the water was still safe for the cultivation or life of the marine animal, while the value of Ammonia exceeded the standard value which means the water is not good enough for cultivation.

Table 1. Type of substrate for each genus of mollusk in Dullah Laut Beach, Moluccas, Indonesia

Genus	Kind of substrate
Atactodea	Sandy substrate
Hippopus	On sandy bottoms of rock substrate
Macrocallista	Seagrass
Mactra	Seagrass

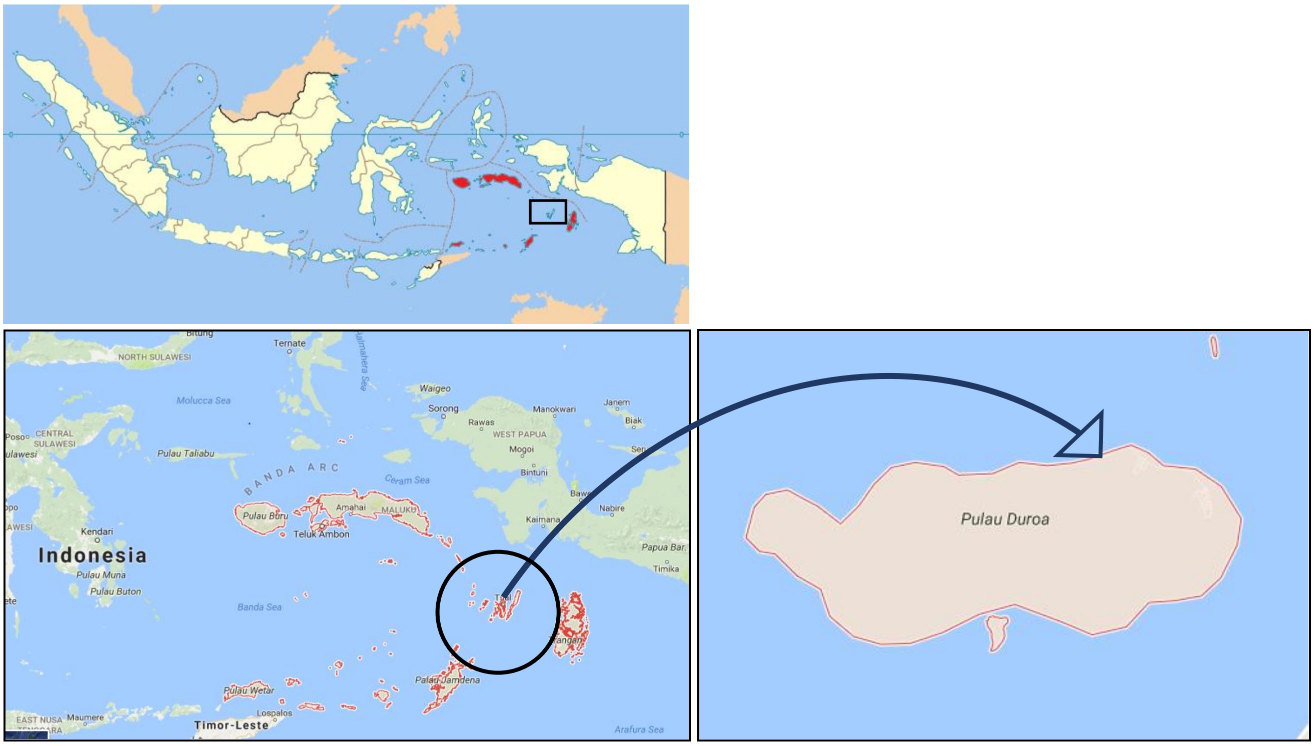

Figure 1. Location of Dullah Laut Beach, Maluku, Indonesia

Table 2. Test results of nitrite and ammonia samples

Test Results of nitrite samples			
Location	Cons	Unit (mg/L)	Evidence
In front of Dullah Laut Junior High School	-	0.01	Still safe for the cultivation or life of marine life, based on (KMNLH 2004 No.51)
The test result of the ammonia sample			
Location	Cons	Unit (mg/L)	Evidence
In front of Dullah Laut Elementary School	-	0.5	Not good enough for cultivation because it does not match the value of water quality standards
Comparison with water quality standards			
The value of water quality standards by government decisions (KMNLH No 51 2004)	Nitrite: its value is not more than 0.1 mg/L Ammonia: its value is not more than 0.3 mg/L'		

Discussion

Generally, Bivalvia is an aquatic organism that lives on the bottom of the sea and buries itself in sand or corals (Nontji 1987). This organism is well distributed on mud and soft sediment due to its feeding characteristic: filter feeder and burrower (Woodin 1976). Therefore, the size of sediment grain would influence the distribution of bivalves. Coarse sediment has lower nutrition for bivalves because organic substances could not settle (Wood 1987). Based on Regulation of Environmental Ministry number 51 (2004), the interval pH for marine organisms is between 7-8.5 mg / L. Therefore, the result of nitrite in the seawater could be nutrition for bivalves because the value is still within limits. In contrast, the result of Ammonia found in this study could be toxic for bivalves. The value of Ammonia exceeds the standard value probably because the waters have been contaminated with either organic or inorganic waste.

Atactodea is an edible dioecious cosmopolitan clam abundant in tropical regions (Baron 1992; McLachlan and Brown 2006; Boxshal et al. 2013). These clams live on fine sand and bury themselves in it to find food, reproduce, and avoid predators (Heryanto and Radjab 2013). The morphological characteristic of *Atactodea* is small, equivalve, and relatively robust. Internal ligament, a poorly-defined umbo, relatively pronounced lateral teeth, grooves, and sculptured concentric ridges. The species is opisthogyrate, whereby the umbones curve toward the posterior rather than the anterior margin of the valve, thereby reversing the usual mode of identification for left and right valves (Chan 2010; Lamprell and Whitehead 1992). This bivalve is a true filter feeder on sandy substrates in the intertidal zone (Baron 1992; Paulay 2000).

Mactra includes to family Mactridae Lamarck. The common name of its species is a wedge-through shell with a size up to 40 mm distributed on Indo-Pacific to Durband (Nel et al. 2012). This species belongs to Kingdom Animalia, Phylum Mollusca, Class Bivalvia, Order Eulamellibranchiata, Family Mactridae, and Genus *Mactra* (Venkatesan et al. 2010). Mactridae was known as the "surf clams" family, consisting of 180 species (Huber 2010).

The characteristic of *Mactra* and *Atactodea* as a member of Mactridae are having shell equivalve, ovate, or trigonal to transversely elongated, closed to somewhat gaping posteriorly. Umbones prosogyrate, more or less prominent; the outer surface is smooth or mostly concentrically sculptured, often with an obvious periostracum. External ligament short and not prominent, just behind the umbones; internal ligament well developed, set in each valve in a deep trigonal pit of the hinge plate and pointing towards the umbo. Hinge characteristic, each valve with two cardinal teeth and smooth or striated, more or less developed, lateral teeth; cardinal teeth of the left valve forming an inverted V-shaped process; delicate additional cardinal lamellae often present in either valve. Interior of shell porcelaneous. Two, often subequal, adductor muscle scars. Pallial line with a well-developed sinus (Carpenter and Niem 1998).

Atactodea, an active burrower in sandy or muddy substrate with feeding type, is filter feeding in the soft bottom ecosystem (Poutiers 1998; Lamprell and Whitehead 1992). Meanwhile, it also filtered water clarity, phytoplankton, and suspended solids in an estuary (Gerritsen et al. 1994). *Mactra* also could be found in mangroves and seagrass beds (Masagca et al., 2010; Mudjiono et al., 1992). Having female and male reproductive organs separate individuals, it is a dioecious animal with free swimming in the larval stage. Generally, these species are considered edible bivalves (Carpenter and Niem 1998). On the outer valves of this Bivalvia, there is a calcified layer. Vaughn and Hakenkamp (2001) explain that algae and other invertebrates could attach to bivalve shells.

The shells of *Mactra* and *Atactodea* are mostly solid, equivalve or subequivalve, obliquely rounded, or ovate to subtrigonal in outline and usually not gaping; inequilateral, with generally prominent, prosogyrate umbones, at or in front of the midline of the shell. Lunule and/or escutcheon are usually present. The sculpture is only concentric or with a radial component. The periostracum is typically inconspicuous. The external ligament, located behind the umbones, is often inserted in a deep groove. The hinge has 3 usually radially disposed of cardinal teeth in each valve (1 or more of which may be grooved or bifid); anterior lateral teeth are sometimes present. The interior of the shell is porcelaneous. The posterior is sometimes slightly larger with two more or less equal adductor muscle scars. The pallial sinus is usually present. Inner margins are smooth to denticulate. Gills of eulamellibranchiate type, with folded branchial sheets; outer demibranch are smaller than the inner, expanded, and almost flat above the axis. The foot is large and rather short, hatchet-shaped, and rarely byssate in the adult. The mantle broadly opens ventrally. Siphons are short to long, naked, fused, or separate, with simple tentacles on tips and inside the inhalent opening to strain large particles (Carpenter and Niem 1998).

Macrocallista is a bivalve belonging to the family Veneridae (Sartori et al., 2015). The shell of *Macrocallista* is mostly solid and transversely elongate-oval with a more or less smooth surface. The shell's interior is porcelaneous, and the pallial sinus is deep with two typically equal adductor muscle scars. Lunule and/or escutcheon at the dorsal view are usually present. The external ligament behind the umbones is often inserted in a deep groove with three hinges that usually radially disposed of cardinal teeth in each valve (one or more of which may be grooved or bifid), and anterior lateral teeth are sometimes present. The mantle broadly opens ventrally. Such as *Mactra*, *Atactodea*, and *Macrocallista* are active borrowers in various soft bottoms among marine growths like seagrass. *Macrocallista* is common in low intertidal to shallow subtidal depths (Carpenter and Niem 1998; Etheridge and Jack 1892).

Hippopus is genera that belong to the family Cardiidae, subfamily Tridacninae (ter Poorten et al. 2014; ter Poorten 2014). Cardiidae is an important and conspicuous mollusk family found throughout the Red Sea and Indo-Pacific, from East Africa to the Eastern Pacific biogeographic region (Rosewater 1965; Othman et al. 2010). *Hippopus* is generally known as giant clam or kima. The shell of the family Cardiidae is equivalve, thick, heavy, and often very large, with strongly scalloped free margins, inequilateral. The body and shell in adults of Tridacnidae appear to be reversed to other bivalves because of their highly specialized mode of life. The valve margins are in the dorsal position, and the umbones, ligament, and hinge are ventral. The outer surface of the shell has strong radial folds. The external ligament is set in a groove of the anteroventral margin. A hinge with a single ridge-like cardinal tooth is present in each valve; two lateral teeth in the right valve and one lamellar lateral tooth in the left valve. The interior is porcelaneous, has a pallial line without a sinus, and internal margins are often more or less crenulated (Carpenter and Niem 1998).

Hippopus is globose in shape, without a well-defined byssal orifice or very slight**.** *Hippopus* is found in sandy bottoms of the rocky substrate, and its foot is relatively large and non-byssate. The mantle of *Hippopus* does not indeed exceed shells. Therefore, the IUCN Red List Category and Criteria Hippopus is of least concern which means a lower risk or conservation dependent (Wells 1996).

The Tridacninae mantle is much larger than other bivalves, and it has various functions such as protecting soft organs, water transport, and maintaining the symbiotic relationship with dinoflagellates. Consequently, the mantle coloration and patterns vary greatly within species. The least variation in the mantle exists in *T. gigas*, *H. hippopus*, and *H. porcellanus* (Calado et al., 2017). Their distribution is Tropical eastern Indian Ocean to western Pacific, from the Andaman Islands to eastern Melanesia; north to southern Japan and south to Queensland, restricted in the tropical western Pacific; known from the southern Philippines, Sulawesi (Celebes), Moluccas, and western Irian Jaya (New Guinea) (Carpenter and Niem 1998).

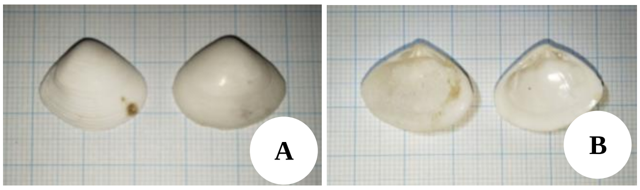

Figure 2. Shell of *Atactodea* (A) exterior view and (B) interior view

Figure 3. The shell of *Mactra* (left) interior view and (right) exterior view

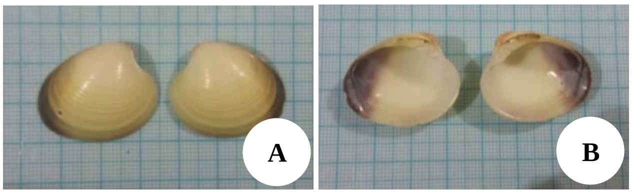

Figure 4. Shell of *Macrocallista* (A) interior view and (B) exterior view

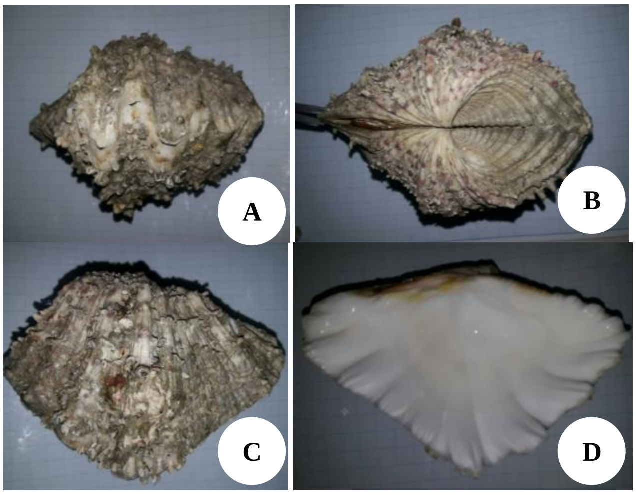

Figure 5. Shell of *Hippopus* (A) dorsal view, (B) ventral view, (C) exterior view, and (D) interior view

Tridacninae is a mixotrophic filter-feeder. The organic carbon sources in the metabolites are supplied by symbionts and by passively filtering live and dead organic compounds from the water column. Tridacninae is the only bivalve that can establish a symbiotic relationship with dinoflagellates. A larger mantle surface allows a larger symbiotic population to perform photosynthesis and secrete larger amounts of organic compounds into the Tridacninae. Such dinoflagellates coupled with accessory pigments can result in intense coloration of its mantle (Calado et al. 2017).

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Species diversity of gastropods (Cypraeidae and Conidae) at Krakal Beach, Gunungkidul, Yogyakarta, Indonesia

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Marine Study Club, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Bulaksumur, Depok, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-580839, ♥email: dea.febiansi@mail.ugm.ac.id.

Manuscript received: 9 October 2017. Revision accepted: 19 June 2018.

Abstract. *Febiansi D, Rahmayanti F, Kurnia RN, Silmi MA, Dewi AK, Millaty NK, Prasetya TA, Roshitafandi DA, Sartika HW, Trijoko. 2018. Species diversity of gastropods (Cypraeidae and Conidae) at Krakal Beach, Gunungkidul, Yogyakarta, Indonesia. Ocean Life 2: 27-32.* Krakal is a beach in Gunungkidul, Yogyakarta, Indonesia which has white sand and coral substrate in the intertidal zone. Cypraeidae and Conidae are families of gastropods found quite varied in the intertidal zone of Krakal Beach. The purpose of this research was to discover the diversity of Cypraeidae and Conidae families in the intertidal zone of Krakal Beach, Gunungkidul, Yogyakarta. The research was conducted on 24 May 2014 and 25 May 2017. The ecological parameters recorded were 26,5ºC for temperature in 2014 and 26ºC for temperature in 2017, ±3.35% for salinity in 2014 and ±3,6% for salinity in 2017, 7.7 for pH. in 2014, and 7 for pH in 2017. The samples were collected using the purposive sampling method, and specimens were identified by determining the morphological characteristics of the shell. This study found 5 species of the Cypraeidae family in the intertidal zone of Krakal Beach: *Cypraea annulus*, *Cypraea bouteti*, *Cypraea moneta*, *Cypraea caputserpentis,* and *Cypraea lynx*. While for the family Conidae, 5 species were found: *Conus coronatus*, *Conus ebraeus*, *Conus capitaneus*, *Conus botulinus,* and *Conus fergusoni*.

Keywords: Conidae, Cypraeidae, diversity, gastropod, Krakal

INTRODUCTION

Indonesia is an archipelago. It can be estimated that the Indonesian archipelago comprises 17,840 islands. According to data published by PBB in 2008, Indonesia has the fourth longest coastline in the world and extends 95,181 km from the western end to the eastern tip of Indonesia (KKP 2009). Long coastlines support many marine flora and fauna species with high abundance and diversity (Pieter et al., 2013). One of the beaches with potential for diversity is Krakal Beach, located in Ngestirejo Village, Tanjungsari Sub-district, Gunungkidul Regency, Special Region of Yogyakarta. Krakal Beach is one of the white sand beaches that has become a famous tourist attraction. The beach is located at the coordinates of S8 ° 8'42.3 "E110 ° 36'8.9"; the length of the coastline reaches over 700 meters with a gentle and wide surface.

The coastal intertidal zone is quite extensive with various substrates in the form of rocks, sandstone, and corals, which are overgrown with algae, seagrass, and sponge. The variety of these substrates causes this location to have a diverse biota community. Various biota communities that can be found are algae communities, coral reefs, fish, and various other invertebrate organisms that include thermodynamics, mollusks, crustaceans, and meiofauna. The high potential of marine resources has begun to be disrupted by a human activity because this beach's intertidal area interacts with human activities, especially tourists. The activities of surrounding communities that often take marine biota for consumption, such as *Ulva* sp., sea urchins, and ornamental fish. The area will be submerged in seawater during tidal conditions and become open areas when sea water recedes. Hence, the area becomes a place that tourists and the surrounding community very easily exploit. The habitat changes significantly impact the survival of flora and fauna from year to year (Satino 2003). One of the organisms that are sensitive to habitat changes is mollusks. Mollusks are soft-bodied animals. Mollusks have three main body parts, i.e., legs, visceral mass, and mantle. Most Mollusks secrete a strong protective pillar made of calcium carbonate. Mollusks mostly live in the sea, although some species inhabit freshwater, and some snails and bare snails live on land (Campbell et al. 2010). Mollusks play an important role in forming marine ecosystems (Arbi 2010). Changes in the structure of the molluscan community can serve as a bioindicator of the aquatic environment. Therefore, a study of the inventory of biodiversity and abundance of Mollusks is required. This research aims to assess the diversity of mollusk species, especially the family Cypraeidae and Conidae, located in the intertidal zone of Krakal Beach. The results of this study are expected to be used as additional information about the biodiversity of Mollusks located in the intertidal zone of Krakal Beach. In addition, it can be used as a reference in determining coastal tourism management policy.

MATERIALS AND METHODS

Study area

The research was conducted on 24 May 2014 and 25 May 2017 at intertidal zone of Krakal Beach, Gunungkidul, Yogyakarta, Indonesia (S8°8’42.3″ E110°36’8.9″). Materials used in this research included Gastropod samples, particularly family Cypraeidae and Conidae, aquadest, 73% $MgCl_2$, and 70% alcohol. Tools used included zip lock plastic, laminated millimeter blocks, paper labels, and a digital camera.

Procedures

Sample collection

Sample collection was conducted using the purposive sampling method. Sampling was done by surveying the coastal intertidal zone by walking from the eastern end to the beach's western end. Along the way, they found gastropod was collected and inserted into a plastic zip lock. The plastic is labeled using a label paper containing the sampling site and time. Substrates where the specimen was found were also noted for supporting data. Before preservation, several morphological characteristics were also observed.

Preservation

Sample preservation began by taking pictures of the shell and aperture of the specimens. Preservation via dry and wet preserves using $MgCl_2$ 73% (relaxation), 96% alcohol (fixation), and 70% alcohol (wet incidence).

Identification

Identification of Gastropods was conducted using the following resources: The Living Marine Resources of The Western Central Pacific Volume 1 and 2 (Carpenter and Niem 1998), The Shell Book (Rogers 1908), Seashells of the World (Abbott 1985), and Compendium of Seashells (Abbott and Dance 1998).

RESULTS AND DISCUSSION

This study found 5 species of the Cypraeidae family in the intertidal zone of Krakal Beach, namely *Cypraea annulus*, *Cypraea bouteti*, *Cypraea moneta*, *Cypraea caputserpentis*, and *Cypraea lynx*. While for the family Conidae, 5 species were found: *Conus coronatus*, *Conus ebraeus*, *Conus capitaneus*, *Conus botulinus*, and *Conus fergusoni*. Cypraea is a gastropod with oval-shaped, smoothly polished, and often brightly colored shells, with a narrow aperture stretching along the whole shell length. Generally associated with coral reefs, Cypraea uses tentacles to sense and capture food. They feed mainly on algae, coral animals, foraminifera, sponge, and small crustaceans. The tentacles were distributed all over the mantle surface to achieve the maximum surface area to capture food. Cypraea move by using the muscular foot tissue. There are two ways for gastropods animal to attach: the peristaltic muscle and the mucus to glide. The thick muscular foot tissue can produce mucus as a lubricant when moving and reduce desiccation. Like most gastropods, Cypraea has a siphon to aid respiration. The siphon is a part of the mantle skirt, and when needed, it curls to form a tubular extension at the anterior. That allows the water current to feed oxygen towards its gills in the mantle cavity. Reproduction is dioecious, and the egg is generally spawned in coral caves, empty shells, or similar dark places. Female gastropods do not leave the eggs after spawning to protect the eggs from predators until the eggs have hatched.

In general, species of Mollusks found in coastal waters of Krakal, Gunungkidul Yogyakarta can be classified as follows :

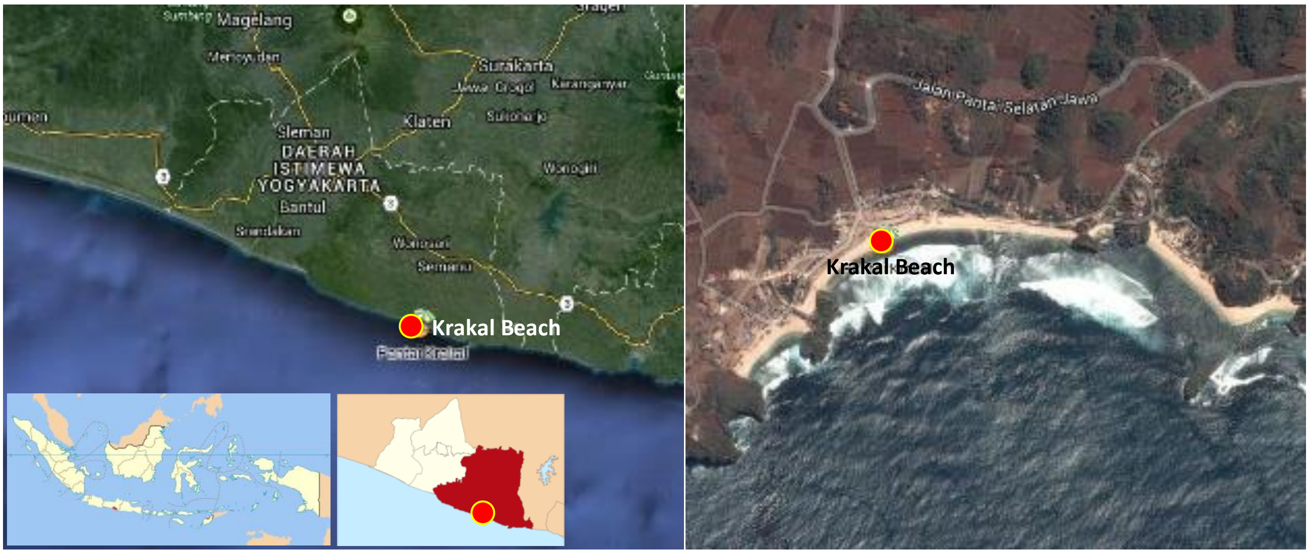

Figure 1. Map showing research location at Krakal Beach, Gunungkidul, Yogyakarta, Indonesia

Cypraea annulus

The gold ring cowry is a marine gastropod in the Cowry family, Cypraeidae. This species is usually 1.5-2 cm long and has a distinctive gold dorsal band on the glossy, cream shell. *C. annulus* is nocturnal and emerges to graze on plants and algae at night with its mottled, brown mantle fully extended over its shell. Habitat preferences include shallow water, tide pools, under stones, or amongst seagrasses. Distribution in Indo-Pacific, tropical Indo-Pacific, and also in Australia. Cypraea annulus live at 24℃-28℃, on salinity 33,67 PSU-35,42 PSU, and depth at 0,5 m – 8 m (Rosenberg 2011).

Cypraea bouteti

Creamy brown colored with lines across the back or upper side of the shell. Habitat in under stones or amongst seagrasses, the intertidal zone. Distribution in Indo-Pacific (Moretzsohn 2012).

Cypraea moneta

Has a shell with a knobbly outline and raised dome. Colour creamy, yellowish or pale green, occasionally with three darker bands. Habitat in shallow seagrass. Distribution: Indo-Pacific, tropical Indo-Pacific. Depth 0m-70m, salinity 33,67 PSU-35,5PSU, and temperature 23-28ºC (Rosenberg 2010).

Cypraea caputserpentis

Rather flattened shell with thickened margins, up to 4 cm. Dorsal surface brown with numerous cream spots. The lower sides and base are dark chocolate-brown. Habitat intertidal zone in rocky or shallow seagrass. Distribution in the West Indian Ocean, tropical Indo-Pacific. *Cypraea caputserpentis* live at 23-28ºC, and salinity 33.7 PSU-37.2 PSU (Rosenberg 2012).

Cypraea lynx

The dorsum surface of these smooth and shiny shells is generally pale brown, pale purple, or grey, densely covered with small and large dark brown or purple dots. The large spots are extended to the edges. These cowries live in shallow tropical water, subtidal and intertidal, usually under rocks or corals up to about 10 meters. Distribution in the Indian Ocean, western Pacific Ocean, western and northern Australia, and tropical Indo-Pacific. *Cypraea lynx* lives in 25-28ºC and salinity on 33.7 PSU-35.8 PSU (Rosenberg 2010).

Conus coronatus

Has distinctive features that distinguish it from other species. This species has a small, squat-heavy shell, up to 4 cm; it has an aperture variably wider at the base than at the shoulder, and the sides of the body whorl convex. Colour light, mottled pinkish-blue with brown dots and blotches. Variously sized brown, black or olive markings, spirally aligned on either side of the subcentral band, separate or fuse into 2 solid color bands. Variably spaced spiral rows of alternating white and dark dots or dashes from base to shoulder. Aperture purple-brown (Richmond 1997; Gmelin 1791). Environmental parameters of *Conus coronatus* are 26.8-28.5°C for water temperature, 1.0-2.88 µmol/L for water silicate concentration, 0.088-0.26 µmol/L for water phosphate concentration, 0.09-0.44 µmol/L for water nitrate concentration, and 4.35-4.67 mL/L for water dissolved O_2 concentration (Bouchet et al. 2015). Habitats in shallow water, often under boulders (Richmond 1997). Abundant in coral reef areas, in sand pockets among corals, or exposed on rocks. Intertidal and shallow sublittoral zones to a depth of about 10 m. Sometimes present in local markets of the northern Philippines. Widespread in the Indo-West Pacific, from East Africa to eastern Polynesia; north to Japan and Hawaii, and south to northern New South Wales (Carpenter and Niem 1998).

Conus ebraeus

Easily recognizable small, squat shell, up to 5 cm, with a rounded, short spire. Body whorl smooth, convex at top third, straight or slightly concave below, sculptured with weak spiral ribs on the lower half, patterned with four spiral bands of blackish squares on a white background, the lower band is compressed at the base. Aperture narrow with colored bands. Siphon and rostrum black, tipped with a narrow red margin (Richmond 1997; Kohn 1959; Beechey 2004). Environmental parameters of *Conus ebraeus* are 1-67 m for depth range, 23.160-28.394ºC for water temperature, 0.983-7.726 µmol/L for water silicate concentration, 0.071-0.526 µmol/L for water phosphate concentration, 0.146-3.658 µmol/L for water nitrate concentration, 4.131 mL/L-4.700 mL/L for water dissolved O_2 concentration, and 33.721-35.125 PPS for salinity (Bouchet et al. 2015). This species is found in intertidal and subtidal habitats to about 3m, on the sand, among or beneath dead corals, and on coral reefs and limestone platforms. It feeds on polychaetes (Rockel et al. 1995). The distribution is Indo-Pacific (Richmond 1997).

Conus capitaneus

The shell of moderate thickness; body whorl encircled by finely punctate striae on the basal half, the striae more distinct and separated by low ridges basally; aperture rather narrow, the sides parallel. Shoulder angular, smooth; spire rather low, obtuse, striate; apex pointed. Color of body whorl variable, yellow or olive-yellow to orange-brown or olive brown, encircled by several dark brown dotted lines and two broad white bands, interrupted by dark brown blotches, at the shoulder and centrally on the body whorl. Closely spaced longitudinal wavy brown lines may also cross the white bands. Young individuals may lack the two white bands. Spire tesselated with alternate brown and white blotches, continuing to form a band on body whorl at the shoulder. Outer lip thin; aperture violet within (Beechey 2004). Environmental parameters of *Conus capitaneus* are 23.25-26.8 ºC for water temperature, 1.0-1.25 µmol/L for water silicate concentration, 0.13-0.16 µmol/L for water phosphate concentration, 0.09-0.22 µmol/L for water nitrate concentration, and 4.67-4.82 mL/L for water dissolved O_2 concentration (Bouchet et al. 2015).

Conus betulinus

Spire of low to moderate height, outline variably concave. Basal third of the last whorl with variably broad spiral ribs. Ground color yellowish tan to orangish brown, less often cream white mottled with yellow or orange; occasionally, ground overlaid with grey. The last whorl generally has spiral rows of brown markings, varying from many closely set rows to the absence of rows. Markings vary from narrow spiral dashes to rectangular bars and from dots to round or squarish spots and axial flecks. Dark markings regularly alternate with white markings, often absent from adapical two-thirds. The basal part of the last whorl may be of darker color. Aperture white, sometimes pale yellow or violet; smaller shells often suffused with violet-brown deep within (Linnaeus 1758). Found in coastal and shallow sublittoral zones to a depth of about 20 m, in sheltered bays and on reefs, inhabiting sand pockets, sand flats, and muddy sand. Sand flats, especially in sheltered areas and near seagrasses. Widespread in the Indo-West Pacific, from East Africa to eastern Polynesia; north to southern Japan, and south to Queensland and New Caledonia (Carpenter and Niem 1998).

Conus fergusoni

Spire whorls slightly concave; large white specimens, small specimens yellow-orange, large (maximum length 153 mm) with widely spaced spiral rows of dark brown spots; spire lacking color pattern. Spire low to moderately elevated; spire outline concave in small specimens to nearly straight in large specimens; shoulder sharply angulate in small specimens, less angulate in large specimens. The aperture was moderately broad. Color light yellow-orange, paler in medium-sized specimens, fading to white in large specimens; small specimens with a distinct lighter spiral band about the middle of the shell and usually a second light band at the shoulder; spire lacking color pattern; small specimens with spiral rows of dark brown dots on body whorl; aperture white within. The periostracum is thin and light-colored in small specimens; its thick and dark brown in large specimens (McLean and Nybakken 1979). This species occurs at 0 to 200 m on sandy and muddy substrates depth (Paredes et al., 2010; Tenorio et al., 2012).

Respiratory System. This animal respires only by branchiae and has the head furnished with two tentacula that bear the eyes near their summit. In addition, they have a narrow mantle and a tube above the head, which gives the water admittance to the respiratory organ. Reproduction System, sexes are separate in *Conus,* and the male has an extendable penis (Kohn 1959).

Digestive System. Their venom is produced in the tubular venom duct and expelled into the proboscis by the contraction of a muscular bulb at the basal end of the venom duct. The proboscis also contains a radula tooth used as both a harpoon and disposable hypodermic needle through which the venom is delivered to the prey. Once the venom has been injected, the prey is immobilized almost instantaneously and engulfed by the cone snail (Halai and Craik 2009).

Figure 2. *Cypraea annulus*

Figure 3. *Cypraea bouteti*

Figure 4. *Cypraea moneta*

Figure 5. *Cypraea caputserpentis*

Figure 6. *Cypraea lynx*

Figure 7. *Conus coronatus*

Figure 8. *Conus ebraeus*

Figure 9. *Conus capitaneus*

Figure 10. *Conus betulinus*

Figure 11. *Conus fergusoni*

This research into the diversity of Mollusca at Krakal Beach found as many as 10 species consisting of 5 species of Cypraeidae members and 5 species of conidae members. Species found *include Cypraea annulus, Cypraea bouteti, Cypraea moneta, Cypraea caputserpentis*, and *Cypraea lynx*. Conidae 5 species are *Conus coronatus*, *Conus ebraeus, Conus capitaneus, Conus botulinus*, and *Conus fergusoni*. The abundance of gastropods in Krakal Beach positively correlates with coastal substrate conditions in the form of rocks. In addition, gastropods have varied diets; some are herbivores, deposit feeders, Polychaeta eaters, scavengers, bivalve eaters, and fellow gastropod eaters. Therefore, gastropods are more easily found in different habitats (Susetiono 2005).

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Short Communication: Caught fish species diversity of South Morotai, North Maluku, Indonesia

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Abstract. *Nabil WA, Habibah I, Aryochepridho, Trijoko. 2018. Caught fish species diversity of South Morotai, North Maluku,* Indonesia. *Ocean Life 2: 33-36.* South Morotai is a part of Morotai Island, an archipelago in eastern Indonesia with high fishery potentials such as fish, sea cucumbers, crabs, shrimp, and algae. Research on fish diversity in South Morotai is needed because there is insufficient data and information about the potential of Morotai Island marine fisheries. This research aimed to collect data on fish species in Morotai, especially South Morotai. This research was conducted by surveys of fish caught by local fishermen in July 2017. Results showed that there were 23 species of fishes belonging to 14 families, with the highest diversity belonging to the Scaridae family (4 species). Serranidae and Acanthuridae each had 3 species identified. Balistidae and Labridae each had 2 species identified. In addition, one species was identified from each family of Rachycentridae, Lethrinidae, Lutjanidae, Sphyraenidae, and Mullidae.

Keywords: Caught fish species, diversity, South Morotai

INTRODUCTION

Morotai Island is located at the northern end of North Halmahera Regency and is part of North Maluku Province. Geographically Morotai Island lies between 2˚00'-2 ˚40 'LU and 128 ˚15'-128 ˚48'BT, bordered by the Pacific Ocean to the North, Halmahera Sea to the East, the Morotai Strait to the South and the Sea of Sulawesi to the west. The total area of Morotai is 2,474.94 km^2 or 10 percent of the land area of the North Maluku Regency (Edward 2015). This region holds great fishery potential, such as reef fish, algae, corals, and other marine invertebrates. These waters are suitable for the development of fishery-based industries as well as marine tourism.

Coral reefs are complex ecosystems in the tropics that contain high biodiversity. Naturally, coral reefs are habitats for many marine species for spawning, nurseries, feeding, and foraging, especially for many species with significant economic value. The high diversity of marine biota in coral reefs makes these ecosystems hotspots of marine biodiversity (Fraser et al., 2003).

The reef fish community is one of the main components of the coral reef ecosystem. It has an important role within this ecosystem, for example, as an indicator of coral condition. Almost all life stages of reef fish directly or indirectly depend on coral reef existence. As a coral-related fish, coral reef destruction affects the diversity of reef fish (Rani et al. 2010).

The diversity of reef fish varies depending on the condition of the waters. This study aims to record the potential of fisheries, especially the diversity of reef fish in the sea of South Morotai. Thus, we aim to obtain data that describes fish species located in the region of South Morotai.

MATERIALS AND METHODS

Study area

The study was conducted in South Morotai, North Maluku, Indonesia (Figure 1). This area was characterized by a sandy substrate consisting of species of seagrasses, seaweed, and coral reefs covering the bottom surface area. The research was carried out in July 2017 during the day.

Procedures

The study collected data on fish species caught by local fishermen. Fishes were identified mainly using Gerald et al. (2003) and fishbase.org. The samples were identified by their morphological characters.

Figure 1. Map of the sea of South Morotai, North Maluku, Indonesia, as a fishing catchment area

Table 1. Species diversity of fish found in South Morotai, North Maluku

Family	Species
Rachycentridae	*Rachycentron canadum*
Serranidae	*Variola louti*
	Epinephelus fasciatus
	Chromileptes altivelis
Lethrinidae	*Lethrinus olivaceus*
Lutjanidae	*Lutjanus madras*
Scaridae	*Bolbometopon muricatum*
	Hipposcarus longiceps
	Cetoscarus ocellatus
	Scarus quoyi
Sphyraenidae	*Sphyraena barracuda*
Labridae	*Cheilinus undulatus*
	Novaculichthys taeniourus
Balistidae	*Rhinecanthus aculeatus*
	Balistapus undulatus
Mullidae	Unidentified
Acanthuridae	*Ctenochaetus striatus*
	Acanthurus triostegus
	Naso cf. *annulatus*
Siganidae	*Siganus canaliculatus*
Carangidae	*Caranx melampygus*
Hemiramphidae	*Hemiramphus far*
Belonidae	*Tylosurus crocodilus*

RESULTS AND DISCUSSION

Fish diversity

The marine fish species observed can be classified into 23 species from 14 Families of Fish (Table 1). Each species has distinct characteristics that distinguish it from other species. The most abundant species found in South Morotai belonged to the family Scaridae with four species, followed by Serranidae and Acanthuridae with three species each, Labridae and Balistidae with two species, Rachycentridae, Lutjanidae, Lethrinidae, Sphyraenidae, Siganidae Carangidae, Hemiramphidae, and Belonidae with one species each. One specimen from the family Mullidae remains unidentified.

Discussion

This paper's term 'species' refers to the morphological species concept. Thus, other concepts of species were not used.

Scaridae, the parrotfishes, are known for their teeth which usually form a pair of beak-like plates in each jaw (Bellwood 2001). Parrotfishes are important to coral ecosystems due to their role as grazers on algae on the reef substrate. Bellwood and Choat (1990) divided the parrotfish community into two functional groups: excavators and scrapers. Excavators take relatively slow and large bites, leaving distinct scars on the substratum. Scrapers take quick, small bites, leaving only a few scrapes on the substratum.

Serranidae, which consists of groupers and sea basses, is one of the most important fish families in Indonesian marine fisheries (Genisa 1999). Serranid fishes have an opercle with 3 (rarely 2) flat spines, and the preopercle's margin is nearly always serrate or with 1 to 4 spines (Heemstra and Randall 2001). Groupers are usually placed in a cage culture by Morotai fishermen to enlarge their body mass before being sold in the market.

Acanthuridae contains surgeonfishes, tangs, and unicornfishes. The distinctive characteristic of this family is the spine (multiple spines in genus *Naso*) located on each side of the base of the tail (Allen et al. 2003).

The Balistidae shares some similarities with Monacanthidae. Both have two-part dorsal fins, in which the front part's first spine is distinctively stout. The difference is the stout spine of Balistidae can be locked in place, which cannot be done by members of Monacanthidae (Allen et al. 2003).

Labridae is a large family of fish with various sizes and body shapes (Allen et al. 2003). Fishes from this family have a terminal and protrusible mouth with teeth in jaws that are usually separate and caniniform (Westneat 2001). Within Labridae, the *Cheilinus undulatus* (humphead wrasse), is endangered species according to IUCN Redlist, was found during this research. The combination of a long time to reach a mature age and overfishing has caused a great decline in the humphead wrasse population in the last 30 years (Russell 2004). This species is a protogynous hermaphrodite and reaches first sexual maturation at about 35-50 cm total length or under 5 years of age (Sadovy et al. 2003). According to local fishermen, this species has a very high market value, but the size and quantity in the local market are highly restricted due to government law. Therefore, local fishermen tend to sell these fish on the black market to make more money.

The Rachycentridae family consists of a single species, *Rachycentron canadum*. This migratory fish occurs in tropical and subtropical seas of the world, except in the central and eastern Pacific Oceans (Shaffer and Nakamura 1989).

Lethrinidae and Lutjanidae are two families that have sloped heads. Lethrinid fishes have a maxilla mostly concealed below infraorbital bones, not articulating broadly, with a distal tip of the premaxilla and toothless vomer and palatines (Carpenter 2001). Lutjanid fishes have a maxilla that slips for most or all of its length under the lacrimal when the mouth is closed and usually have a vomer and palatines with teeth (Anderson dan Allen 2001). Several species of these two belong to economically important fishes in Indonesia, especially the genera *Lethrinus* and *Lutjanus* (Genisa 1999). Morotai fishermen usually sell fish of these two families as salted fish.

Members of the Carangidae family are popular as game fish. They are strong open water swimmers that occasionally form large schools (Allen et al. 2003). These family members have two dorsal fins, with the first in a moderate height or very low and arched (or elevated) above the pectoral fins but straight posteriorly, extending onto the caudal fin (Smith-Vaniz 2001).

Sphyraenidae only contains a single genus, *Sphyraena*. The members of this family are often called barracudas. The long jaw of a barracuda is filled with an array of sharp pointed teeth (Allen et al., 2003). Fish from this family become a threat to swimmers and divers due to their aggressive behavior.

Hemiramphidae (halfbeak) and Belonidae (needlefish) belong to the order of Beloniformes. Both have a slender and elongated body with an unequally forked tail. This special feature on the tail allows them to jump out of the water and briefly 'run' on the surface when frightened. For example, we observed a needlefish jump out of the water when pursuing a jumping prey (probably a halfbeak).

Siganidae, the rabbitfishes, is a family of venomous fish which can inflict extremely painful wounds (Allen et al. 2003). The venom is injected through spines on the dorsal (13 spines), ventral (2 spines), and anal fin (7 spines). Spines on the ventral fins are separated by 3 soft rays, which is unique to this family (Woodland 2001). Local fishermen bite rabbitfish tail and swallow it raw to relieve pain from its sting.

Mullidae is a family of bottom feeders called goatfishes. A pair of barbels extending from the chin of this fish is responsible for its common name. These barbels are used as chemoreceptors in the search for food (Allen et al. 2003). Unfortunately, the specimen observed in this research remains unidentified due to poor pictures and other data.

We believe there are many more unobserved fish species in Morotai seas. Thus, there is still a long way before we can truly understand the potential of Morotai Island marine fisheries. There remains a wide area of sea that needs to be explored in South Morotai alone to determine further information on the marine biodiversity of this important area.

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