Uca annulipes photo by Bernard Dupont
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. 2017. 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, Palerospora muriacta, 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, were 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 31.4°C in February 2009 triggered the bleaching event observed in May 2009 at the site. Acroporids showed the first sign of bleaching and paling as from January 2009 when mean SST was at 30°C. Branching coral (P. eydouxi) and solitary coral (Fungia sp.) exhibited only 15% of their colonies showing paling by April 2009. A. cytherea, A. hyacynthus, and A. muriacta showed varying bleaching conditions [Pale (P), Partially-bleached (PB) and Bleached (B)] at onset of the bleaching event whilst 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. fasciularis indicated a quick coloration recovery from P to NB after the bleaching event, although their maximum quantum yield post bleaching data indicated a differential recovery in visual coloration and PSII functioning among the corals. A. muriacta 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 order of recovery 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 occurs as a stress response in reef-building corals exposed to acute short-term stress or to long-term exposure to environmental stress, from both natural and anthropogenic sources. Muscatine et al. (1981) reported that in the symbiotic relationship between coral host and its symbiont, the host can obtain up to 100% of its daily carbon requirements from photosynthesis in its endosymbiont. The coral bleaching process takes place due to 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. The cellular processes and/or mechanisms for the 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 affects 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 a number of 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 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 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; Bhagoji 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 Woensel et al. (2011) revisited the winners and losers of coral bleaching over a 14-year period. 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 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, taxa that are highly integrated cannot contain the damage and rates of whole colony mortality are therefore high (e.g. *Acropora*) (McCowan et al. 2012).

The present study was carried out to investigate recovery of eight species of reef-building corals of Mauritius following a major single bleaching event recorded in May 2009 at the lagoon of Belle Mare, Mauritius (Mattan-Moogawara et al. 2012). The study aimed the following: (i) to investigate the bleaching susceptibilities prior to and 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**

**Field work**

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 for an assessment of bleaching and subsequent recovery over time. The twenty coral colonies were first tagged in October 2008 for monitoring of bleaching. 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. Coral colonies were tagged with fluorescent tapes for easy identification during the span of the study, and the tapes were removed after completion of study.

**Sample collection and preparation**

Colonies of eight reef-building corals, *Acropora cytherea*, *Acropora hyacinthus*, *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 whole colony looks healthy and normal colour; Pale (P) condition indicates overall paling of colony; Partially-bleached (PB) condition indicates colony which exhibits less that 30% bleaching/whitening; Bleached (B) condition indicates colony which exhibits >90% bleaching/whitening. Coral tips of 2-3 cm were collected and kept in seawater in 250 ml sampling bottles. Coral samples were brought to laboratory and dark-adapted to allow the photosynthetic endosymbionts to relax all their PSII reaction centres before measurement of maximum quantum yield, Fv/Fm.

**Chlorophyll a 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 (3000 μmol quanta m-2 s-1, 0.8 s duration) was applied to determine maximal fluorescence (Fm) when all PSII centres were closed. Ratio of change in fluorescence (Fm/Fo) caused by the saturating pulse to the maximal fluorescence (Fm) in a dark-adapted sample, is correlated to the maximum quantum yield of
PSII and thus represent the PSII functioning (Genty et al. 1989).

**In-situ measurements of physical parameters**

Temperature (OAKTON pH/mv/ºC 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 on a monthly basis 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 highest mean salinity recorded in August 2009 at 35.25‰ and lowest at 33.25‰ recorded in April 2009. Mean dissolved oxygen fluctuated over the study period with 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 mean lowest temperatures recorded at 23.9ºC in winter month of September 2009 and mean highest temperatures recorded 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) after which it decreased gradually to 27.5ºC in May 2009. Lowest mean sea surface temperatures were recorded in October 2009 at 24.1ºC.

**Percentage bleaching occurrence in sampled colonies**

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%) and *Acropora* sp. (25%), and in *A. cytherea* (15%) and *A. hyacynthus* (20%) (Figure 2).

All (100%) colonies of *Fungia* sp. and *P. eydouxii* 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, and in May 2009, percentage of colonies showing paling increased up to 25% in *P. damicornis* and up to 30% in *G. fascicularis*.

*A. cytherea* colonies showed an increasing occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (60%). 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 increasing occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (65%). 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 increasing occurrence of bleached (B) conditions from January 2009 (0%) to May 2009 (35%). In February 2009, *A. muricata* showed all four visual conditions at 25% NB, 50% P and 25% PB, as 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, the occurrence of 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 event in May 2009 until October 2009. Figure 3 shows a comparison of the bleaching condition in the eight studied species. Results indicated that all NB coral colonies of the eight species remained as 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) as 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, to the exception of *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 a lapse of two months, i.e. in July 2009. Complete recovery in these two species to non-bleached (NB = 1) condition occurred in August 2009.

**Chlorophyll fluorescence ratio, Fv/Fm**

Figure 4 indicated chlorophyll fluorescence ratio of *in hospite* zooxanthellae cells from coral samples collected from tagged coral colonies showing different visual
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). This 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 as compared to 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 post-bleaching months 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). Observations made over 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, whereas PSII functioning indicated slower recovery in A. cytherea from 0.45 in May 2009 to 0.6 in October 2009. In May 2009, only three species of corals exhibited the bleached (B) condition, namely A. cytherea, A. hyacynthus, A. muricata. PSII functioning recorded during May 2009 in bleached (B) colonies were as low as 0.14 in A. cytherea and A. hyacynthus, and 0.19 recorded in A. muricata. A gradual recovery of PSII functioning was observed in all three species post bleaching in May 2009 (Fv/Fm = 0.14-0.19) to October 2009 (Fv/Fm = 0.6). However, results indicated that A. muricata recovered fastest in the month of July 2009 with highest chlorophyll a fluorescence ratio of 0.57 as compared to the other two Acroporids.

**Discussion**

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

**Physical parameters**

It is observed that an increase of sea surface temperatures up to a maximum of 31.4°C in February 2009 has triggered the bleaching event in May 2009. 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 2A. Percentage bleaching occurrence (% out of 20 colonies) in the eight studied coral species over 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 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 one-year study period 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 as from January 2009 when mean sea surface temperatures rose above 30 °C, 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; Rieg 2002; Jones 2008; McCowan et al. 2012). Families of corals that are mostly characterised by branching growth forms (e.g. Acroporidae and Pocilloporidae) are considered to be most susceptible to bleaching and experience highest rates of mortality once bleached (Baird and Marshall 2002; Jones 2008). 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. They more frequently experience partial mortality rather than whole colony mortality (Brown and Suharsono 1990; McClanahan 2000; Baird and Marshall 2002; Rieg 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. This 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 thermally most robust among the eight studied corals, with only 15% of their colonies showing paling in April 2009 prior to 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 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. This differential susceptibility among Acroporids have been explained by the following factors 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).

Percentage bleaching occurrence in sampled coral species post-bleaching event

and age (Loya et al. 2001); differential susceptibility and adaptive mechanisms of 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 colour and normal chlorophyll a 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, to the exception of A. cytherea which took slightly longer to recover. Moreover, all PB colonies recorded in May 2009 for A. muricata recovered to NB as from June 2009 onwards indicating a rapid recovery, to the exception of 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, as compared to B colonies for A. cytherea, A. hyacynthus and A. muricata which showed no complete recovery until the month of August 2009.

The above observations suggest that tabular corals A. cytherea were more affected by bleaching event 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/colour of coral may be linked to a recovery of PSII functioning in zooxanthellae. Rodrigues et al. (2008) have demonstrated that M. capitata bleached six days earlier than P. compressa, and that PSII repair recovered 6.5 months earlier in the latter than in M. capitata. The authors suggested that 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, although pale in appearance, showed no remarkable change or improvement in PSII functioning, suggesting that paling may have involved loss of pigments rather than damage to 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 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 as compared to other two Acroporids, *A. cytherea* and *A. hyacinthus*, showing a faster recovery of functioning of Chl a in its zooxanthellae symbionts in the months of June and July 2009. All P, PB and B samples in this study gained in coloration by October 2009, at same time 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. hyacinthus*). 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. hyacinthus*, and their slower recovery in terms of PSII functioning was also observed. Jones and Yellowlees (1997) have reported the manner by which 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. The authors suggested that algal cell size in their study appeared invariant of seasonal change, zooxanthellae density, chl a concentration and division frequency. If 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 a 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); zooxanthellae 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 Clannah 2005); morphological variability on surface skeleton of host as a means for photoprotection (Bhagooli 2012b); and host strategies during recovery, for example, stored energy reserves and photosynthetically acquired carbon and/or heterophically 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). Resilience of zooxanthellae symbiont and/or resilience of coral host are also important in determining rate of recovery (Rodrigues et al. 2008).

Anthony et al. (2009) reported that survival following bleaching was also strongly influenced by remaining lipid reserves, rates of heterotrophy, and rates of photopigment (or symbiont) recovery. However, these factors were not measured within this study, and provide an opportunity for further investigation to look into effect of zooxanthellae size, density, clades and gene expression in both 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 results of the study highlight a differential recovery among eight studied species of reef-building corals, in terms of recovery of colour (visual condition) and recovery of the PSII functioning of zooxanthellae endosymbionts, post-May 2009 bleaching event. The order of recovery was as follows: Massive-like/ solitary corals > Branching and semi-bulbous corals > tabular corals. Rate of recovery was fastest in *P. damicornis* and *G. fascicularis*, followed by *Acropora sp.*, and lastly by *A. cytherea* and *A. hyacinthus*. Normally, a recovery in bleaching condition, i.e. gain in coloration from B to PB, PB to P and P to NB, also indicated a recovery of PSII functioning a coral to the exception of *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. 2017. Short-term effects of heavy metal and temperature stresses on the photo-physiology of *Symbiodinium* isolated from the coral *Fungia repanda*. *Ocean Life* 1: 11-20. This study aimed to investigate the effects of the heavy metals, copper, zinc and lead, on the photosynthesis of the symbiotic dinoflagellate *Symbiodinium* isolated from the coral *Fungia repanda*. Freshly isolated *Symbiodinium* found to belong 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*/*F*₀), relative maximum electron transport rate (*rETR*ₘₐₓ) and maximum non-photochemical quenching (*NPQ*ₘₐₓ) of the photosystem II (PSII). An increase in non-photochemical quenching accompanied by a decrease in photosynthetic capacity was noted for copper at a concentration of 50 µg/L for both temperatures. The *F*/*F*₀ 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 non-photochemical quenching accompanied by a decrease in photosynthetic capacity. Pb had the most profound effect on all of the isolates. The *F*/*F*₀ 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*/*F*₀: maximum quantum yield; *rETR*ₘₐₓ: relative maximum electron transport rate; *NPQ*ₘₐₓ: maximum non-photochemical quenching; PSII: photosystem II; rpm: revolutions per minute; RLCs: rapid light curves; *F*₂: initial fluorescence; *F*ₘₐₓ: 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). Release of heavy metals to the marine environment mainly results from atmospheric and river inputs, direct discharges, industrial dumping and sewage sludge, 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). Heavy metals are known to 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 sublethal temperatures (Iglesias-Prieto et al. 1992) leads to photoinhibition of photosynthetic processes in marine organisms. Elevated temperature has been found to cause damage to the 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 pose a threat to 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*, which is 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 (Bieltman 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 a 14-day period and Oukarroum et al. (2012) worked with cultured microalgae over a 24-hour period. To the best of our knowledge, there is a dearth 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 in order 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 (*Fv/Fm*) of PSII, the maximum relative electron transport rate (*rETR*ₘₐₓₚₐₓ) and the maximum non-photochemical 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) prior to further processing. *Symbiodinium* cells were obtained from *F. repanda* by blasting the coral with filtered sea water (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 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 re-suspended with FSW before a 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 of above 10⁵ 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](image-url)
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 period, 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; Mattanmoorgawa 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 sea water 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 levels of heavy metals reported in coral reefs areas (e.g. Ali et al. 2011) and set toxic thresholds (ANZECC 1992). Five millilitres of each heavy metal solution was added to McCartney bottles followed by subsequent addition of 1 Millipore filter with adsorbed symbionts per vial. The McCartney bottles were then immersed in two waterbaths, one set at 28 °C and the other one 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 \(F_0\) was determined by applying a weak pulse-modulated measuring light (< 0.1 µmol quanta m\(^{-2}\) s\(^{-1}\)) when the PSII reaction centres are open. The maximum fluorescence \(F_{\text{m}}\) was determined after applying a saturating pulse (> 6000 µmol quanta m\(^{-2}\) s\(^{-1}\)) when the reaction centres are closed. The ratio of the change in fluorescence to maximum fluorescence \((F_{\text{m}}-F_o)/F_{\text{m}}\) gives the dark-adapted photosynthetic parameter \(F_o/F_{\text{m}}\), which is a good proxy of the maximum quantum yield of PSII (Genty et al. 1989). Samples were dark-adapted for 15 minutes prior to measurement. After the dark-adapted measurement, the samples were light adapted for 5 minutes, followed by 30 s dark period, and initial and maximum fluorescence \(F_o'\) and \(F_{\text{m}}'\) respectively) were determined again. The product of the ratio of change in fluorescence to maximum fluorescence of light-adapted samples \((F_{\text{m}}'-F_o')/F_{\text{m}}'\) also known as the effective quantum yield) and the photosynthetically active radiation (PAR) gives the parameter relative Electron Transport Rate (rETR). The non-photochemical quenching \(\text{NPQ}\) parameter, which regulates 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 \(\text{NPQ} = [F_{\text{m}}-F_o']/F_{\text{m}}'\) (Bilger and Björkman 1990).

The rETR and the NPQ were derived from the rapid light curves (RLCs) obtained after light adapting the samples. The RLCs determines the physiological flexibility of the symbionts to adapt their photosynthetic apparatus to rapidly changing light intensities. Rapid irradiances occurred at an interval of every 10 s and gave fluorescence measurements which were fitted as an exponential decay curve. The \(r\text{ETR}_{\text{max}}\) was obtained by fitting rETR curves in the Sigma Plot software using the Platt et al. (1980) equation. \(\text{NPQ}_{\text{max}}\) represents the highest non-photochemical 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 10min at 4000 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 4000 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, treated for 1-2 hrs at 65°C and stored at room temperature for later analyses. DNA extraction, was carried out using slightly modified method of Rowan and Powers (1991). Proteinase-K was added to the *Symbiodinium* suspension and incubated for 2-3 hrs at 55°C. 64 µl of 5M NaCl was added followed by 60 µl of 10% cetyltrimethylammonium bromide (CTAB) and was toped up to 600µl with sterile distilled water. The lysate was then heated for 30min at 65°C followed by addition of 600µl of chloroform. The lysate was subject to chloroform extraction once and phenol extraction twice. 900µl of cold ethanol was then 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 re-suspended 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 visualize in agarose gel.

Statistical analyses

Chlorophyll fluorescence data was arcsine transformed prior to 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, \(F_o/F_{\text{m}}, r\text{ETR}_{\text{max}}\) and \(\text{NPQ}_{\text{max}}\). Differences between groups were determined by the Post Hoc Tukey HSD test.
RESULTS AND DISCUSSION

Genotyping results showed that *F. repanda* harboured the *Symbiodinium* Clade C (Figure 2). An increase in temperature, heavy metal concentrations, and exposure time reduced the maximum quantum yield (Fv/Fm) of the symbionts significantly (P < 0.05). The maximum non-photochemical quenching (NPQmax) 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 rETRmax 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, Fv/Fm, rETRmax and NPQmax with Cu concentrations over three and eighteen-hour treatments. Zooxanthellae isolated from *F. repanda* had initial Fv/Fm values of 0.593 ± 0.011. At 28 °C, no change in Fv/Fm was noted (P > 0.05), the light-adapted parameters were affected significantly; 50 µg/L copper reduced the rETRmax significantly for all isolates (P < 0.001 for 3 h; P < 0.01 for 18 h) and increased the NPQmax 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 rETRmax (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 decrease in Fv/Fm (P < 0.05 for 3 h; P < 0.01 for 18 h) and rETRmax (P < 0.01 for 18 h; no change for 3 h) and significant increase in NPQmax (P < 0.001 for both 3h and 18 h).

**Effects of Zn**

Zn exposure on isolated *Symbiodinium* (Figure 4) showed no significant change in Fv/Fm for all treatments. However, the rETRmax 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 NPQmax (P < 0.01) was noted for the same treatment. At 32°C, zinc concentrations of 50µg/L and 100µg/L caused significant increase in NPQmax (P < 0.001 for 50 µg/L; P < 0.01 for 100 µg/L) but not the other measured photosynthetic parameters.

![Image](Image 333x378 to 544x572)

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

| Source of variation | Fv/Fm | NPQmax | rETRmax |
|---------------------|-------|--------|---------|
|                     | 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  | 0      | 0.120 | 2     | 0.005  | 7.93   | 0.001 | 2     | 0.007  | 6.88   | 0.001 |
| HM*Time             | 4     | 0.000  | 0      | 0.047 | 4     | 0.002  | 3.93   | 0.005 | 4     | 0.008  | 7.46   | 0.000 |
| Conc*Time           | 6     | 0.000  | 0      | 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 |

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 Fv/Fm, NPQmax and rETRmax. 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)
Figure 3. Maximum quantum yield of PSII ($F_v/F_m$), maximum relative electron transport rate ($rETR_{max}$) and non-photochemical quenching ($NPQ_{max}$) of zooxanthellae isolated from F. repanda under Cu treatments at 28°C (A, B and C) and 32°C (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. Maximum quantum yield of PSII ($F_v/F_m$), maximum relative electron transport rate ($rETR_{max}$) and non-photochemical quenching ($NPQ_{max}$) of zooxanthellae isolated from F. repanda under Zn treatments at 28°C (A, B and C) and 32°C (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 ($n = 3$).
Maximum quantum yield of PSII ($F_{v}/F_{m}$), maximum relative electron transport rate ($rETR_{max}$) and non-photochemical quenching ($NPQ_{max}$) of zooxanthellae isolated from $F. repanda$ under Pb treatments at 28°C (A, B and C) and 32°C (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; $P < 0.01$ for 18 h; 50 µg/L: $P < 0.01$ for 3 h, $P < 0.01$ for 18 h). The $rETR_{max}$ was significantly reduced after 18 h at lead concentrations of 50mg/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, $P < 0.01$ 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. This suggests that 50 µg/L Cu decreased the photosynthetic capacity of the *Symbiodinium* and the excess energy was effectively dissipated. This is consistent with Yruela et al. (1992) who noted that Cu inhibits electron transfer at the level of pheophytin Q$_A$-Fe domain of the PSII reaction centre and Han et al. (2008) who noted no significant change in photosynthetic yield at Cu concentrations 25-50 µg/L but a higher Cu concentration of 250 µg/L did reduce the $F_{v}/F_{m}$ significantly. A large body of research has shown that Cu is toxic to the photophysiology of marine organisms and cause 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 to be dose-dependent, a high Cu concentration is expected to cause 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 *Symbiodinium* (CCMP 2467) isolated from the coral *Stylophora pistillata*. They reported no significant change in $F_{v}/F_{m}$ and slight but not significant increase in maximum rate of photosynthetic electron transport ($P_{max}$) up to 2 days of exposure to 50µM Cu at 25°C. However, after 3 days exposure, was observed significant decline in both $F_{v}/F_{m}$ and $P_{max}$. They also reported that the time of electron transport between photosystems ($t_{PSII-PSI}$) 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-Penalver 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 O2-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 (H2O-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). This 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 and hence it may not be a good indicator of Zn stress (Joshi and Mohanty 2004). Baumann et al. (2009) reported significant reduction in yield of macroalgae when exposed to Zn concentration of 10 \( \mu \)g/L after 4 days. In the latter study, Zn was reported to irreversibly bind to the test macroalgal species, causing 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 \( Symbiodinium \) and found no significant change in \( F_v/F_m \) and but significant increase in maximum rate of photosynthetic electron transport (\( P_{max} \)) up to 2 days of exposure to 100\( \mu \)M Zn at 25°C. After 3 days 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 photosynthetic reduction cycle (Stiborova et al. 1986). Moreover, PSII has been found to be 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 centre of PSII (Joshi and Mohanty 2004). This is in accordance with a decrease in \( rETR_{max} \) with increasing concentration of Pb. While in the present study \( F_v/F_m \) of \( Symbiodinium \) was severely reduced when exposed to a Pb concentration of 50 \( \mu \)g/L, Baumann et al. (2009) reported Pb to be 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 of macroalgae at 10 \( \mu \)g/L possibly because the macroalgae were tolerant to moderately high Pb concentration (Strömgren 1980; Lamai et al. 2005). However, Hussain et al. (2006) found drastic reduction in yield parameters when mussel 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) and this corresponds to the effects of Pb in our study. As a result, the system significantly increased its non-photochemical quenching to safely harness the excitation energy. Kuzminov et al. (2013) documented Pb toxicity in a cultured \( Symbiodinium \) and reported no significant change in \( F_v/F_m \) up to 3 days of exposure to 50\( \mu \)M Pb at 25°C but slight decrease in maximum rate of photosynthetic electron transport (\( P_{max} \)) up to 2 days of exposure. After 4 days exposure a significant decline in both \( F_v/F_m \) and \( P_{max} \) was observed. However, \( \psi_{tsil-psi} \) was the first parameter to be affected.

Under stress conditions such as combined heavy metal and thermal stress, the higher capacity for non-photochemical quenching helps to provide protection to the photosynthetic organism. In the present study, all heavy metal treatments carried out at the elevated temperature (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. It is likely the alternative sources of protons such as the PS I cyclic electron transfer and/or chlororespiration are 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 on marine life forms (Cairns et al. 1978; Sokolova and Lannig 2008; Oukarroum et al. 2012). As noted by Oukarroum et al. (2012), 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. The Mauritian waters is not spared from both metal contamination (Duby 2006) and elevated thermal anomalies (Mattan-Moorgawa et al. 2012; Bhagooli and Taleb-Hossenkhah 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 at high concentrations and these heavy metals 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 as well as the concentration of heavy metals. Mitchelmore et al. (2007) showed that the coral \( Pocillopora damicornis \) could accumulate Cu 3-fold and
30-fold at 5 and 50 μg/L, respectively, after 4d of exposure, with the in hospite Symbiodinium accumulating 1.5-fold of Cu in 5μg/L treatment higher than that in the control. Bielmyer et al. (2010) investigated the effect of exposure of Cu on the coral A. cervicornis for 5 weeks and observed that Cu exposure and accumulation may affect the symbiont by reducing CO2 available for photosynthesis. Kuzminov et al. (2013) also reported exposure time-dependent 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 and hence, it is important to understand the mechanisms of action of these heavy metals to better evaluate the effects of heavy metal stress. It is also noteworthy that the accumulation of heavy metals for an effective concentration resulting in significant photophysiological changes may be time-dependent and thus short-term exposures in hours may need to be extended to days of exposures to be able to thoroughly evaluate impacts of heavy metals on Symbiodinium.

Rise in the surface sea water temperature is expected to cause mass bleaching events leading to ‘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 demonstrated a reduction in 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 Fm/F∞, 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 the elevated temperature. These include the oxygen-evolving complex (Havaux 1993), the reaction centre (Heckathorn et al. 1998) as well as the connectivity between the light harvesting complex and the reaction centre of PSII (Schreiber and Armond 1978). Warner et al. (1999) and Lesser and Farrell (2004) have shown that the main site of photoinhibitory damage at the PSII is the D1 proteins, the loss of which is correlated with reductions in Fm/F∞. 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. This enforces the suggestion that thermal stress exacerbates the pathway of cellular damage that occurs as a result of 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 in 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 different Symbiodinium clade types, namely A3, C1 and D1a. The coral species harboring Symbiodinium D1a exhibited 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 Kaulyssing 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 to 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 difference in Symbiodinium clade types or sub-types. Further studies on the sub-clade types, 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. 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, the near coast areas are also places where higher levels of both essential and non-essential metals may occur. 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 conclusions, 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 higher temperature. The present study showed that a higher temperature enhanced the harmful effect of heavy metals and this lead to 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 which may be thermally robust and are either resistant or resilient to thermal anomaly events, may be rendered 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, Nashurrrokhman 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 high-levels 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 Bivalve class in the intertidal zone of Dullah Laut Beach, Southeast Molucca related with 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 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 namely Atactidea, Macrourilla, 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 megadiversity countries, Indonesia is a maritime country that has abundant marine wealth. Various types of beaches that exist in Indonesia support various kinds of marine life. Various marine commodities can be cultivated today such as fish, crustaceans, molluscs, echinoderms, and seaweed. One of the most live animals includes well-exploited molluscs such as meat oysters, pearl oysters, green shells, blood clams, abalone, and kima (Sadradjah 2015). One of the abundant marine biotas in Indonesia is Mollusca. Molluscs are suitable to live in sandy beach environments and coral fragments (Romimoharto and Suhardjono 1999). Molluscs are covered with an exoskeleton. Eastern Indonesia is one part of Indonesia that has various types of beaches that are still natural. The beach consists of various substrates such as algae, seagrass, sand, and rocks suitable for Mollusca habitat. Dullah Laut Village is a small island in Southeast Moluccas that has beaches with various substrate. The island is located at the coordinate point -5.533990 LS / 748941 BT and obscured by other small islands. These sandy and rocky beach characteristics are very suitable for habitat for Molluscs, especially bivalves. In accordance with the origin of the word (bi = two, valve = kaleal kalkareus), bivalves are a type of Mollusca that has 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 the self-enters the sand or mud using its 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). In this study, bivalves took based on different substrate types will be analyzed. In addition, with the existence of bivalves that are 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 become an archipelago becomes an exciting reason for proper research. This results in Mollusca being a good environmental indicator. This study was conducted to determine the diversity of bivalves in the coastal village 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 -5.53990L/132.748941BT) (Figure 1). Sampling was conducted in 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
Collection of molluscs was done by purposive sampling method in various substrates within the intertidal zone. If the substrate was sandy, the collection of molluscs 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 18.00 pm WIT. All bivalve specimens found alive were collected and placed into a plastic container. The collection of mollusc samples mollusc was assisted by local residents. Samples were preserved by washing, boiling, then cleaning the contents. The samples of molluscs were documented on mm block paper and were brought to the Faculty of Biology, Universitas Gadjah Mada, Indonesia for identification. Preserved samples of mollusc were identified with the help of FAO, Worms, EOL, and other literature. The environmental parameters like water temperature was tested four times in two months with a thermometer. 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 Figure 2-5. The samples of mollusc (bivalve) were identified belonging to genus Atactodea, Hippopus, Macrocallista, Mactra, and Tridana.

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

The sample of water from Dullah Laut Beach was brought to LIPI to test the nitrate and ammonia content using KIT nitrate and ammonia. The result of nitrite content is 0.01 mg/L and ammonia is 0.5 mg/L. The value of nitrite contained when it compared with water quality standards from government decisions (KMLH No 51 2004) it is not more than the standard value which means the water was still safe for the cultivation or life of 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 mollusc 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

| 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 (KMNHLH 2004 No.51) |

| 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 with the value of water quality standards |

| Comparison with water quality standards | 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 which 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, which is filter feeder and burrower (Woodin 1976). The size of sediment grain would influence the distribution of bivalves. A 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 organism between 7-8.5 mg / L. The result of Nitrite contain on the seawater could be nutrition for bivalve because the value is still within limits while 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 which its very abundant on tropical region (Baron 1992; McLachlan and Brown 2006; Boxshall et al. 2013). These clams live on fine sand and bury itself in it to find food, reproduce, and avoid predators (Heryanto and Radjab 2013). The morphological characteristic of Atactodea is small, small, equivalue and relatively robust. Internal ligament, a poorly-defined umbo, relatively pronounced lateral teeth and 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 at intertidal zone (Baron 1992; Paulay 2000).

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

The characteristic of Mactra and Atactodea as a member of Mactridae are having shell equivale, ovate or trigonal to transversely elongated, closed to somewhat gaping posteriorly. Umbones prosogyrate, more or less prominent. Outer surface 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 known as 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 had a role as filtering water clarity, phytoplankton, and suspended solids on estuary (Gerritsen et al. 1994). Mactra also could be found in mangroves and seagrass beds (Masagca et al. 2010; Mudijono et al. 1992). Having female and male reproductive organs in separates individual, it is a dioecious animal with free swimming in the larval stage. Generally, these species are considered edible bivalves (Carpenter and Niem 1998). On outer valves of this Bivalvia, there is a calcified layer. Vaughn and Hakenkamp (2001) explain that algae and other invertebrates could attach on bivalve shells.

The shells of Mactra and Atactodea are mostly solid, equivale or subequivale, 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 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. Two more or less equal adductor muscle scars, the posterior is sometimes slightly larger. Pallial sinus is usually present. Internal 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 out 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 interior of the shell 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* and *Atactodea*, *Macrocallista* are also active borrowers in various soft bottoms among marine growths like seagrass. *Macrocallista* are common in low intertidal to shallow subtidal depths (Carpenter and Niem 1998; Etheridge and Jack 1892).

*Hippopus* is genera that belongs to the family Cardiidae, subfamily Tridacinaceae (ter Poorten et al. 2014; ter Poorten 2014). Cardiidae is an important and conspicuous mollusc 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 family Cardiidae is equivale, thick, heavy and often very large, with strongly scalloped free margins, inequilateral. The body and shell in adults of Tridacinidae appear to be reverse to other bivalves because of their highly specialized mode of life. The valve margins are in dorsal position and the umbones, ligament, and hinge are situated ventrally. The outer surface of the shell has strong radial folds. The externak 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 rocky substrate, its foot is relatively large and non-bysstate. The mantle of *Hippopus* does not indeed exceed from shells. The IUCN Red List Category and Criteria Hippopus is of least concern which means a lower risk or conservation dependent (Wells 1996).

Tridacinacea mantle is much larger than that of other bivalves and it had various functions such as protecting soft organs, water transport and maintenance of the symbiotic relationship with dinoflagellates. The mantle coloration and patterns vary greatly within species. The least variation in the mantle exists in *T. gigas, H. hippocus*, 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](image2.png)

![Figure 3. Shell of Mactra (left) interior view and (right) exterior view](image3.png)

![Figure 4. Shell of Macrocallista (A) interior view and (B) exterior view](image4.png)

![Figure 5. Shell of Hippopus (A) dorsal view, (B) ventral view, (C) exterior view, and (D) interior view](image5.png)
Tridacnae is a mixotrophic filter-feeder. The sources of organic carbon in the metabolites are supplied by symbionts and also by passively filtering live and dead organic compounds from the water column. Tridacnae is the only bivalve that can establish a symbiotic relationship with dinoflagellates. A larger mantle surface allows a larger symbiotic population that will perform photosynthesis and secrete larger amounts of organic compounds to the Tridacnae. The presence of 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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Abstract. Febiansi D, Rahmayanti F, Kurnia RN, Silmi MA, Dewi AK, Millaty NK, Prasetya TA, Roshitafandi DA, Sartika HW, Trijoko. 2018. Diversity species 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 May 24, 2014 and May 25, 2017. The ecological parameters recorded were 26.5°C for temperature in 2014 and 26°C for temperature in 2017, ±3.5% 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 purposive sampling method, and specimens were identified by determining the morphological characteristics of the shell. This study found 5 species of Cypraeidae family in the intertidal zone of Krakal Beach, those are Cypraea annulus, Cypraea boucheti, Cypraea moneta, Cypraea caputerpentis and Cypraea lynx. While for family Conidae 5 species were found, those are Conus coronatus, Conus eboraes, 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 a wide range of marine flora and fauna species with high abundance and diversity (Pieter et al. 2013). One of the beaches that has potential for diversity is Krakal Beach in Gunungkidul Regency, Special Region of Yogyakarta. Krakal Beach is located in Ngéstirejó Village, Tanjunsgari 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°42.3’ E110°36.9’, the length of the coastline reaches over 700 meters with a gentle and wide surface. The coastal intertidal zone is quite extensive with a variety of substrates in the form of rocks, sandstone, and corals, which are overgrown with algae, seagrass, and sponge. The variety of these substrate causes this location to have a diverse community of biota. Various biota communities that can be found are algae communities, coral reefs, fish, and various other invertebrate organisms that include thermodynamics, molluscs, crustaceans, and meiofauna. The high potential of marine resources has begun to be disrupted by human activity. This is because the intertidal area of this beach has a lot of interaction with human activities, especially tourists. Coupled with 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 sea water during tidal conditions and will become open areas when sea water recedes so the area becomes a place that is very easily exploited by tourists and the surrounding community. The habitat changes have a significant impact on the survival of flora and fauna from year to year (Satino 2003). One of the organisms that are sensitive to habitat changes is molluscs. Molluscs are a soft-bodied animal. Molluscs have three main parts of the body, i.e., legs, visceral mass, and mantle. Most molluscs secrete a strong protective pill made of calcium carbonate. Molluscs mostly live in the sea, although some species inhabit fresh water, and some snails and bare snails live on land (Campbell et al. 2010). Molluscs play an important role in the formation of 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 molluscs is required. The purpose of this research is to assess the diversity of mollusc species especially 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 molluscs 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 (8°8′42.3″ E110°36′8.9″). Materials that were used in this research included Gastropod sample particularly family Cypraeidae and Conidae, aquadest, 73% MgCl₂, 70% alcohol. Tools that were used included zip lock plastic, laminated millimeter blocks, paper labels, and digital camera.

Procedures

Sample collection

Sample collection was conducted using purposive sampling method. Sampling was done by surveying the coastal intertidal zone by walking from the eastern end to the western end of the beach. Along the way, the gastropod that was found was collected and then inserted into a zip lock plastic. The plastic is labeled using a label paper containing sampling site and sampling 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 shell and aperture of the specimens. Preservation via dry and wet preserves using MgCl₂ 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 Cypraeidae family in the intertidal zone of Krakal Beach, namely Cypraea annulus, Cypraea bouteti, Cypraea moneta, Cypraea caputserpentis and Cypraea lynx. While, for family Conidae 5 species were found, those were Conus coronatus, Conus ebraeus, Conus capitaneus, Conus botulinus, and Conus fergusoni. Cypraea is a gastropod that has an 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 or coral animals, also 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, using the peristaltic muscle and the mucus to glide. The thick muscular foot tissue can produce mucus as lubricant when moving and reduce desiccation. Like most of the 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. This allows the water current to feed oxygen towards its gills in the mantle cavity. Reproduction dioecious, the egg is generally spawned in coral caves, empty shells or similar dark places. Female gastropods do not leave the eggs after spawning in order to protect the eggs from predators until the eggs have hatched.

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

![Map showing research location at Krakal Beach, Gunungkidul, Yogyakarta, Indonesia](Image)
Cypraea annulus

The gold ring cowry, is a marine gastropod in the Cowry family, Cypraeidae. This species is usually 1.5-2 cm in length 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, also in Australia. Cypraea annulus live at 24°C–28°C, 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, intertidal zone. Distribution in Indo-Pacific (Moretzsohn 2012).

Cypraea moneta

Has a shell with knobby 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 23-28°C (Rosenberg 2010).

Cypraea caputserpentis

Rather flattened shell with thickened margins, up to 4 cm. Dorsal surface brown with numerous cream spots. Lower sides and base dark chocolate-brown. Habitat intertidal zone in rocky or shallow seagrass. Distribution in the West Indian Ocean, tropical Indo-Pacific. Cypraea caputserpentis live at 23-28°C, 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 tropical shallow 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 25-28°C 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; has aperture variably wider at base than at shoulder; and the sides of body whorl convex. Colour light, mottled pinkish-blue with brown dots and blotches. Various sized markings of brown, black or olive, spirally aligned on either side of subcentral band, either separate or fusing 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 O₂ concentration (Bouchet et al. 2015). Habitat 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 lower half. Patterned with four spiral bands of blackish squares on a white background, the lower band compressed at 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, 23.160-28.394°C 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 O₂ concentration, and 3.721-35.125 PPS for salinity (Bouchet et al. 2015). This species is found in intertidal and subtidal habitats to about 3m, on sand, among or beneath dead corals and on coral reef and limestone platforms. It feeds on polychaetes (Rockel et al. 1995). The distribution is Indo-Pacific (Richmond 1997).

Conus capitaneus

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, striae; 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. The white bands may also be crossed by closely spaced longitudinal wavy brown lines. Young individuals may lack the two white bands. Spire tesselated with alternate brown and white blotches, continuing to form band on body whorl at shoulder. Outer lip thin; aperture violet within (Beechey 2004). Environmental parameters of Conus capitaneus are 23.25-26.8 °C 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 mLL/L for water dissolved O₂ concentration (Bouchet et al. 2015).
Conus betulinus

Spire of low to moderate height, outline variably concave. Basal third of 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. Last whorl generally with spiral rows of brown markings, varying from a great number of closely set rows to 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 alternate regularly with white markings that are often absent from adapical two-thirds. Basal part of 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 littoral 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 specimens white, small specimens yellow-orange, shells 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. Aperture 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. Periostracum thin and light colored in small specimens, thick and dark brown in large specimens (McLean and Nybakken 1979). This species occurs at depths of 0 to 200 m on sandy and muddy substrates (Paredes et al. 2010; Tenorio et al. 2012).

Respiratory System. This animal respires only by branchiae, and have the head furnished with two tentacula, which bear the eyes near their summit. They have a narrow mantle, and a tube above the head, by which the water gains 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 radular tooth which is 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).
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, those are *Conus coronatus*, *Conus ebraeus*, *Conus capitaneus*, *Conus botulinus*, and *Conus fergusoni*. The abundance of gastropods in Krakal Beach is positively correlated 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, 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, Aryocepirdho, 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 fisheries potential such as fish, sea cucumbers, crabs, shrimp, and algae. Research on fish diversity in South Morotai is needed because there is no sufficient data and information about the potential of Morotai Island marine fisheries. The goal of this research was 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. 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' '40"LU and 128°15' '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 km² or 10 percent of the land area of 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 habitat for many marine species for spawning, nurseries, feeding, and foraging, especially for a number of species that have 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 and has an important role within this ecosystem, for example, as an indicator of coral condition. Almost all life stages of reef fish are directly or indirectly dependent on coral reef existence. As a coral-related fish, coral reef destruction affects 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 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 was conducted by collecting data of 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.
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 characters that distinguished it from other species. The most abundant species found in South Morotai belonged to 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 family Mullidae remains unidentified.

Discussion

The term ‘species’ in this paper 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 substratum. Scrapers take rapid, small bites, leaving only few scrapes on 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 margin of the preopercle 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 on genus Naso) located on each side of the base of the tail (Allen et al. 2003).

Balistidae shares some similarities with Monacanthidae. Both have two-part dorsal fins, which the first spine of the front part 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, which has various sizes and body shapes (Allen et al. 2003). Fishes belonging to this family have a terminal and protrusible mouth with teeth in jaws usually separate and caniniform (Westneat
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 of 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 value at market, but the size and quantity in the local market is highly restricted due to government law. To make more money, local fishermen tend to sell this fish on the black market.

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 Ocean (Shaffer and Nakamura 1989).

Lethrinidae and Lutjanidae are two families which have sloped heads. Lethrinid fishes have a maxilla which is mostly concealed below infraorbital bones, not articulating broadly, with a distal tip of premaxilla and toothless vomer and palatines (Carpenter 2001). Lutjanid fishes have a maxilla which 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 for the genera *Lethinus* and *Lutjanus* (Genisa 1999). Fishes of these two families are usually sold by Morotai fishermen 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 fin 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).

*Sphyraena* 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 its 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 water and briefly ‘run’ on the surface when frightened. 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 in order 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). The specimen observed in this research remains unidentified due to insufficient 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 potentials of Morotai Island marine fisheries. There remains a wide area of sea that needs to be explored in South Morotai alone to further determine information on the marine biodiversity of this important area.

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GUIDANCE FOR AUTHORS

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Book: Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book: Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of the wetland plants. In: TW. Schnitzer (ed) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract: Assaeed AM. 2007. Seed production and dispersal of Rhazya stricta. 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding: Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds) Toward Mount Lawu National Park; Proceeding of National Seminar and Workshop on Biodiversity Conservation to Protect and Save Geodiversity in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000 [Indonesian].

Thesis, Dissertation: Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in an Agroforestry System based on Sengon. [Dissertation] Universitas Brawijaya, Malang [Indonesian].

Information from internet: Bierman NK, Song H, Orakl 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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