INDO PAC J OCEAN LIFE
Volume 7, Number 1, June 2023
Pages: 16-26

P-ISSN: 2775-1961
E-ISSN: 2775-1953
DOI: 10.13057/oceanlife/o070102

Diplosoma simile (Ascidiacea: Didemnidae) distribution and its photosynthetic thermal stress responses from Mauritius: Implications for invasive or opportunistic behavior

ANGELA LIU YEW FAI[1], DEEPEEKA KAULLYSING[1,2], SRUTI JEETUN[1], MOUNESHWAR SOONDUR[1,2], RANJEET BHAGOOLI[1,2,3,♥]

[1]Department of Biosciences and Ocean studies, Faculty of Science and Pole of Research Excellence in Sustainable Marine Biodiversity, University of Mauritius. Réduit 80837, Mauritius. Tel.: +230-4037916, ♥email: r.bhagooli@uom.ac.mu
[2]The Biodiversity and Environment Institute. Réduit, Republic of Mauritius
[3]The Society of Biology. Réduit, Republic of Mauritius

Manuscript received: 9 September 2022. Revision accepted: 17 October 2022.

Abstract. *Fai ALY, Kaullysing D, Jeetun S, Soondur M, Bhagooli R. 2023.* Diplosoma simile *(Ascidiacea: Didemnidae) distribution and its photosynthetic thermal stress responses from Mauritius: Implications for invasive or opportunistic behavior. Indo Pac J Ocean Life 7: 16-26.* This study aimed to investigate the morphological identity of the ascidian *Diplosoma*, its distribution, experimental thermal stress responses, and its characteristics of being potentially invasive or opportunistic in Mauritian waters. The *Diplosoma* species were anesthetized in menthol, fixed, and preserved in a formalin-seawater solution. Isolated zooids and embryos were examined under a microscope for distinct morphological characteristics, which revealed their identity as *D. simile* (Sluiter, 1909). The *D. simile* percentage cover studied at seven sites in 2019 around Mauritius tended to be high at the near-reef zone, with a high percentage of dead coral cover per 0.5 m^2. At Flic en Flac, long-term observations in 2008, 2010, 2016, and 2019 indicated a significant decrease and increased from 61.25±3.31 to 6.15±0.60 % and 0.08±0.05 to 2.50±090% for the percentage covers of live coral and *D. simile*, respectively. The *D. simile* was recorded on five coral species, and its percentage occurrence increased from 2008 to 2019 in *Acropora muricata*, *A. cytherea*, *Pocillopora damicornis*, and *Montipora aequituberculata* with the highest levels occurring in *Acropora* while *Porites lutea* was not found to be covered by *D. simile* during the study. Visual observations from the thermal exposures at 27ºC, 30ºC, and 33ºC during 19 hrs revealed that *D. simile* was more affected by increasing temperature and exposure time. Using four observed conditions of normal, bleached/paled, tissue sloughing, and mortality, *D. simile* suffered from only bleaching/paling at 33ºC at 19 hrs exposure. Variable visual responses were noted for corals, with *P. lutea* appearing normal at all trials while *A. cytherea* was suffering from mortality both at 30ºC and 33ºC at 19 hrs exposure. The effective quantum yield (ΦPSII), measured using a Diving-PAM, declined significantly at 33ºC treatments at 6 and 19 hrs of exposure. *D. simile* generally tended to be more thermally tolerant than corals like *P. lutea*, *P. damicornis*, *M. aequituberculata*, *A. muricata*, *A, cytherea*, though the ΦPSII thermal responses were variable among the tested corals. When considering the reported behavioral characteristics of *D. simile*, namely the lack of broad dispersal range and wide environmental tolerance, it is tempting to deduce that the species is potentially invasive. However, in this study, *D. simile*'s significant distribution on dead corals, its presence in zones of high anthropogenic activities, and its relatively more robust thermal stress responses than corals colonized suggest an opportunistic behavior.

Keywords: Ascidian, corals, *Diplosoma simile*, distribution, diving-PAM, Mauritius, opportunistic behavior, thermal tolerance

INTRODUCTION

Ascidians emerge from the kingdom Animalia and belong to the class Ascidiacea, which is considered the largest class of the Subphylum Tunicata (Phylum Chordata) (Hirose and Hirose 2013). These organisms have been subject to many studies regarding their bioactive compounds and broad physico-chemical tolerance, which provides for their complex behavioral patterns (Hirose and Hirose 2013; Hirose and Nozawa 2010). Furthermore, ascidians have further been reported to display characteristics that significantly increase risks of invasion outbreak, that is, their globally widespread distributions and broad environmental tolerances (Hirose and Hirose 2013; Akram et al. 2015; Koplovitz et al. 2015; Villalobos et al. 2017).

However, knowing that these organisms have extensive dispersal ranges and are easily overlooked, it would not be surprising that many occurring species are left uncharted (Goodbody 2000; Hirose et al. 2012; Villalobos et al. 2017). Around Mauritius Island, for instance, only 32 species have been reported around the Island (Table 1) with 2 species being endemic, namely: *Pseudodistoma mauritiana* and *Polycarpa nigricans*. However, the discovery of another ascidian species with high morphological similarity to the genus *Diplosoma* around Mauritius Island has led to considering the high possibility of a documentation gap regarding ascidians. With increasing evidence of coral vulnerability worldwide, it is crucial to understand and address any further potential threats posed by ascidians (Carilli et al. 2010; Pandolfi et al. 2011; Bhagooli and Taleb-Hossenkhan 2012).

Coral bleaching and reef degradation rates are escalating worldwide and locally, mostly due to increasing global temperatures and exposure to anthropogenic-related stressors (Mattan-Moorgawa et al. 2012; Louis et al. 2016; 2020; Hughes et al. 2017; Bhagooli and Kaullysing 2019; Bhagooli et al. 2021a) and some reefs are predicted to be "locally extinct" (Sheppard 2003; Bhagooli and Sheppard 2012). It is generally considered that corals bleach, whereby they lose their zooxanthellae and/or the zooxanthellae photosynthetic pigments (Glynn 1993), and this disassociation between the coral animal and its dinoflagellate zooxanthellae occurs in most cases due to the zooxanthellae photosynthetic dysfunctioning under thermal and light stressors (Iglesias-Prieto et al. 1992; Warner et al. 1996; 1999; Jones and Hoegh-Guldberg 1998; Bhagooli 2013). Knowing that such phenomena are indicators of decreasing coral reef health status, the latter is more at risk of being subject to our competition by reef-associated organisms, which would further reduce chances of coral recovery (Mattan-Moorgawa et al. 2018; Bhagooli and Kaullysing 2019). Moreover, the urgency of in-depth studies on ascidian-related threats is even more pressing given that ascidian-induced-bioinvasion has been increasingly reported worldwide, especially following mass thermal-induced coral bleaching (Dijkstra et al. 2007; Tebbett et al. 2019). It is noteworthy that several local studies on photo-physiological responses to thermal and other stressors have been conducted on scleractinian corals (Mattan-Moorgawa et al. 2015, 2018; Kaullysing et al. 2016; Louis et al. 2020; Bhagooli et al. 2021b), seaweeds (Narain et al. 2023; Bhagooli et al. 2021c) and seagrasses (Bhagooli et al. 2021c), microalgae (Sadally et al. 2016; Soondur et al. 2021, 2022) ascidians thermal stress responses are yet to be investigated.

Several authors further mentioned the high invasiveness of the ascidian genus *Diplosoma* (Sommer et al. 2010; Rodriguez-Martinez et al. 2012; Hirose et al. 2012), some reporting it to smother and kill live *Acropora* spp., which are the preferred host species, following their settlement on areas of dead corals (Littler and Littler 1995; Li et al. 2016). To avoid any confusion arising from the controversial and often misleading nature of the similar terms "invasive" and "opportunistic" species, the present study referred to Colautti and MacIsaac (2004) definition of invasive species and Whitlatch and Zajac's (1985) definition of opportunism.

The present study aimed at investigating the extent of the ascidian species' invasive potential around Mauritius Island. The objectives included: (i) Morphological characterisation of the ascidian species; (ii) Assessment of the distribution of the ascidian around Mauritius relative to the status of the coral health; (iii) Evaluation of the thermally-induced photosynthetic stress response of the ascidian species relative to some of its coral hosts; and (iv) Inference from these investigations on the potential invasive or opportunistic behavior of the ascidian in the Mauritian context.

MATERIALS AND METHODS

Field surveys

Field surveys were conducted at Flic Flac to determine the long-term percentage cover of live coral and *Diplosoma* at Flic en Flac in October/November 2008, 2010, 2016, and 2019. Four stations along the reef flat at Flic en Flac were surveyed using five 1 m x 1 m quadrats per station. *Diplosoma* on five coral species, namely *Acropora muricata*, *A. cytherea*, *Pocillopora damicornis*, *Montipora aequituberculata*, and *Porites lutea* by observing 20, 10, 15, 5, and 5 coral colonies during each year's survey and expressed as percentage occurrence out of the observed colonies.

The percentage ascidian cover (%AC), dead coral cover (%DCC), and healthy coral cover (%HCC) were determined through field surveys in the summer between October 2019 and March 2020 at several sites around Mauritius. Following Leujak and Ormond (2007), the survey was performed at 7 selected sites around Mauritius Island: Balaclava, Trou aux Biches, Flic en Flac, Bel Ombre, Blue Bay Marine Park, Belle Mare, and Flat Island (Figure 1). Three zones were also selected at each site (based on their respective distance to the shore), namely: near-coast (20 m), lagoon (60 m), ad near-reef (150 m) zones. At each zone, a 20 m transect line was laid parallel to the shoreline, and a 50 cm x 50 cm PVC-made quadrat was photographed perpendicularly to the substratum at 0.5 m intervals along the line.

Microscopic observations

The methods described are based on Lafargue and Wahl (1987) and Marks (1996) protocols for examination under an optical microscope. The ascidian species samples were collected at Flic en Flac while still attached to their substrate. They were maintained in seawater-filled Ziploc® bags until reaching the University of Mauritius. First, the colonies were placed in a glass container in an aquarium with running water and left for 1 day to promote zooid relaxation. Next, 3.5 g/L menthol crystals were added and left unstirred to cause the least mechanical stress until the colonies were fully anesthetized (approximately 2 hrs). Next, the container was left in a freezer for 3hrs until the ice began to form before transferring to a solution of 10% formalin-seawater for 24 hrs. Finally, the colonies were transferred to a 7% formalin-seawater solution and kept for 4 weeks before microscopic observation.

Masson's acid Hemalum staining agent was prepared following Lafargue and Wahl (1987). First, 5 g of Aluminium potassium sulfate KAl(SO4)2 was dissolved in 100 mL of distilled water. Next, 0.2 g of Hematoxylin was added to the boiling solution and left to boil for a few more seconds before being cooled to room temperature. The solution was filtered and 2% acetic acid was added until a color change from dark purple to red was noticed. The staining agent was kept in the absence of light and would be valid a month post-preparation.

Fixed colony samples were thoroughly rinsed in distilled water before adding Masson's acid hemalum. First, the translucent colonial ascidian was immersed in the staining agent until the desired level of stain was achieved (~5 minutes). Next, the sample was rinsed with distilled water to remove all excess staining agents. Next, individual zooids and embryos were isolated from the colony and examined for morphological characteristics under an inverted microscope. The presence of calcareous spicule was further tested by immersing the colonial ascidian in 4% Hydrochloric acid (HCl), which would decalcify the calcareous content hence leaving small bubbles to form on the surface of the colony.

Table 1. List of ascidian species and their respective taxonomic classifications reported around Mauritius and other distribution locations in the Indo-West-Pacific Region

Suborder, family	Species name	Location	References
Aplousobranchia			
Synoicidae	*Pseudodistoma mauritiana**	Flic en Flac (Mauritius)	Vasseur (1967)
Didemnidae	*Didemnum candidum*	Australia, Malaysia, Indian Ocean, Trou aux Biches (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	D. molle	Ile D'Ambre (Mauritius)	Mattan-Moorgawa et al. (2015)
	Atriolum robustum	Mayotte, Mauritius, Indian ocean	Monniot and Monniot (2001); Vasseur (1967)
	Trididemnum sansibaricum	Zanzibar, Mozambique, Flic en flac (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	T. natalense	Australia, South Africa, Trou d'eau Douce (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	T. neridionale	Trou aux Biches (Mauritius)	Monniot and Monniot (2001); Vasseur (1967)
	Diplosoma multifidum	Mauritius, India, Indonesia	Millar (1975)
Polycitoridae	*Eudistoma rhodopyge*	South Africa, South-west of Madagascar, Ilot Barkly (Mauritius)	Monniot and Monniot (2006); Vasseur (1967)
	E. mobiusi	Mozambique, Tanganyika, Zanzibar, South of Madagascar, Pointe d'Esny (Mauritius)	Monniot and Monniot (2006); Vasseur (1967)
	E. atrum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	E. reginum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Polycitorella pallida	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Cystodytes solitus	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	C. dellechiajei	Mauritius, Philippines, Australia	Millar (1975)
Clavelinidae	*Clavelina enormis*	Mauritius	Millar (1975)
Polyclinidae	*Aplidium convergens*	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	Polyclinum constellatum	Mauritius	Monniot and Monniot (2006); Vasseur (1967)
	P. festum	Mauritius	Millar (1975)
Phlebobranchia			
Ascidiidae	*Ascidia sydneiensis*	South Africa, Australia, Flic en Flac (Mauritius)	Palomino-Alvarez et al. (2019); Vasseur (1967)
	A. munda	North Australia, Flic en Flac (Mauritius)	Palomino-Alvarez et al, (2019); Vasseur (1967)
Stolidobranchia			
Styelidae	*Symplegma viride*	East and South Africa, Madagascar, Australia, Port Louis (Mauritius)	Vasseur (1967); Monniot and Monniot (2007)
	Amphicarpa inhacae	Mozambique, Ilot Barkly (Mauritius)	Vasseur (1967)
	Polyandrocarpa anguinea	South Africa, Port Louis (Mauritius)	Vasseur (1967); Monniot and Monniot (1994)
	P. tincta	Mozambique, Port Louis (Mauritius)	Vasseur (1967)
	*Polycarpa nigricans**	Cassis (Mauritius)	Vasseur (1967); Monniot (2002)
	P. madagascariensis	Mauritius, Mozambique, Madagascar	Monniot (2002)
	Cnemidocarpa madagascariensis	South-West Madagascar, Flic en Flac (Mauritius)	Vasseur (1967); Monniot (2002)
	Styela partita	Port Louis (Mauritius)	Vasseur (1967)
Pyuridae	*Pyura pulla*	Trou d'eau Douce (Mauritius)	Vasseur (1967)
	P. momus	Port Louis (Mauritius)	Vasseur (1967)
	Microcosmus exasperatus	Port Louis (Mauritius)	Vasseur (1967)
	Cynthia pallida	Mauritius, Jamaica, western pacific	Monniot (2002)
Molgulidae	*Molgula natalensis*	Ilot Barkly (Mauritius)	Vasseur (1967)

Note: **P. mauritiana* and *P. nigricans* are endemic ascidian species to Mauritius Island

Thermal stress experiment

Forty-five fragments, nine per each of the 5 colonies, of the ascidian *Diplosoma* on dead coral part and five coral species, namely *P. lutea*, *M. aequituberculata*, *Acropora muricata*, and *A. cytherea* were collected at Flic en Flac on 17th January 2020. Nine aquaria were set up and arranged in 3x3 order and labeled as 27°C (control), 30°C and 33°C in dim light conditions. Oxygen pumps were provided for each aquarium supplied with 5 fragments per species. All the tanks were further equipped with thermometers and representative tanks with Hobo temperature and light data loggers to monitor temperature and light levels.

Chlorophyll-a fluorescence measurement

The effective quantum yield at photosystem II (ΦPSII) was determined using a Diving-PAM (Pulse amplitude Modulator). A saturating pulse of 4000 µmol quanta m^{-2} s^{-1} and a weak light emission of <1 µmol quanta m^{-2} s^{-1} were used to measure the maximum (Fm) and minimum (Ft) fluorescence, respectively. ΦPSII was then calculated as (Fm-Ft)/ Fm (ΔF/ Fm) (Genty et al. 1989).

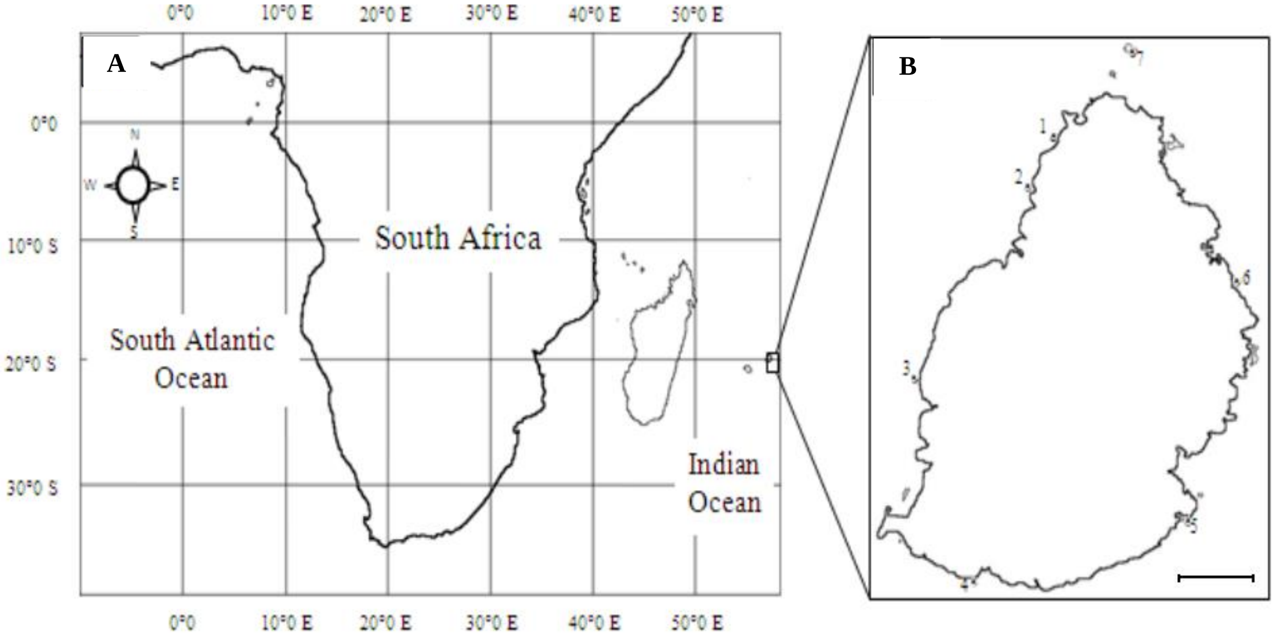


Figure 1. A. Map of the Indian Ocean showing the location of Mauritius (Scale = 10 km); B. Map of the study area. The numbers represent the snorkeling sites around Mauritius Island. 1. Balaclava, 2. Trou aux Biches, 3. Flic en Flac, 4. Bel Ombre, 5. Blue Bay, 6. Bellemare, 7. Flat Island

Ascidians and coral samples were left to shortly acclimatize for 3 hrs in seawater at room temperature (~27°C) prior to exposures at 27°C, 30°C and 33°C for a duration of 19 hrs. It is noteworthy that previous coral thermal stress studies have employed temperatures of 33ºC (Ralph et al. 2001) and 34, 36, and 38ºC (Warner et al. 1996) over periods of hours to days. ΦPSII was measured at times T_0, T_3, T_6, and T_{19} for initial 3 hrs, 6 hrs, and 19 hrs exposures, respectively, for all temperature trials. ΦPSII is one of the four main chlorophyll fluorescence parameters used in many studies investigating the photophysiology of sea plants and photosynthetic symbiotic marine invertebrates (Bhagooli et al. 2021d).

Data analysis

Using the software package PASW Statistics, the Shapiro-Wilk Normality test was used to assess the data distribution pattern. Correlations between variables were investigated through Spearman Rho's non-parametric test since none of the variables were normal. Principal Correspondence Analysis (PCA) was further performed on data regarding the species' distribution to investigate any correlation between percentage ascidian cover (%AC) and their location around the Island. Kruskal-Wallis and comparative Wilcoxon rank-sum test were computed to compare the percentage of ascidian cover relative to the percentage of dead coral cover (%DCC), percentage of healthy coral cover (%HCC), and distance from the shore. Similar statistical tests were run to compare the species ΦPSII relative to increasing temperature and exposure time.

RESULTS AND DISCUSSION

Ascidian morphological features: External features

The ascidian colonies were mostly found on *Acropora* sp., distributed on the underside of the coral structures already covered by seaweeds (indicating that these were dead before settlement). Although well hidden, they were spotted by their bright green color, which is due to the presence of *Prochloron*, a cyanobacterial symbiont having a dominant concentration of chlorophyll-a (Hirose et al. 2004; Hirose et al. 2012; Stalin et al. 2016). The 1mm-thick ascidian colonies varied in terms of colony size per coral structure. When exposed to a bright light source, they seemed to reflect as bright blue spots (Figure 2).

Ascidian morphological features: Internal features

No bubbles of gas were spotted on the surface of the colony sample upon exposure to (HCl) which implied that no spicules were present in the colony. Zooids were about 700 µm long (Figure 3) and were evenly distributed throughout the colony on the upper part of the tunic. Four rows of stigmata were also noticed, and the stigmal arrangement of each of the four rows from top to bottom formed the pattern "6-6-6-5". The species also lacked an atrial aperture in its thorax while having a two-lobed testis in its lower abdomen region. Furthermore, the ascidian's retractor muscle separated almost directly from under its thorax. Embryos of various sizes were also isolated (with the largest ones being 500-600 µm long), and only their sensory vesicle was noticed. Contrary to zooids, present mostly in the upper-tunic region, the embryos were unevenly present on the underside of the tunic (i.e., closest to the coral substrate).

Field surveys of live corals and ascidians at Flic en Flac

Field surveys conducted at Flic Flac for the years 2008, 2010, 2016, and 2019 revealed a significant decrease and increase from 61.25±3.31 to 6.15±0.60 % and 0.08±0.05 to 2.50±090% for the percentage covers of live coral and the ascidian species, respectively (Figure 4A). The survey on the occurrence of the latter ascidian on five coral species indicated that the percentage occurrence of the ascidian increased from 2008 to 2019 in *A. muricata*, *A. cytherea*, *P. damicornis*, and *M. aequituberculata* with the highest levels found in both the *Acropora* while *P. lutea* was not found to be covered by the ascidian during the study (Figure 4B). The *D. simile* occurred in four out of the five studied corals (Figure 5).

D. simile distribution pattern

Principal Correspondence Analysis (PCA) (Figure 6) showed the highest % ascidian cover at near-reef zones as well as at sites known to harbor high anthropogenic activity, namely: Flat Island, Flic en Flac, and Balaclava (in decreasing order of correlation strength).

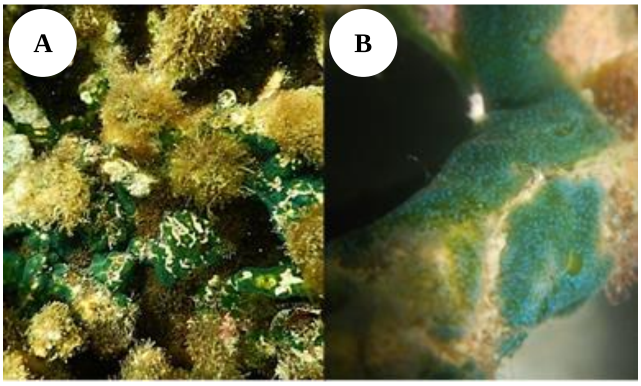


Figure 2. Ascidian species are growing on *Acropora* at Flic en Flac; A. Sample species in situ before collection; B. Sample species in a laboratory, showing natural bright green color after exposure to a camera flash

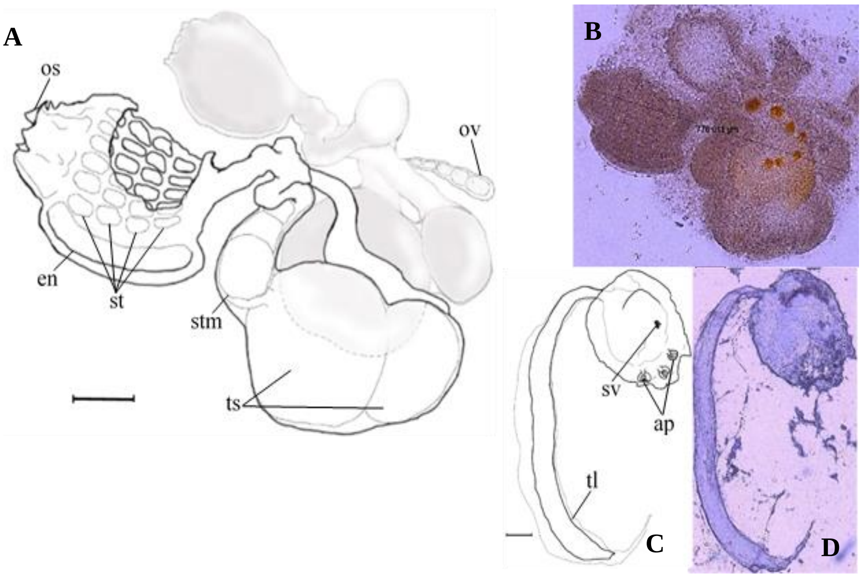
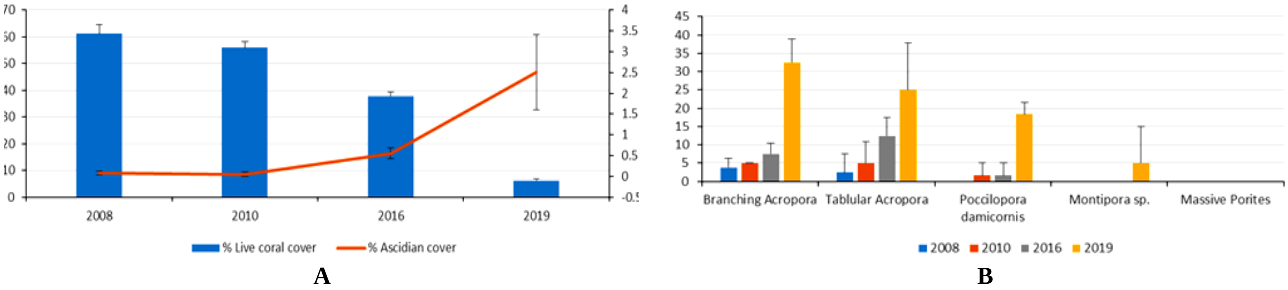


Figure 3. Isolated ascidian species at various life stages (Scale = 100 µm). A. Line drawing of an adult zooid; with one of the two individuals being shaded to facilitate morphological differentiation; B. Adult zooid (778 µm long); C. Line drawing of embryo; D. Embryo (459 µm long); (st) Stigmata; (os) Oral siphon; (en) Endostyle; (stm) Stomach; (ov), Ovary; (tl) Tail; (ad) Adhesive papillae; (sv) Sensory vesicle



A

B

Figure 4. Percentage live coral and *D. simile* cover (A) and percentage occurrence of *D. simile* on coral species including branching *Acropora* (*A. muricata*), tabular *Acropora* (*A. cytherea*), cauliflower coral (*Pocillopora damicornis*), foliose coral (*Montipora aequituberculata*), and massive coral (*Porites lutea*) at Flic-en-Flac in years 2008, 2010, 2016 and 2019 (B)

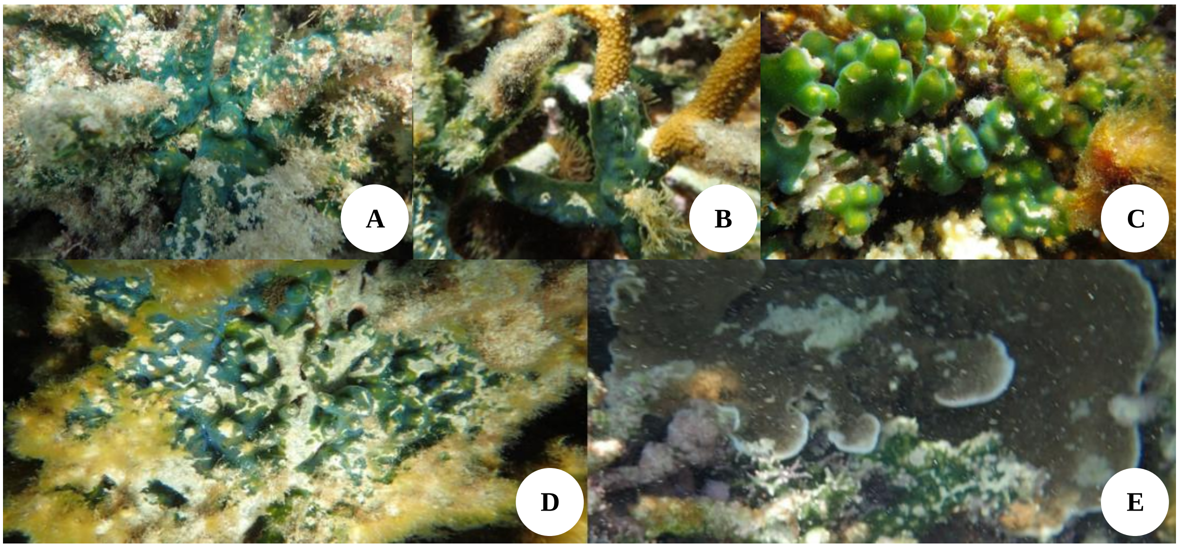


Figure 5. *D. simile* occupying completely dead corals and/or dead parts of live corals. A. Completely dead *Acropora muricata*; B. Live *A. muricata*; C. *Pocillopora damicornis*; D. Completely dead *A. cytherea*; and E. *Montipora aequituberculata*

The highest mean of percentage ascidian cover (%AC) was recorded at Flat Island (10.34 ± 1.16) and the lowest one at Blue Bay Marine Park (0.01 ± 0.01). The correlation between %AC and distance from shore yielded an R-value (+0.478) and a Kruskal-Wallis ($P< 0.001$), which indicated the presence of a significant correlation between both variables. PCA further revealed an interesting relationship between %AC, %DCC and %HCC, whereby %AC is positively related to %DCC and negatively related to %HCC. These relationships were confirmed with yielded R-values (+0.697) and (-0.359) for both tests, respectively as well as Kruskal-Wallis ($P<0.05$) in both cases. Such a finding suggests a highly significant correlation between %AC/%DCC and %AC/%HCC.

Effective quantum yield responses to thermal stress

The three thermal trials had temperature means and light intensity maximums of 26.8±0.5°C and 1000.0lux; 30.4±0.3ºC and 882.6lux; and 33.7±0.6C and 958lux, respectively (Figure 7). The effective quantum yield at Photosystem II (ΦPSII) of *D. simile* significantly declined at 3 hr, though slightly, and at 19 hr in 33ºC trials (Figure 8A). The ΦPSII responses to thermal stress were variable among corals tested (Figure 8B-F), with *Acropora* (Figure 8E, F) being the most susceptible and *P. lutea* (Figure 8B) the least susceptible to thermal stress. Decreases in ΦPSII in *D. simile* were lower at 30ºC compared to *A. muricata*, *M. aequituberculata*, and *P. lutea* after 19hrs (Figure 9A). Generally, *D. simile* appeared to be more resistant than the test corals to thermal stress exposure in this study.

Thermal stress responses: Visual observations

The colonies' external color remained constant during the first 6hrs of the experiment at all temperatures; Only those maintained at 33°C showed a slight decrease in the level of blue reflection. However, at time T=19 hrs, ascidians were surprisingly discovered to turn translucent grey from a bright green color. The colonies' surface areas having changed color were also estimated to be proportional to the temperature they were exposed to. Ascidians at 27°C had a minimal <5% while those at 33°C had about 50% change in color. When looking at four observed conditions of normal, bleached/paled, tissue sloughing, and mortality, *D. simile* suffered from only bleaching/paling at 33ºC at 19 hrs exposure (Figure 9B). The corals had variable visual responses, with *P. lutea* appearing normal at all trials while *A. cytherea* was suffering from mortality at 30ºC and 33ºC at 19 hrs exposure (Figure 9B).

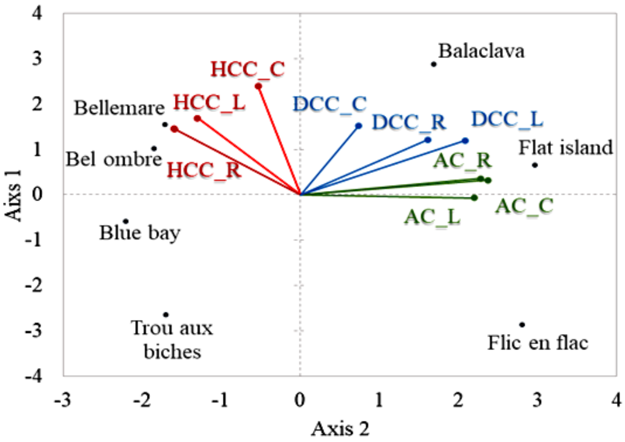
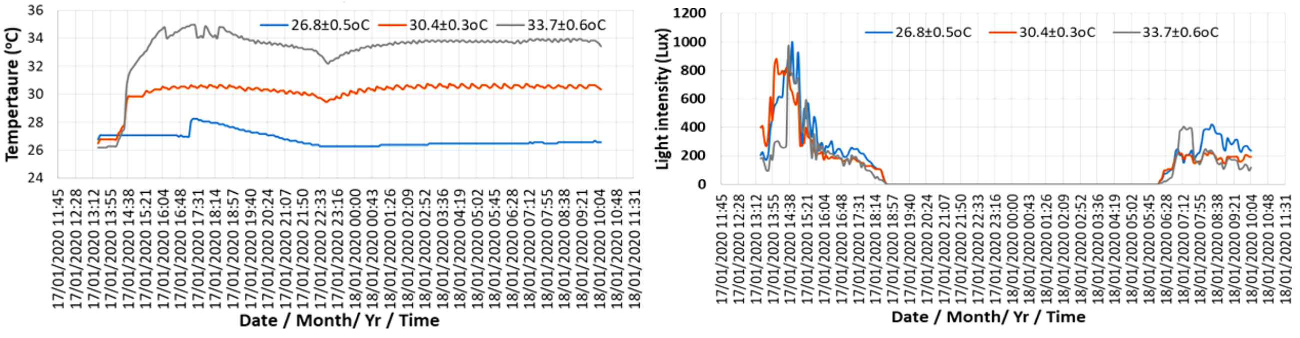
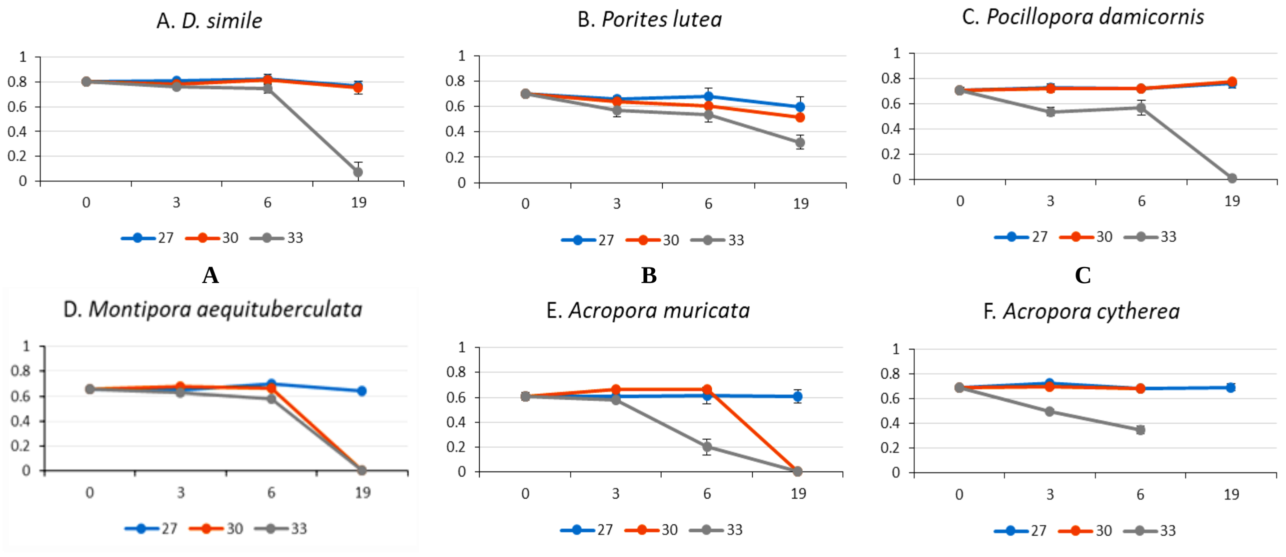


Figure 6. PCA indicates the relationships between variables like%AC, %DCC, %HCC, distance from shore, and location around the Island. (AC_C: %AC at coast, AC_L: %AC at lagoon, AC_R: %AC at reef)



A B

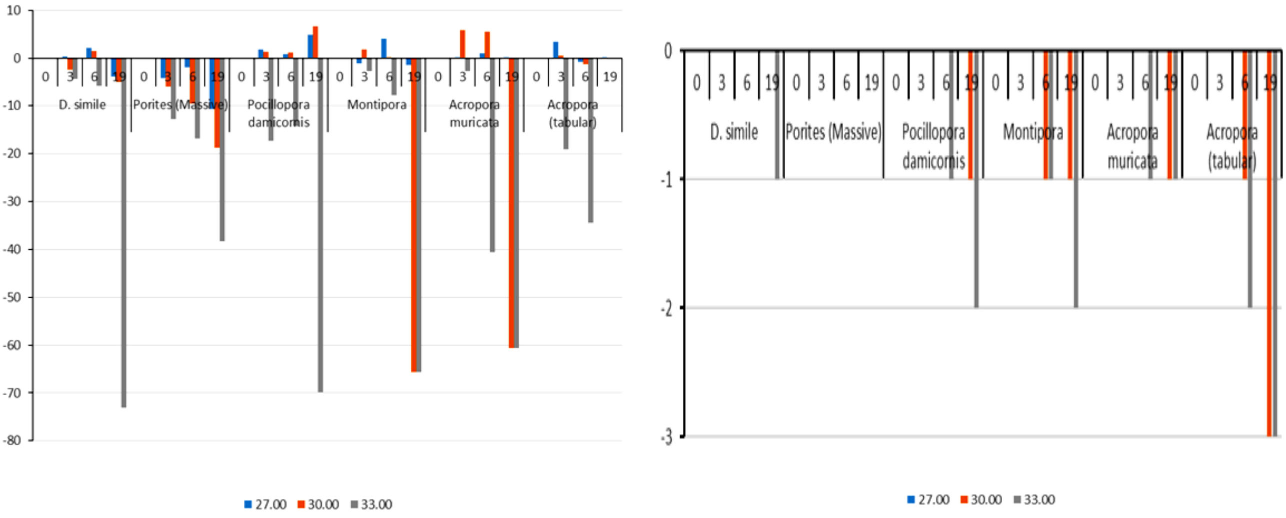
Figure 7. Temperature (A) and light exposure (B) during experimental trials



A B C

D E F

Figure 8. Quantum yield at PSII for the ascidian *D. simile* (A), and corals *Porites lutea* (B), *Pocillopora damicornis* (C), *Montipora aequituberculata* (D), *Acropora muricata* (E) and *Acropora cytherea* (F) exposed to temperature trials of 27, 30 and 33ºC at time 0, 3, 6 and 19 hrs of exposure



A B

Figure 9. A. Percentage change in mean effective quantum yield at PSII and B. Visual health conditions of treated specimens at 27, 30, and 33ºC at times 0, 3, 6, and 19 hrs. Healthy-looking / Normal: 0; Bleached: -1; Tissue Sloughing: -2; Dead: -3

Discussion

Diplosoma *species identification*

The species' viviparous embedding in a common sheet-like colonial tunic suggested its belonging to the family Didemnidae (Class Ascidiacea; Order Aplousobranchia) (Hirose and Hirose 2013; Goodbody 2000). The further noticed segregated individual arrangement (with zooids on top and embryos at the bottom of the tunic), as well as the presence of 4 rows of stigmata and the lack of atrial aperture, were further features that confirmed its belonging to this genus (Kott 2001; Oka et al. 2005; Stalin et al. 2016). Despite the mention of intra-species polymorphism by Lafargue and Wahl (1987) as being a site-specific adaptive response to the dynamic changes present in the tunicates' environment, which could hinder correct species-level identification (based on morphological features only), accurate identification to genus-level was nonetheless mentioned possible (Lafargue and Wahl 1987; Oka et al. 2005; Koplovitz et al. 2015).

The species, for instance, showed very similar characteristics to the genera *Diplosoma* with its external bright green color and the lack of calcareous spicules mentioned as the most determining features (Kott 1982; Lafargue and Wahl 1987; Hirose and Hirose 2013). The presence of 4 rows of stigmata and the lack of atrial aperture were further features that confirmed its belonging to this genus (Kott 2001; Oka et al. 2005; Hirose and Hirose 2013; Stalin et al. 2016). Despite the high risk of wrong identification triggered by the concept of intra-species polymorphism, the species nonetheless showed further characteristics that seemed to point the direction of identification towards a certain species.

The species stigmata pattern being noticed to follow the arrangement 6-6-6-5 and the presence of a two-lobed testis considerably narrowed down the list of matching species to two, namely: *Diplosoma simile* (Sluiter, 1909) (Sluiter 1909) and *Diplosoma virens* (Hartmeyer, 1909) (Hartmeyer 1909-1911; Koplovitz et al. 2015; Hirose and Nozawa 2010; Goodbody 2000; Oka and Hirose 2008). Despite their outstanding resemblance, authors agreed on some morphological differences, which included the length of the species' embryo, colonies' thickness, and the point of separation of the zooid's retractor muscles (Kott 2001; Hirose and Hirose 2009; Hirose et al. 2009; Hirose and Nozawa 2010; Koplovitz et al. 2015). The *D. virens* is known to have a thick colony (2-5 mm) and harbor a large embryo of approximately 1.5 mm in length, having a small tail (in proportion to the head size) that can wrap only halfway around to the top of the head (Kott 2001; Koplovitz et al. 2015). The *D. simile*, on the other hand, is known to have thinner colonies (1-2 mm) with a smaller embryo (about 800 µm long) with a rather long tail that wraps to the top of its head (Kott 2001). Furthermore, the position where the retractor muscle separates from the thorax tends to be the determining feature between the two species, while in *D. simile*, the muscle emerges directly from underneath the thorax in a T-like shape, in *D. virens*, the muscle separates at a much lower position halfway down the zooid's esophageal neck in an N-like shape (Hirose and Hirose 2009; Hirose et al. 2009). Since the present species' colony was about 1-2 mm thick, had a small embryo (778 µm), which tail wrapped around the top of the head, and had a T-shaped retractor muscle, it is concluded that the studied species is a *D. simile*.

Diplosoma simile *thermal tolerance*

In the last decades, authors agreed on the negative impact of increasing temperatures on marine ecosystems; coral reefs in particular, which are susceptible to undergoing mass bleaching (Bruno et al. 2007; Bhagooli 2009; Barshis et al. 2013; Obura et al. 2017). This phenomenon was proved right after mass bleaching occurred as a result of the ENSO event in 2016, causing up to 60% coral bleaching around Mauritius Island (Obura et al. 2017). The most affected reef-building coral genus being, *Acropora*, which comprised 80% of the coral bleaching, was explained by their low thermal tolerance (~1-2°C higher than normal; in Mauritius being 27-28°C) (Middlebrook et al. 2008; Schoepf et al. 2015). However, the present study showed that *D. simile* has optimal photosynthetic efficiency at temperatures far exceeding the thermal threshold of *Acropora* corals.

Photosymbiotic *Prochloron* sp. (known to be thermo-resistant) was, for instance, recorded to display higher ΦPSII at high temperatures varying between 20°C-40°C when tested in vitro (Dionisio-sese et al. 2001). The reason for the recorded host ascidian bleaching within the optimal temperatures for *Prochloron* sp., however, remains uncertain; either being the result of the stressed *D. simile* colony releasing *Prochloron* sp. in the same way, corals bleach as an adaptive response or due to the photosymbiont itself moving out of the stressed organism. Since the extent of their interdependence is still not fully understood, it is safe not to attribute anything to the reason for ascidian bleaching with rising temperature.

However, the overall liability of the thermal stress experiment in establishing *D. simile* invasiveness remains unclear since it did not encompass the array of factors that affect a species' thermal resistance and hence cannot be used to measure the true extent of the species' thermal tolerance accurately. Time factor certainly was one of them, as indicated in this study. Furthermore, this study did not consider the "environmental condition," "photosymbiotic ultraviolet resistance," or "species' adaptive mechanism" factors that are also known to influence overall fitness (Naranjo et al. 1996; Grimsditch and Salm 2006; Hirose et al. 2004; Pineda et al. 2012; Sensui and Hirose 2018). Bussapakorn et al. (2018) revealed, for instance, the evolution of coral response to thermal stress bringing the multi-dimensional aspect associated with a single species thermo-resistance, the latter being affected by a variety of site-specific conditions (e.g., nutrient load, depth, wave action intensity) as well as regional and international conditions (e.g., ENSO events, earthquake).

The Diplosoma simile *behavior inferred from its distribution pattern*

With the records of mass coral bleaching due to the recent ENSO events, as well as the previous mentions of *D.*

simile characteristics, it can be deduced that the latter species represents a potential threat to Mauritian coral reefs. (Vargas-Ángel et al. 2009; Kremer and Rocha 2011; Hirose and Hirose 2013; Koplovitz et al. 2015; Kaullysing et al. 2016; Obura et al. 2017; Gudka et al. 2018). The notion of invasion, however, also includes an aspect of geographical belonging, whereby a species is unlikely to develop invasive behavior in its native environment (due to ecological integration). Since there is no record of *D. simile* being native to Mauritius, in addition to the records stating its restricted occurrence to shallow waters, it is highly possible that this species was introduced to the Mauritian waters through anthropogenic means (Koplovitz et al. 2015).

The possibility of an introduction by marine transport can be investigated through the species' distribution pattern. It was seen that the highest species occurrence happened in the vicinity of ports or regions with high boat activity (i.e., Balaclava, Flat Island, and Flic en Flac). Furthermore, the region with the lowest species occurrence also seemed to harbor some level of boat activity; Belle Mare and Blue Bay Marine Park are renowned snorkeling spots. An interesting contrast between Balaclava and Blue Bay Marine Parks also seemed to relate to %AC and anthropogenic level. It was further found that among the top 5 ranked beaches in Mauritius, 4 harbors the highest %AC around the Island. Moreover, *D. simile* seemed to occur away from the shore, in regions where most boat activities normally involve recreational purposes. These findings reinforce the probability of an introduction by boat to the Mauritian waters as well as a potential link between the *D. simile* outbreak and the level of anthropogenic activities.

Despite their "higher thermal tolerance," "higher reported resilience," and "resistance to anthropogenic stressors" (contrary to coral communities), the labeling of *D. simile* as an invasive threat to Mauritian reef-building corals remains unclear (Jimenez et al. 2012; Brown et al. 2019). It is to be highlighted that the nature of the reported species' invasive behavior in some countries does not necessarily imply the same pattern to be found in the Mauritian context due to differences in distribution-influencing conditions (Shenkar and Swalla 2011; Koplovitz et al. 2015). Furthermore, the notions of "opportunistic," "invasive," and "dominant" species, often being mixed up in relevant literature, also brought uncertainty to the question, which hence required reference to specific authors in determining *D. simile* behavior. Therefore, since the ascidian was only reported on dead corals, with no means of quantifying its rate of introduction, and since it is still at an early introduction stage (judging by its small population % cover around the Island), the species does not fit Colautti and MacIsaac (2004) description of an invasive species. The *D. simile* seemed to adopt the behavior mentioned by Whitlatch and Zajac (1985) as opportunistic, hence being more apparent to an "opportunistic species" in the Mauritian context.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Biosciences and Ocean Studies, Faculty of Science, and the Pole of Research Excellence in Sustainable Marine Biodiversity, University of Mauritius, Mauritius for logistic support. We are also grateful to the then Ministry of Ocean Economy, Marine Resources, Fisheries and Shipping, Republic of Mauritius, for granting permit for coral reefs studies and sample collection. RB and DK acknowledge the funding (T0721 and T0105) from the Higher Education Commission (HEC) to work on corals and coral reefs studies. The authors are grateful to the reviewers for insightful comments that improved the manuscript.

REFERENCES

Akram AS, Arshan MLK, Jaffar Ali HA. 2015. Occurrence of alien ascidians in V.O Chidambaranar Port, Thoothukudi. Intl J Sci Human 1 (1): 209-216.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR. 2013. Genomic basis for coral resilience to climate change. Proc Natl Acad Sci USA 110 (4): 1387-1392. DOI: 10.1073/pnas.1210224110.

Bhagooli R, Kaullysing D. 2019. Seas of Mauritius. World Seas: An environmental evaluation 253-277: DOI: 10.1016/b978-0-08-100853-9.00016-6.

Bhagooli R, Mattan-Moorgawa S, Kaullysing D, Chumun PK, Klaus R, Munbodhe V. 2021a. Status and sustainability of reefs and shorelines of the Republic of Mauritius (2021). In: Gunputh RP (eds). Sustainable Development Goals. Chapter 8, Star Publications Pvt. Ltd., New Delhi, India.

Bhagooli R, Mattan-Moorgawa S, Kaullysing D, Louis YD, Gopeechund A, Ramah S, Soondur M, Pilly SS, Beesoo R, Wijayawanti DP, Bachok ZB, Monrás VC, Casareto BE, Suzuki Y, Baker AC. 2021d. Chlorophyll fluorescence - a tool to assess photosynthetic performance and stress photo-physiology in symbiotic marine invertebrates and seaplants. Mar Pollut Bull 165: 112059. DOI: 10.1016/j.marpolbul.2021.112059.

Bhagooli R, Sheppard CRC. 2012. Prediction of recurrences of mass coral bleaching/mortality and vulnerability of reef-building corals to climate change in Mauritian and Japanese waters. Univers Mauritius Res J Special Issue: Sustain Mar Environ A 18: 105-121.

Bhagooli R, Soondur M, Ramah S, Gopeechund A, Jeetun S, Kaullysing D. 2021b. Photo-physiology of healthy and bleached corals from the Mascarene Plateau. Special Issues 2/2021-Studies on the Mascarene Plateau. WIO J Mar Sci 2: 109-120. DOI: 10.4314/wiojms.si2021.2.8.

Bhagooli R, Soondur M, Ramah S, Gopeechund A, Kaullysing D. 2021c. A first study on the variable photo-physiological performance of macroalgae and seagrasses from Saya de Malha and Nazareth Banks, Mascarene Plateau. Special Issues 2/2021-Studies on the Mascarene Plateau. WIO J Mar Sci 2: 95-108. DOI: 10.4314/wiojms.si2021.2.7.

Bhagooli R, Taleb-Hossenkhan N. 2012. Thermal spatial heterogeneity and coral bleaching: implications for habitat refuges. Proc 12th Inter Coral Reef Symp, 9D Refuges for Corals in Time and Space. 6pp.

Bhagooli R. 2009. Symbiont-dependent thermal bleaching susceptibility in two reef-building corals, *Stylophora pistillata* and *Platygyra ryukyuensis*. Univers Mauritius Res J 15: 607-626.

Bhagooli R. 2013. Inhibition of Calvin-Benson cycle suppresses the repair of photosystem II in *Symbiodinium*: Implications for coral bleaching. Hydrobiologia 714: 183-190. DOI: 10.1007/s10750-013-1535-4.

Brown BE, Dunne RZ, Somerfield PJ, Edwards AJ, Simons WJF, Phongsuwan N, Putchim L, Anderson L, Naeije MC. 2019. Long-term impacts of rising sea temperature and sea level on shallow water coral communities over a 40 year period. Sci Rep 9: 8826. DOI: 10.1038/s41598-019-45188-x.

Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, Harvell CD, Sweatman H, Melendy AM. 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5 (6): 1-7. DOI: 10.1371/journal.pbio.0050124.

Bussapakorn U, Petchporn C, Sompop R. 2018. An effect-analysis method for species-dependent coral health status in temperature and ammonia: a case study of *Acropora* sp., *Turbinaria* sp., and *Porites* sp. E3S Web Conf 93: 01002. DOI: 10.1051/e3sconf/20199301002.

Carilli JE, Norris RD, Black B, Walsh SM, Mcfield M. 2010. Century-scale records of coral growth rates indicate that local stressors reduce coral thermal tolerance threshold. Glob Change Biol 16: 1247-1257. DOI: 10.1111/j.1365-2486.2009.02043.x.

Colautti RI, MacIsaac HJ. 2004. A neutral terminology to define 'invasive' species. Divers Distrib 10: 135-141. DOI: 10.1111/j.1366-9516.2004.00061.x.

Dijkstra J, Harris LG, Westerman E. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. J Exp Mar Biol Ecol 342: 61-68. DOI: 10.1016/j.jembe.2006.10.015.

Dionisio-sese ML, Maruyama T, Miyachi S, 2001. Photosynthesis of *Prochloron* as affected by environmental factors. Mar Biotechnol 3: 74-79. DOI: 10.1007/s101260000062.

Genty B, Briantais JM, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica Biophysica Acta 990: 87-92. DOI: 10.1016/S0304-4165(89)80016-9.

Glynn PW. 1993. Coral reef bleaching: Ecological perspectives. Coral Reefs 12: 1-17. DOI: 10.1007/BF00303779.

Goodbody I. 2000. Diversity and distribution of ascidians (tunicata) in the pelican cays. Belize. Atoll Res Bull 480: 301-333. DOI: 10.5479/si.00775630.480.

Grimsditch GD, Salm RV. 2006. Coral Reef Resilience and Resistance to Bleaching. IUCN, Gland, Switzerland.

Gudka M, Obura D, Mwaura J, Porter S, Yahya S, Mabwa R. 2018. Impact of the 3rd Global Coral Bleching Event on the Western Indian Ocean in 2016. Global Coral Reef Monitoring Network (GCRMN)/Indian Ocean Commission, pp 1-67.

Hartmeyer R. 1909-1911. Ascidien. In: Bronn HG (eds). Klassen und Ordnungen des Tierreichs 3 (1): 81-98; 1281-1773.

Hirose E, Iskandar BH, Wardiatno Y. 2014. Photosymbiotic ascidians from Pari Island (Thousand Islands, Indonesia). ZooKeys 422 (422): 1-10. DOI: 10.3897/zookeys.398.5176.

Hirose E, Nozawa Y. 2010. Photosymbiotic ascidians from Kenting and Lyudao in Taiwan. Zool Stud 49 (5): 681-687.

Hirose E, Ohtsuka K, Ishikura M, Maruyama T. 2004. Ultraviolet absorption in ascidian tunic and ascidian-Prochloron symbiosis. J Mar Biolog Assoc 84: 789-794. DOI: 10.1017/S0025315404009956h.

Hirose E, Oka AT, Hirose M. 2009. Two new species of photosymbiotic ascidians of the Genus *Diplosoma* from the Ryukyu Archipelago, with Partial Sequences of the COI Gene. Zool Sci 26: 362-368. DOI: 10.2108/zsj.26.362.

Hirose E, Turon X, López-Legentil S, Erwin PM, Hirose M. 2012. First records of didemnid ascidians harbouring *Prochloron* from Caribbean Panama: Genetic relationships between Caribbean and Pacific photosymbionts and host ascidian. Syst Biodivers 10 (4): 435-445. DOI: 10.1080/14772000.2012.735716.

Hirose M, Hirose E. 2009. DNA BARCODING in photosymbiotic species of *Diplosoma* (Ascidiacea: Didemnidae), with the description of a new species from the Southern Ryukyus, Japan. Zool Sci 26: 564-568. DOI: 10.2108/zsj.26.564.

Hirose M, Hirose E. 2013. Photosymbiotic ascidians from oceanic islands in the tropical Pacific as candidates of long-dispersal species: morphological and genetic identification of the species. Aquat Inv 8 (3): 271-280. DOI: 10.3391/ai.2013.8.3.03.

Hughes TP, Kerry JT, Álvares-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Berger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, antin NE, Comeau S, Conoly, SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gillmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs PA, Hoogenboom MO, Kennedy EV, Kuo CY, Lough JM, Lowe RJ, Liu G, McCulloch MT, Malcom HA, McWilliam MJ, Pandolfi JM, Perals RJ, Pratchett MS, Schoepf V, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK. 2017. Global warming and recurrent mass bleaching of corals. Nature 543: 373-377. DOI: 10.1038/nature21707.

Iglesias-Prieto R, Malta LJ, Robins WA, Trench R. 1992. Photosynthetic response to elevated temperature in the symbiotic dinollagellate *Symbiodinium microadriaticum* in culture. Proc Natl Acad Sci USA 89: 10302-10305. DOI: 10.1073/pnas.89.21.10302.

Jimenez IM, Larkum AWD, Ralph PJ, Kühl M. 2012. In situ thermal dynamics of shallow water corals is affected by tidal patterns and irradiance. Mar Biol 159 (8): 1773-1782. DOI: 10.1007/s00227-012-1968-8.

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U. 1998. Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. Plant Cell Environ 21: 1219-1230. DOI: 10.1046/j.1365-3040.1998.00345.x.

Kaullysing D, Gopeechund A, Mattan-Moorgawa S, Taleb-Hossenkhan N, Kulkarni, B, Bhagooli R. 2016. Increased density of the corallivore *Drupella cornus* on *Acropora muricata* colonies overgrown by *Padina boryana*. Proceedings of the 13th International Coral Reef Symposium, Honolulu.

Koplovitz G, Hirose E, Hirose, Shenkar N. 2015. Being green in the Red Sea - the photosymbiotic ascidian *Diplosoma simile* (Ascidiacea: Didemnidae) in the Gulf of Aqaba. Syst Biodivers 13 (2): 131-139. DOI: 10.1080/14772000.2014.978410.

Kott P. 1982. Didemnid-algal symbioses: Host Species in the Western Pacific with Notes on the Symbiosis. Micronesica 18 (1): 95-127.

Kott P. 2001. The Australian Ascidiacea, part 4, Aplousobranchia (3), Didemnidae. Mem Queensl Mus 47: 1-408.

Kremer LP, Rocha RM. 2011. The role of *Didemnum perlucidum* F. Monniot, 1983 (Tunicata,Ascidiacea) in a marine fouling community. Aquatic Invasions 6 (4): 441-449. DOI: 10.3391/ai.2011.6.4.09.

Lafargue F, Wahl M. 1987. The Didemnid ascidian fauna of France. Annales de l'Institut Océanographique 63 (1): 1-46.

Leujak W, Ormond RFG. 2007. Comparative accuracy and efficiency of six coral community survey methods. J Exp Mar Biol Ecol 351: 168-187. DOI: 10.1016/j.jembe.2007.06.028.

Li S, Chen T, Xu L, Hu M. 2016. Ascidians (*Diplosoma* sp.) kill *Acropora* corals in a deteriorating reef environment (Luhuitou, Sanya, northern South China Sea). Bull Mar Sci 92 (4): 527-528. DOI: 10.5343/bms.2016.1072.

Littler MM, Littler DS. 1995. A colonial tunicate smothers corals and coralline algae on the Great Astrolabe Reef, Fiji. Coral Reefs 14(3): 148-149. DOI: 10.1007/BF00367232.

Louis YD, Bhagooli R, Kenkel C, Baker AC, Dyall S. 2016. Gene expression biomarkers of heat stress in scleractinian corals: Promises and limitations. Comp Biochem Physiol 191: 63-77. DOI: 10.1016/j.cbpc.2016.08.007.

Louis YD, Bhagooli R, Seveso D, Maggioni D, Galli P, Vai M, Dyall SD. 2020. Local acclimatisation-driven differential gene and protein expression patterns of Hsp70 in *Acropora muricata*: implications for coral tolerances to bleaching. Mol Ecol 29 (22): 4382-4394. DOI: 10.1111/MEC.15642.

Marks JA. 1996. Three sibling species of didemnid ascidians from northern Norway: *Didemnum albidum* (Verrill, 1871), *Didemnum polare* (Hartmeyer, 1903), and *Didemnum romssae* sp.nov. Can J Zool 74: 357 -379. DOI: 10.1139/z96-043.

Mattan-Moorgawa S, Bhagooli R, Rughooputh SDDV. 2012. Thermal stress physiology and mortality responses scleractinian corals of Mauritius. Proc 12th Inter Coral Reef Symp, 9A Coral Bleaching and Climate Change. 6pp.

Mattan-Moorgawa S, Poonet G, Bhagooli R, Rughooputh S. 2015. Differential effects of thermal and chemical stressors on tissue balls from scleratinian corals. Afr J Mar Sci 36 (4): 439-447. DOI: 10.2989/1814232X.2014.974208.

Mattan-Moorgawa S, Rughooputh SDDV, Bhagooli R. 2018. Variable PSII functioning and bleaching conditions of tropical scleractinian corals pre- and post-bleaching event. Ocean Life 2 (1): 1-10. DOI: 10.13057/oceanlife/o020101.

Middlebrook R, Hoegh-Guldberg O, Leggat W. 2008. The effect of thermal history on the susceptibility of reef-building corals to thermal stress. J Exp Biol 211: 1050-1056. DOI: DOI: 10.1242/jeb.013284.

Millar RH, 1975. Ascidians from the Indo-West-Pacific region in the Zoological Museum, Copenhagen (Tunicata, Ascidiacea). Steenstrupia. Zool Mus 3 (20): 205-336.

Monniot C, Monniot F. 1994. Additions to the inventory of eastern tropical Atlantic ascidians; Arrival of cosmopolitan species. Bull Mar Sci 54 (1): 71-93.

Monniot C, Monniot F. 2007. Records of ascidians from Baharain, Arabian Gulf with three new species. J Nat Hist 31 (11): 1623-1643. DOI: 10.1080/00222939700770871.

Monniot C. 2002. Stoliobranch ascidians from the tropical western Indian Ocean. Zool J Linn Soc 135: 65-120. DOI: 10.1046/j.1096-3642.2002.00017.x.

Monniot F, Monniot C. 2001. Ascidians from the tropical western Pacific. Zoosystema 23 (2): 201-383.

Monniot F, Monniot C. 2006. Ascidies (Polyclinidae, Pseudodistomidae et Polycitoridae) de l'ouest de l'océan Indien. Zoosystema 28 (1): 113-156.

Narrain D, Baulroop J, Bhagooli R, Bahorun T. 2023. Differential photosynthetic, phytochemical and antioxidative responses of three macroalgae *Ulva lactuca, Gracilaria salicornia* and *Turbinaria ornata* exposed to thermal and irradiance conditions. Indo Pac J Ocean Life 7: 1-15. DOI: 10.13057/oceanlife/o070101

Naranjo SA, Carballo JL, García-Gómez JC. 1996. Effects of environmental stress on ascidian populations in Algeciras Bay (Southern Spain). Possible marine bioindicators? Mar Ecol Prog Ser 144: 119-131. DOI: 10.3354/meps144119.

Obura D, Gudka M, AbdouRabi F, Gian SB, Bijoux J, Freed S, Maharavo J, Mwaura J, Porter S, Sola E, Wickel J, Yahya S, Ahamada S. 2017. Coral reef status report for the Western Indian Ocean. Global Coral Reef Monitoring Network (GCRMN)/ International Coral Reef Initiative (ICRI), pp 144.

Oka AT, Hirose E. 2008. Photosymbiotic Ascidians from Nakanoshima Island and Takarajima Island (the Tokara Islands, Ryukyu Archipelago, Japan) with remarks on the status of *Diplosoma midori* (Tokioka, 1954). Publ Seto Mar Biol Lab 40 (5/6): 85-92. DOI: 10.5134/72816.

Oka AT, Suetsugu M, Hirose E. 2005. Two new species of *Diplosoma* (Ascidiacea: Didemnidae) bearing prokaryotic algae *Prochloron* from Okinawajima (Ryukyu Archipelago, Japan). Zool Sci 22: 367-374. DOI: 10.2108/zsj.22.367.

Palomino-Alvarez LA, Rocha RM, Simões N. 2019. Checklist of ascidians (Chordata, Tunicata) from the southern Gulf of Mexico. ZooKeys 832: 1-33. DOI: 10.3897/zookeys.832.31712.

Pandolfi JM, Connolly SR, Marshall D, Cohen AL. 2011. Projecting coral reef futures under global warming and ocean acidification. Science 333 (6041): 418-423. DOI: 10.1126/science.1204794.

Pineda MC, Turon X, López-Legentil S. 2012. Stress levels over time ion the introduced ascidian *Styela plicata*: The effects of temperature and salinity variations on hsp70 gene expression. Cell Stress Chaperones 17: 435-444. DOI: 10.1007/s12192-012-0321-y.

Rodriguez-Martinez RE, Jordan-Garza AG, Baker DM, Jordan-Dahlgren E. 2012. Competitive interactions between corals and *Trididemnum solidum* on Mexican Caribbean reefs. Coral Reefs 31: 571-577. DOI: 10.1007/s00338-011-0871-y.

Sadally SB, Taleb-Hossenkhan N, Bhagooli R. 2016. Microalgal distribution, diversity and photo-physiological performance across five tropical ecosystems around Mauritius Island. Western Indian Ocean J Mar Sci 15 (1): 49-68.

Schoepf V, Stat M, Falter JL, McCulloch T. 2015. Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. Sci Rep 5 (17639): 1-14. DOI: 10.1038/srep17639.

Sensui N, Hirose E. 2018. Cytoplasmic UV-R absorption in an integumentary matrix (tunic) of photosymbiotic ascidian colonies. Zool Stud 57: 1-11. DOI: 10.6620/ZS.2018.57-33.

Shenkar N, Swalla BJ. 2011. Global diversity of Ascidiacea. PLoS ONE 6 (6): 1-12. DOI: 10.1371/journal.pone.0020657.

Sheppard CRC. 2003. Predicted recurrences of mass coral mortality in the Indian Ocean. Nature 425: 294-297. DOI: 10.1038/nature01987.

Sluiter CP. 1909. Die tunicaten der Siboga-Expedition. II : Die merosomen Ascidien. Siboga Exped 56: 1-112.

Sommer B, Harrison PL, Scheffers SR. 2010. Aggressive colonial ascidian impacting deep coral reefs at Bonaire, Netherlands Antilles. Coral Reefs 29: 245. DOI: 10.1007/s00338-009-0579-4.

Soondur M, Boojhawon R, Lowe R, Kaullysing D, Casareto BE, Suzuki Y, Bhagooli R. 2022. Rainfall-driven nutrient loading affects coastal phytoplankton in the southwestern Indian Ocean: A lagoon at Mauritius Island. Afr J Mar Sci 44 (2): 153-169. DOI: 10.2989/1814232X.2022.2066722.

Soondur M, Ramah S, Boojhawon R, Kaullysing D, Bhagooli R. 2021. Variations in abundance, diversity, photo-physiology and estimated productivity of micro-phytoplankton with depth at the Saya de Malha Bank, Mascarene Plateau. Special Issues 2/2021 – Studies on the Mascarene Plateau. Western Indian Ocean J Mar Sci 53-68. DOI: 10.4314/wiojms.si2021.2.4.

Stalin C, Anantan G, Raghunathan C. 2016. A new record of photosynthetic ascidians from Andaman & Nicobar Islands, India. Indian J Geo Mar Sci 46 (11): 2393-2398.

Tebbett SB, Streit RP, Bellwood DR. 2019. Expansion of a colonial ascidian following consecutive mass coral bleaching at lizard Island, Australia. Mar Environ Res 144: 125-129. DOI: 10.1016/j.marenvres.2019.01.007.

Vargas-Ángel B, Godwin LS, Brainard RE. 2008. Invasive didemnid tunicate spreading across coral reefs at remote Swains Island, American Sāmoa. Coral Reefs 28: 53. DOI: 10.1007/s00338-008-0428-x.

Vasseur P. 1967. Contribution à L'étude des ascidies De L'ile Maurice (Archipel Des Mascareignes, Océan Indien). Recueil des Travaux de la Station marine d'Endoume suppl 6: 101-139.

Villalobos SM, Lambert G, Shenkar N, López-Legentil S. 2017. Distribution and population dynamics of key ascidians in North Carolina harbours and marinas. Aquat Inv 12 (4): 447-458. DOI: 10.3391/ai.2017.12.4.03.

Warner ME, Fitt WK, Schmidt GW. 1999. Damage to photo-system II in symbiotic dinoflagellates: A determinant of coral bleaching. Proc Natl Acad Sci USA 96: 8007-8012. DOI: 10.1073/pnas.96.14.8007.

Warner ME, Fitt WK, Schmidt, GW. 1996. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: A novel approach. Plant Cell Environ 19: 291-299. DOI: 10.1111/j.1365-3040.1996.tb00251.x.

Whitlatch RB, Zajac RN. 1985. Biotic interactions among estuarine infaunal opportunistic species. Mar Ecol Prog Ser 21: 299-311. DOI: 10.1023/A:1017032200173.