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Expansion of a colonial ascidian following consecutive mass coral bleaching at Lizard Island, Australia

Short Title:

Expansion of a colonial ascidian

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Abstract

Mass coral bleaching is challenging today's coral reefs. However, our understanding of dynamics in benthic space holders, following such disturbances, is limited. To address this, we quantified successional dynamics of the ascidian, *Didemnum* cf. *molle* using a series of temporally and spatially matched photoquadrats across both the 2016 and 2017 mass coral bleaching events on the Great Barrier Reef. Unlike corals, *D.* cf. *molle* appeared to flourish in the warm temperatures and rapidly expanded. Indeed, colony density increased nearly 6-fold over two years with one quadrat experiencing an increase of over 1000 ind. m^{-2}. However, this increase did not simply track the increase in space due to coral mortality, but may have benefitted from reduced predation or increased nutrient availability following mass coral mortality. This study highlights the potential of *D.* cf *molle* to expand under bleaching conditions and to become a more prominent component of future reef configurations.

Keywords:

Benthos; Coral Reef; *Didemnum molle*; Ecosystem Change; Great Barrier Reef; Marine Ecology; Reefs

1. Introduction

Coral reefs are amongst the most biodiverse ecosystems on Earth, with intense competition for benthic space (Jackson, 1977) and disturbances causing shifts in species composition (Hughes et al., 2018). Unfortunately, large-scale disturbances are becoming more prevalent on coral reefs (Hughes et al., 2019). In the wake of these events, new reef configurations are emerging with fewer coral species and lower coral cover (Hughes et al., 2018; Norström et al., 2009). Indeed, during the austral summers of 2016 and 2017 Australia's Great Barrier Reef (GBR) experienced unprecedented back-to-back mass coral bleaching and coral mortality (Hughes et al., 2019). Such mortality provides free space which is often rapidly colonised by algae, especially turfing forms (Diaz-Pulido and McCook, 2002). However, while post-bleaching dynamics of corals (e.g. Hughes et al., 2018; Torda et al., 2018; Wismer et al., 2019) and to a lesser extent algae (e.g. Diaz-Pulido and McCook, 2002) have received attention in the literature, our understanding for other benthic space holders is relatively limited (reviewed in Norström et al., 2009).

One common, yet often overlooked group of benthic organisms on coral reefs are ascidians. Ascidians are a unique group of basal chordates, which have tadpole-like larvae with a notochord which is lost once the larvae settle (Lambert, 2005). This life history allows them to actively disperse over short distances, with the potential to colonize suitable down-stream sites (Olson, 1985). This expansion is facilitated by the ability of some ascidians to move short distances over the benthos and reproduce asexually through division (Cowan, 1981). Indeed, expansions of ascidians on coral reefs have been noted previously (e.g. Bak et al., 1996; Oren and Benayahu, 1998; Roth et al., 2018; Shenkar et al., 2008), yet their successional dynamics following coral mortality on such unprecedented scales is currently unclear. The aim of this study, therefore, was to utilise a series of temporally and spatially matched photoquadrats to examine the successional dynamics of a widespread ascidian across both the 2016 and 2017 mass coral bleaching events on the GBR.

2. Materials and methods

2.1 Study site and species

The abundance and coverage of the ascidian *Didemnum* cf. *molle* was studied in the Lagoon and on Loomis Reef at Lizard Island (14°40′8.04″S, 145°27′33.84″E; ESM Fig. S1), in the northern Great Barrier Reef, Australia. These sheltered reefs at Lizard Island were some of the worst affected by the 2016 bleaching event (Wismer et al., 2019), but were largely protected from two cyclones which struck the exposed side of the island in the years prior (2014/2015) to the bleaching (Brandl et al., 2016; Khan et al., 2017). Coral bleaching was particularly pronounced at Lizard Island during the 2016 event as a result of >8 degree heating weeks by April 2016 (Hughes et al., 2019; Wismer et al., 2019). From January 2016 – April 2016 average water temperature at 0.6 m depth regularly exceeded 30˚C and maximum photosynthetically active radiation often exceeded 2000 µ moles s^{-1} m^{-2} (ESM Fig. S2). It must be noted that DNA barcoding is recommended for the correct identification of ascidians (Jaffarali et al., 2018), and without this certainty the conservative nomenclature *D.* cf. *molle* is used herein. Indeed, *Atriolum robustum* is often misidentified as *D. molle* (see ESM Text S1 and Fig. S3 for details).

2.2 Sampling

Along the reef edge (1-3 m depth), in both the Lagoon and on Loomis Reef (ESM Fig. S1), 27 and 36 quadrats, respectively, were examined to assess changes in *D.* cf. *molle* abundance. Quadrats (1 m^2) were initially placed haphazardly (~5 m apart) along the reef edge in February 2016, prior to major bleaching, and photographed from above using a Nikon Coolpix W300 digital camera. The same quadrat areas were re-photographed in April 2016 (during peak bleaching), October 2016 (approximately six months post-bleaching) and January 2018 (approximately 21 months post 2016 bleaching and 10 months post 2017 bleaching). Quadrats were not left fixed in place. They were placed in the same locations on each trip using previous photographs loaded onto an additional digital camera (Nikon Coolpix W300) as a guide (see Wismer et al., (2019) for full details and a sensitivity analysis of methods). It should be noted that the present study represents a focused examination of *D.* cf. *molle* dynamics on these two specific reef locations. These locations were selected as both had *D.* cf. *molle* populations prior to the bleaching events. Other sites around Lizard Island, especially exposed locations, generally lacked *D.* cf. *molle*. This lack of *D.* cf. *molle* at other sites is likely to be a consequence of the previous cyclones that scoured the benthos in exposed localities (details above). In addition, limited connectivity among sites is likely to be due to the influence of water currents on the dispersal abilities of *D.* cf. *molle* larvae (see Olson, (1985) for a detailed study of *D. molle* dispersal at Lizard Island).

2.3 Image processing

To assess the abundance and benthic coverage of *D.* cf. *molle* photographs were examined in two ways. Firstly, quadrats in the photographs were systematically searched for *D.* cf. *molle* and the number of individual *D.* cf. *molle* were recorded. Due to the propensity of *D.* cf. *molle* to form dense, clustered colonies, and their ability to reproduce asexually via fission, this method may overestimate *D.* cf. *molle* abundance as it was not always clear if two *D.* cf. *molle* were completely separated or were in the process of dividing. Secondly, therefore, the proportion of benthic space occupied by *D.* cf. *molle*, as well as other benthic organisms, was calculated by generating 50 random points in each quadrat using the software photoQuad V1.4 (Trygonis and Sini, 2012). Each point was categorised as either *D.* cf. *molle*, crustose coralline algae (CCA)/algal turfs, hard corals, soft corals, sand/small rubble, macroalgae (algae > 2cm high), cyanobacterial mat, and other (predominantly *Tridacna* clams). As this method of quadrat examination can undercount rare benthic cover categories, the coverage of *D.* cf. *molle* is likely to be a conservative estimate.

2.4 Statistical analysis

Initially, changes in the entire benthic assemblage over time were examined using a permutational multivariate analysis of variance (PERMANOVA) based on a Euclidean distance matrix of standardised data. In the PERMANOVA sampling trip was treated as a fixed factor, while site and individual quadrat identity nested within site were treated as random factors to account for the repeated measures design of the study, any variability between sites, and the lack of spatial independence among replicate quadrats. Following the PERMANOVA, pair-wise tests were performed to determine within-factor differences. The assumption of homogeneity of dispersions in the PERMANOVA was assessed using a permutational analysis of multivariate dispersions (PERMDISP), which revealed homogeneity. A canonical analysis of principle co-ordinates (CAP) was used to explore/visualise specific differences among sampling periods. All multivariate analysis was performed in Primer 6.0 PERMANOVA+.

To examine specific variation in *D.* cf. *molle* numbers and benthic coverage generalised linear mixed effects models (GLMMs) were used. GLMMs were based on the same fixed and random factors as above. *D.* cf. *molle* abundance was examined using a negative binomial distribution to account for the overdispersed nature of the count data. The proportion of benthic space occupied by *D.* cf. *molle* was examined using a binomial distribution. The fits and relevant assumptions of all models were assessed using residual plots, all of which were satisfactory. All statistical modelling was performed in the software R (R Core Team, 2017) using the *lme4* (Bates et al., 2015) package.

3. Results and discussion

Following the bleaching events the average cover of hard and soft corals at the two study sites declined by 36% and 90%, respectively (Fig. 1, Table S1). Concomitantly, there was a 1.4-fold increase in the coverage of CCA/algal turfs (Table S1). Overall significant changes in the composition of the benthic community were detected between all sampling trips apart from between April 2016 and October 2016 (Fig. 1d, Tables S2, S3). While these changes align with general expectations following a bleaching event, especially a decrease in living corals (e.g. Diaz-Pulido and McCook, 2002; Hughes et al., 2018; Torda et al., 2018), there was also a particularly marked increase in the ascidian *D.* cf. *molle* on these two reefs (Fig. 1).

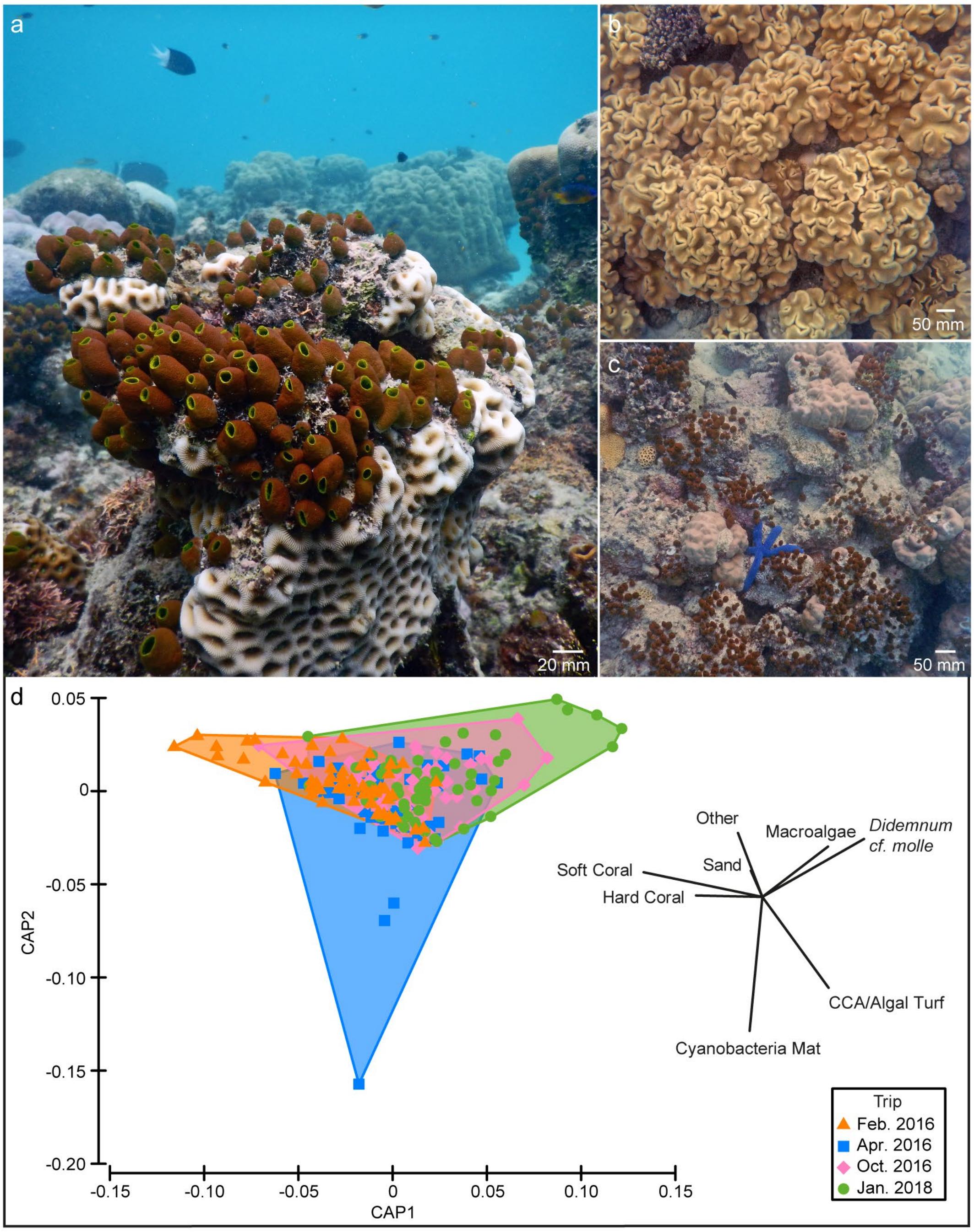

Fig. 1 a) A dense cluster of the ascidian *Didemnum* cf. *molle* growing on top of a coral outcrop at Lizard Island on the Great Barrier Reef, b) the soft coral *Sarcophyton* sp. covering the benthos at Lizard Island prior to mass bleaching (February 2016), c) the same site approximately two years later (January 2018) revealing a marked increase in

the cover of *D.* cf. *molle*. d) Canonical analysis of principle (CAP) coordinates ordination based on a Euclidean distance matrix of standardised benthic cover data for each trip. Vectors show the relationship among benthic components and how they influence the position of data points in the CAP. Vectors were calculated using a multiple correlation model. Coloured polygons are to aid visual interpretation and do not denote significant groupings (April 2016 and October 2016 do not differ significantly). CCA = crustose coralline algae.

Unlike corals, *D.* cf. *molle* appeared to be unaffected by the warm temperatures, and instead flourished, rapidly expanding and occupying space (Fig. 1). Over two years the mean abundance of *D.* cf. *molle* increased nearly 6-fold on average from 32.1 ± 6.6 to 181.9 ± 30.6 individuals m^{-2} (± SE) (Fig. 2a). This was statistically significant (GLMM; $p < 0.001$; Table S4). Indeed, in one of the quadrats an increase of over 1000 ind. m^{-2} was recorded. Similarly, the percentage of benthic space occupied by *D.* cf. *molle* increased 7.7-fold, from 0.6 ± 0.2% to 4.6 ± 0.8% (Fig. 2b) (GLMM; $p < 0.001$; Table S4). Thus the increase in *D.* cf. *molle* was not merely tracking the increase in 'empty' benthic space (i.e. a 1.4-fold increase in CCA/algal turf cover) following coral mortality, but showed a disproportional 7.7-fold increase.

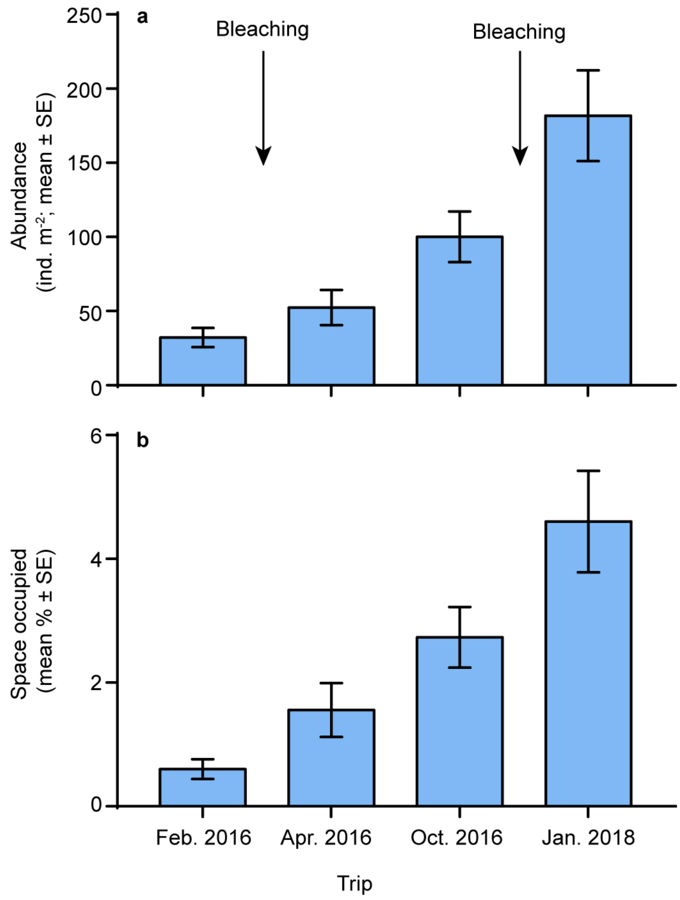

Fig. 2 Change in a) the abundance of, and b) the benthic coverage by, *Didemnum* cf. *molle* at Lizard Island on the Great Barrier Reef, Australia over a two year period. *n* = 63 quadrats in each sampling period.

The rapid increase in *D.* cf. *molle* cover was not due to the 'canopy effect' whereby the loss of coral cover suddenly exposes hidden components of the benthos underneath (sensu Goatley and Bellwood, 2011). Instead, we found a steady increase in *D.* cf. *molle* over the 21 month period, following loss of coral cover (Fig. 2). Indeed, *D. molle* predominantly live in locations exposed to sunlight, rather than under coral canopies (Olson, 1985). The preference for *D. molle* to live in sunlit locations is due to their symbiosis with the prokaryotic alga, *Prochloron* sp. (Kott, 2001; Olson, 1986), which appears to enhance growth (Olson, 1986).

The symbiosis between some ascidians and *Prochloron* sp. is unique in that it is the only known example of an obligate photosymbiont in the Chordata (Hirose and Fukuda, 2006). Unlike the symbiosis between corals and their photosynthetic symbionts (zooxanthellae, *Symbiodinium* spp.) that breaks down with heating leading to coral bleaching (Hughes et al., 2018), the symbiosis between *D. molle* and *Prochloron* sp. appears to be more resilient to heat stress (Figs. 1, 2). Indeed, adult *D. molle* colonies even utilise mycosporine-like amino acids as UV-absorbing substances to protect *Prochloron* sp. from UV radiation (Hirose et al., 2006). The resilience of this *D. molle/Prochloron* symbiosis appears to allow *D. molle* colonies to persist through heat stress and expand.

As ascidians can overgrow most benthic organisms, including corals and macroalgae (Bak et al., 1996; Littler and Litter, 1995; Roth et al., 2018), this suggests ‘available space’ is not a limiting factor and, as such, the expansion of *D.* cf. *molle* may not be directly due to coral mortality opening up new space to occupy. While the direct increase in *D.* cf. *molle* following the bleaching event is unlikely to be coincidental, the mechanistic basis underpinning this increase is currently unclear. One influential factor could be altered predation rates due to a decrease in the abundance of particular fishes following the bleaching event at Lizard Island (for details on the fish assemblage see Wismer et al., 2019). For example, some planktivorous damselfishes declined by nearly 40% between February 2016 and October 2016 (Wismer et al., 2019) and these fishes have been shown to prey heavily on dispersing ascidian larvae with the potential to limit recruitment rates (Olson and McPherson, 1987; Stoner, 1990). Furthermore, predation on settled ascidians by larger fishes can limit their establishment (Kremer and da Roch, 2016; Roth et al., 2017; Russ 1980) and this may have been altered as a result of the changing fish assemblage, although such changes are unlikely to be as marked as for the planktivorous damselfishes (Wismer et al., 2019).

In addition to predation, another factor may lie in the flux of nutrients and particulate organic matter released into the water following the mass-mortality of corals. When corals bleach and/or die, excess nutrients and organic matter are released (Niggl et al., 2009), which may be utilised by a range of organisms. Indeed, during and shortly after the 2016 mass-bleaching event, cyanobacterial mats, which are known to benefit from nutrient fluxes (Ford et al., 2018), were a widespread feature at Lizard Island (Wismer et al., 2019). As filter-feeders, the growth and expansion of *D*. cf. *molle* is also likely to be enhanced under these conditions, with several studies revealing enhanced growth of ascidians, including *Didemnum* sp., under eutrophic conditions (Muñoz et al., 2015; Roth et al., 2017; Shenkar et al., 2008). While nutrient fluxes may have had some effect (unfortunately these were not quantified in the current study) any fluxes are likely to be temporally limited to the weeks immediately following coral mortality. Although the mechanistic basis was not assessed in the current study, our data revealed a clear and progressive increase in *D.* cf. *molle* on bleaching-impacted reefs.

The bleaching events and subsequent expansion of *D.* cf. *molle* could have a two-fold impact on coral recovery. With reduced adult coral populations, coral recruit densities could be suppressed, limiting recruits available for recolonization. Furthermore, coral recruitment is dependent on the availability of suitable settlement locations and cues (Hughes et al., 2018). Evidence suggests the availability of microtopographic refuges that are not grazed heavily (Brandl and Bellwood, 2016), contain low sediment loads (Ricardo et al., 2017), and are not shaded by algae (Brandl and Bellwood, 2016) are critical for the establishment of scleractinian corals. Therefore, by rapidly expanding post-bleaching, the proliferation of *D.* cf. *molle* could affect future recovery trajectories, with microtopographic refuges becoming occupied or shaded, limiting the ability of corals to exploit such refugia.

The extent of any spatial competition between *D.* cf. *molle* and corals will depend on how long the expansion of *D.* cf. *molle* persists. While expansion of *D.* cf. *molle* appears to be previously unreported, expansion of other ascidian species on coral reefs has been noted (e.g. Littler and Littler, 1995; Roth et al., 2018; Vargas-Ángel et al., 2009). For example, *Trididemnum solidum* in the Caribbean (Bak et al., 1996) and *Botryllus eilatensis* in the Red Sea (Shenkar et al., 2008). These expansions range in temporal persistence from seasonal e.g. *B. eilatensis* (Shenkar et al., 2008), to over 15 years e.g. *T. solidum* (Bak et al., 1996). Herein, abundances of *D.* cf. *molle* have increased continuously over two years (Fig. 2). This expansion could be ephemeral and reversed by direct ascidian removal through physical disturbance (Olson, 1985), colony senescence (Olson, 1985), or by predation by fishes, nudibranchs, or other molluscs (Lambert, 2005; Paul et al., 1990). However, for now, it appears that in some locations *D.* cf. *molle* will be a more prominent component of changing reef environments. Further understanding of the organisms, such as *D.* cf. *molle*, which succeed in these changing ecosystems, will be a rich area for future investigation.

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