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RESEARCH NOTE

REVISED Polyp bailout in *Pocillopora damicornis* following thermal stress [version 2; peer review: 2 approved]Alexander J Fordyce¹, Emma F Camp², Tracy D Ainsworth¹¹ARC Centre of Excellence for Coral Reef Studies, James Cook University, Queensland, Australia²Climate Change Cluster, University of Technology Sydney, New South Wales, Australia

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Abstract

Polyp bailout is an established but understudied coral stress response that involves the detachment of individual polyps from the colonial form as a means of escaping unfavourable conditions. This may influence both the mortality and asexual recruitment of coral genotypes across a range of species. It has been observed in response to numerous stressors including high salinity and low pH. Polyp expulsion in association with thermal stress has once been described in a geographically restricted, temperate species. We therefore cannot reliably apply this observation to tropical coral reefs around the world, which are increasingly under threat from thermal stress events. We present the first qualitative observation of polyp bailout following acute temperature shock in a near-natural mesocosm experiment. Detached polyps show similar characteristics to those described in previous studies, including the retention of endosymbiotic zooxanthellae and the ability to disperse across short distances. This finding strongly suggests that polyp bailout occurs in tropical coral reef environments and warrants further detailed research into the implication of this response in terms of individual survival, rapid migration into cooler micro-habitats and local recruitment within the reef environment and its coral community.

Keywordspolyp bailout, thermal stress, coral bleaching, *Pocillopora damicornis***Open Peer Review****Approval Status**

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version 1 17 May 2017		

1. **Sylvain Agostini**, University of Tsukuba, Shizuoka, Japan

2. **Dan Tchernov**, University of Haifa, Haifa, Israel

Hagit Kvitt, University of Haifa, Haifa, Israel

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Fordyce AJ:** Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Camp EF:** Methodology, Visualization, Writing – Review & Editing; **Ainsworth TD:** Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 1

We would like to thank the reviewers for their insightful comments and suggestions that contributed towards significant improvement of the manuscript. We also thank Dr Shapiro for highlighting information regarding settlement success in their 2016 study; this has been included in the 'Implications and future research' section following mention of the results of Sammarco (1982).

Regarding the review of Dr Agostini, the 'Implications and future research' section has been amended (second paragraph) to more accurately reflect our results in discussing the possibility of resettlement following polyp bailout.

Regarding the review of Dr Tchernov and Dr Kvitt, the following changes have been made in line with their comments.

- References to the paper by Kružić (2007) have been included throughout the manuscript and the claim regarding polyp bailout having not been previously observed in association with thermal stress removed.
- Regarding the lack of experimental proof to support the claim of coral bleaching, fluorometric and photographic data was collected and is currently in use for a different publication. When it becomes publicly available, a link to that study will be included in the article in order to provide proof of coral bleaching.
- A description of degree heating days was included in the 'Results' section.
- Whilst a better quality image is not available, Supplementary Material 2 has been added to better show a coiled, extended filaments. Phrasing has been changed to reflect the presumption of adhesive properties of the filaments; their identity as mesenteries is probable due to the visual similarity and findings of Richmond (1985).
- The reference to Goreau & Goreau (1959) has been removed and a reference to the study by Kramarsky-Winter *et al.* (1997) has been included.

See referee reports

Introduction

Coral reefs around the world are facing increasingly frequent acute thermal stress events (Ainsworth *et al.*, 2016; Hughes *et al.*, 2017). As such there has been a corresponding increase in research into how corals respond to high temperatures, how these responses vary within individuals and communities, and how variation influences the resilience, recovery and structure of coral communities. Understanding this variation helps predict patterns of bleaching-induced mortality and reef-wide degradation. As the possibility of frequent, severe bleaching events increases (van Hooijdonk *et al.*, 2016), it is important to understand the drivers of variability in order to improve management and target restoration efforts. Polyp bailout is a possible source of variation that may influence the survival of individual genotypes and recruitment at local scales.

Polyp bailout has been observed in at least six species of tropical scleractinian coral (Serrano *et al.*, 2017) and involves the withdrawal of individual polyps from the coenosarc followed by their detachment from the skeleton (Sammarco, 1982). The detachment of individual polyps from a parent coral colony has previously been recorded in response to poor water quality

(Sammarco, 1982; Serrano *et al.*, 2017), large changes in pH (Kvitt *et al.*, 2015), changes in salinity (Shapiro *et al.*, 2016) and following competition from macroalgae (Sin *et al.*, 2012). As polyps often retain their endosymbiotic dinoflagellates (zooxanthellae) and are able to re-settle, polyp bailout is thought to be a generalised escape response from detrimental conditions (Kvitt *et al.*, 2015; Sammarco, 1982). It may therefore constitute rapid migration away from local sources of mortality.

Despite increasing temperatures being arguably the most significant threat to tropical coral reefs (Hughes *et al.*, 2017), no tropical species have been observed to respond to thermal stress in this way. Kružić (2007) briefly described polyp expulsion in the temperate species *Cladocora caespitosa* following severe thermal stress. However, given the relatively small range of this species, small sample size (n=2) used and lack of information regarding experimental set up, the results are not necessarily applicable to tropical coral reefs around the world. Here we present the first qualitative observation of polyp bailout following thermal stress in *Pocillopora damicornis*, a common reef-forming tropical species (Hoeksema *et al.*, 2014), during an *ex situ* mesocosm study.

Methods

Colonies of *P. damicornis* (n=32) were collected from the Heron Island reef flat in January 2017, from a maximum depth of two metres. Each colony was quartered to give a total of 128 fragments. They were housed in four 500 litre aquaria as part of an outdoors, semi-closed system supplied by a continuous flow of unfiltered seawater from the reef flat. During the week preceding simulated thermal stress, all fragments were acclimated to the aquaria and subjected to ambient conditions (*ca.* 7.980 – 8.020 pH; conductivity of 53 – 54 µS/m; temperature of 26 – 30°C; and PAR of 0 – 3875 K). Following this, temperature was gradually increased in two mesocosms (n=64) on top of natural variation for six days up to a peak daytime temperature of 34°C to simulate a severe bleaching event (as previously reported by Ainsworth *et al.*, 2016). Two control mesocosms continued to be exposed to ambient conditions, differing from treatments in temperature only. Fragments were monitored throughout the day and when polyps were observed to bail out, they were collected using a wide-ended pipette and examined under an Olympus SZX16 stereomicroscope.

Results

On the fifth day of the simulated bleaching event, polyps were observed to begin bailing out at approximately 09:30 (Figure 1; Dataset 1, Fordyce *et al.*, 2017). At this time, peak daytime temperature was 33°C, equivalent to 13 degree heating days (DHDs). DHDs are a measure of accumulated heat stress that is calculated as the cumulative temperature increases above the mean monthly maximum across a period of time. The more common measure, Degree Heating Weeks (DHWs), is used in the prediction and assessment of mass bleaching events (e.g. Ainsworth *et al.*, 2016). By the end of day six, at peak temperature of 34°C, reflecting 18 degree heating days, all polyps had detached (Dataset 1, Fordyce *et al.*, 2017). At the end of the bailout period, polyps began to detach in sheets rather than as individuals. This suggests that thermal stress was too severe to allow successful withdrawal of all polyps from the coenosarc. In contrast,

fragments in the control mesocosms showed no signs of bleaching or polyp bailout (Supplementary Material 1).

Bailed polyps were slightly negatively buoyant, sinking slowly, but could easily be re-suspended with mild disturbance. The detached, individual polyps retained their zooxanthellae and many were observed to extend what are likely to be mesenteric filaments (as described in Richmond, 1985; Figure 2; Supplementary Material 2). Clusters of detached polyps were

Figure 1. Photographs of polyps dropping off the skeleton of *Pocillopora damicornis*. Macrophotograph of polyps, having withdrawn from the connective coenosarc, dropping off the skeleton of the fragments of *Pocillopora damicornis*. Photograph taken with an Olympus Stylus Tough TG-4.

Figure 2. Single polyps and clusters of polyps with zooxanthellae and extended filaments. Micrograph of single polyps and clusters of polyps placed in a glass petri dish, taken using an Olympus SZX16 stereomicroscope with a computer-linked 2.0x objective lens. Total magnification is 17.0x. Small brown dots in the polyp tissue are endosymbiotic zooxanthellae. Coiled filaments are thought to be mesenteries which may aid in resettlement, through adhesion, or feeding.

also observed, however these lacked calcified tissue and so did not resemble the larval clusters described by Richmond (1985) (Figure 2).

Dataset 1. Table of qualitative observations of polyp bailout in control and heat-treated mesocosms

<http://dx.doi.org/10.5256/f1000research.11522.d161213>

Indicated is the peak daytime temperature ($\pm 0.5^\circ\text{C}$) of the four treated mesocosms, the accumulated heat stress corals are exposed to and any observations during the four day bleaching period, including at the beginning and end of polyp bailout.

Implications and future research

In past observations of polyp bailout, corals have largely been subjected to extreme aquarium conditions such as high salinity (up to 54 parts per thousand; Shapiro *et al.*, 2016), low pH (7.2; Kvitt *et al.*, 2015) or little to no water replacement resulting in anoxic and low nutrient conditions (Serrano *et al.*, 2017; Sin *et al.*, 2012). Kružić (2007) on the other hand examined a temperate Mediterranean species. This makes it difficult to apply these results to the context of natural tropical reefs and elucidate the possible role of this response during environmental stress. The present observation was in aquaria with near-natural conditions, using an important reef coral species common across the Indo-Pacific. The peak daily temperature of 34°C was sustained over several days, reflecting severe thermal stress. However, accumulated heat stress amounted to between two and three degree heating weeks (14–21 degree heating days). This is becoming widely reported during bleaching events on the Great Barrier Reef (Ainsworth *et al.*, 2016; Hughes *et al.*, 2017). Therefore, this observation suggests that polyp bailout may be widespread in natural reef environments, in response to currently observed temperature increases. For coral species that utilise this response, the present record has implications for coral recruitment and recovery on local scales, and also suggests that these processes can occur independent of sexual reproduction and the impact of thermal stress on reproductive potential.

Resettlement of detached polyps was not observed, however they appear to be viable and may be able to settle near the parent colony or be dispersed short distances within a reef environment. Sammarco (1982) previously described low ($< 5\%$) settlement and survival rates of detached polyps on settlement tiles contained in jars but survival of individual polyps within a simulated or natural reef environment has yet to be investigated, particularly how it is affected by reef degradation. If polyps were to 'escape' into cooler conditions with higher food availability, it may boost their survival and settlement success rates beyond the 5% observed by Sammarco. Indeed, Shapiro *et al.* (2016) reported far higher rates of resettlement ($\geq 50\%$) when settling detached polyps under more favourable conditions, indicating that the low settlement observed by Sammarco can be partly explained by the environment in which resettlement was tested. In the current study, we noted that each fragment detached a large population of

individual polyps (>50 per fragment), showing that a survival of 50% has the potential to allow for some immediate and significant re-seeding of the local reef habitat. Furthermore, an obvious source of nearby refugia are neighbouring reef slope and mesophotic reef environments (Bridge *et al.*, 2013; Smith *et al.*, 2014), down to which these negatively buoyant polyps may slowly sink. This is particularly relevant given that Kramarsky-Winter *et al.* (1997) only observed polyp expulsion, associated with asexual reproduction, in waters ≤ 7 m; in the present study, colonies were collected from waters ≤ 2 m. However, light attenuation may limit viable settlement depths as individual polyps would need to rapidly acclimate to lower light conditions in order to settle and successfully begin asexual division.

Clearly, extensive future research is needed to explore the survival of individual polyps in both simulated refugia and within the reef habitat following thermal stress events. This will lead to greater understanding of the ecology and wider implications of this stress response and its potential role in coral recruitment and reef recovery following bleaching events. We additionally lack *in situ* observations of this phenomenon. Time-intensive ecological surveying during a predicted bleaching event is needed to reveal whether this is a widespread response to thermal stress. Subsequent use of mass mark and recapture of coral polyps, tagged with stable heavy isotopes, would then allow the tracking of the fate of coral polyps following bailout. Despite being a well-established response to stress, little research has focused on how polyp bailout may influence the survival and recovery of local coral populations.

Supplementary material

Supplementary Material 1. Comparative photographs of control and bleached fragments to show bleaching. Photographs were taken using an Olympus Stylus Tough TG-4 camera. A) Photograph of control fragment taken on 31/01/17; B) Photograph of bleached fragment, with visibly withdrawn individual polyps still attached to the skeleton, taken on 30/01/17.

[Click here to access the data.](#)

Supplementary Material 2. Micrograph of a single polyp with an extended filament. Micrograph of a single polyp taken using the same techniques and equipment used for Figure 2, more clearly showing a coiled, extended filament. Based on the findings of Richmond (1985), this is thought to be a mesentery.

[Click here to access the data.](#)

Data availability

Dataset 1. Table of qualitative observations of polyp bailout in control and heat-treated mesocosms. Indicated is the peak daytime temperature ($\pm 0.5^\circ\text{C}$) of the four treated mesocosms, the accumulated heat stress corals are exposed to and any observations during the four day bleaching period, including at the beginning and end of polyp bailout.

DOI, [10.5256/f1000research.11522.d161213](https://doi.org/10.5256/f1000research.11522.d161213) (Fordyce *et al.*, 2017)

Author contributions

TDA made the initial observation of polyp bailout. EFC photographed bailout for Figure 1. AJF photographed polyps for Figure 2 and prepared the first draft of the manuscript. All authors contributed to subsequent revisions of the manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Dan Tchernov

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Hagit Kvitt

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It is properly revised, and therefor approved for publication.

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 24 July 2017

<https://doi.org/10.5256/f1000research.12446.r22841>

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Dan Tchernov

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Hagit Kvitt

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General remarks:

1. In the Abstract the authors claim: "However, polyp bailout has not previously been

described in association with thermal stress and the coral bleaching response..."

a. This is completely inaccurate-polyp bailout or expulsion in response to thermal stress has been reported in 2007 by Kruzic P.

b. Moreover, the claim that bleaching is also involved: "in association with thermal stress and the coral **bleaching response**" is misleading - there is not a single experimental proof or measurement of bleaching in the current report, merely some remarks on "Paling observed in coral tissue" and "All corals fully bleached" (Dataset 1.). Moreover, the polyps are clearly detaching with the zooxanthellae, (Figure 2: "Small brown dots in the polyp tissue are endosymbiotic zooxanthellae"). So where is the bleaching? In the coenosarc? If so - experimental evidence should be provided. Or the sentence: "...the coral **bleaching response**" should have been omitted.

2. In the Results, the use of the term "heating days" is unclear, and should have been explained ("At this time, peak daytime temperature was 33°C, which is the **equivalent of 13 degree heating days, a measure of accumulated heat stress used in the prediction of mass bleaching events**").
3. Figure 2 is unclear, especially the presumed "coiled filaments are adhesive mesenterial filaments, presumed to aid in rapid settlement". I would expect, under current technologies available, for a better image to convince that indeed these are "adhesive mesenterial filaments".

The literature is not correctly cited.

1. In the Abstract, the paper by Goreau & Goreau (1959) does not clearly describe polyp bailout or expulsion. It deals with calcification all over, and it mentions merely in the Discussion, as unpublished results, that polyps can detach. This citation should have been omitted from the Abstract and put elsewhere.
2. The paper of Kramarsky-Winter *et al.* 1997 on polyp expulsion is not cited.
3. The paper of Kruzic P. 2007 on polyp expulsion under thermal stress, which is the most relevant paper to this report, is not cited.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 19 June 2017

<https://doi.org/10.5256/f1000research.12446.r23072>

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Sylvain Agostini

Shimoda Marine Research Center, University of Tsukuba, Shizuoka, Japan

This paper reports on the qualitative of polyp bailout for the coral species *Pocillopora damicornis* under heat stress in mesocosms.

The study remains qualitative and the authors are clear on this point.

The only remark I would have is in the discussion. The sentence "Detached polyps appear to be viable, able to settle near the parent colony and capable of being dispersed short distances in a reef environment." sounds like that resettlement of the bailed polyp were observed. However it was not observed and remain a speculation. I would suggest to rephrase it to something like that: "While resettlement of the bailed out polyps could not be observed, the polyps appear to be viable, and could be able to settle near the parent colony and to be dispersed accross short distances in a reef environment."

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Comments on this article

Version 1

Author Response 05 Jun 2017

Alexander Fordyce, James Cook University, Queensland, Australia

Dear Dr Shapiro,

Thank you for highlighting this, very relevant in addressing the questions highlighted in this research note. We'll be sure to update the manuscript to include this information which will undoubtedly improve its quality.

Thank you again,

Best wishes,

Alexander Fordyce

Competing Interests: No competing interests were disclosed.

Reader Comment 03 Jun 2017

Orr Shapiro, Volcani institute, ARO, Israel

Please note that in the experiments reported in our "Coral on a chip" paper (Shapiro *et al* 2016) settling rates were often above 50%, much higher than the 5% reported by Sammarco. The low settling rates in that work may be due to poor incubation conditions, as he reports transferring bailed-out polyps to new jars, with new seawater but no flow. I would thus expect initial survival and settlement in the natural reef environment to be considerably higher than that reported by

Sammarco.

Competing Interests: No competing interests were disclosed.

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