UNIVERSITY OF CALIFORNIA, SAN DIEGO

The life and death of perforate corals: micro-community structure within the skeleton.

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Marine Biology with a specialization in Interdisciplinary Environmental Research

by

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2017

The Dissertation of Kathryn Anne Furby is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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2017

DEDICATION

To Palmyra Atoll and the indefinitely wild sea

EPIGRAPH

My drawing was not a picture of a hat. It was a picture of a boa constrictor digesting an elephant. But since the grown-ups were not able to understand it, I made another drawing: I drew the inside of a boa constrictor, so that the grown-ups could see it clearly. They always need to have things explained.

Antoine de Saint Exupéry

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ABSTRACT OF THE DISSERTATION

The life and death of perforate corals: micro-community structure within the skeleton.

by

Kathryn Anne Furby

Doctor of Philosophy in Marine Biology with a specialization in Interdisciplinary Environmental Research

University of California, San Diego, 2017

Stuart Sandin, Chair

Coral reefs have been part of the earth's oceans since the Mesozoic Era, over 200 million years ago. In recent decades, however, reefs have been progressively suffering as a result of human activities. However, coral species vary in their response to ecological disturbance. The work described here examines aspects of the biology of two central

Pacific species: *Porites superfusa*, a small encrusting coral, and *Acropora cytherea,* a dynamic, massive table coral. Both species are perforate corals, with skeletons permeated with an extensive canal system partially lined with living coral tissue. Thus far, the question of whether the perforate coral condition conveys functional advantages lacking in corals with imperforate (relatively dense) skeletons has been overlooked. The present work supported with field work and sample collections at Palmyra Atoll, Line Islands, USA, during approximately annual visits of several weeks each from 2012 to 2016. The reef is protected from fishing and local pollution but still exposed to wide-scale disturbances like climate change (especially evident in warm water bleachings in 2009, 2015, 2016). This dissertation explores the importance of coral regrowth, endoliths and adjacent epiliths to reef recovery dynamics.

Long-term changes in coverage of *Porites superfusa* were followed in a time series of high-resolution photoquadrats that demonstrated new settlement as well as changes in existing colonies (growth, partial mortality, death, and "death" followed by resurrection). Partial mortality and survival was usually observed in larger individuals, whereas smaller individuals tended to grow progressively or apparently die out completely. However, quite often a new small individual would appear in the area where a colony had died previously in the time series. Although settlement of planula larvae from the water column could not be completely ruled out, the source of new growth could have been from a small amount of living tissue cryptically surviving in or on the "dead" colony. Further work would be required to see if new growth was seeded by small regions of viable tissue.

For the other perforate coral in this dissertation, *Acropora cytherea*, the

distribution of living tissues was studied in the canals permeating the skeleton in healthy and evidently dead (algae-covered) regions of the colony. Core samples were studied by scanning electron and light microscopy. In healthy regions, the living coral tissues lined only the intraskeletal canals to a depth of several millimeters from the surface of the colony. In healthy parts of the colony, the canals more than a few millimeters deep in the colony were bounded not by living coral tissues, but with calcareous skeleton. Although the coral skeleton was riddled with endoliths (algae, fungi and bacteria), they were relatively rarely observed in the canal space. In regions where the living part of the colony was covered over with turf algae and other invading organisms, these endoliths were detected, along with abundant sediment, packing the most superficial intracellular canals; surprisingly the canals deeper beneath the overgrown region were free of such extraneous material. In the light of these results, one can speculate that fluid (driven by flagella in the relatively superficial healthy part of the colony) might percolate throughout the intraskeletal canal system, even in regions underlying places overgrown with algae and other organisms. Such a flow could conceivably distribute nutrients or coral cells to regions where they might influence the repair of the locally overgrown parts of the colony overlying them.

Light microscopic and molecular techniques (internal transcribed spacers, ITS and 18S cDNA) were combined to provide an overview of the overgrowing and endolithic organisms associated with *A. cytherea.* The sampled regions of the colony were living, recently dead (near living coral tissue) or long dead (far from living coral tissue). The data permitted mostly genus-level identification of macroalgae, endolithic and epilithic algae, and endolithic fungi. A comparison between living, recently dead, and long dead

samples demonstrated a succession in community composition of the algae. Even so, there were notable similarities in the genera of endolithic fungi present in the skeleton of living and dead regions of the colony. The endolithic communities within the coral may be a link between live and dead coral in the calcium carbonate structures. This might reflect a uniformity and connectivity maintained by the deep circulation within the unobstructed lumens of the intraskeletal canal system throughout the colony, even beneath superficially overgrown areas.

In sum, the results of the different parts of this dissertation point to the need for further studies of the recently neglected perforate skeleton character and connecting endoliths and their possible relationship to resilience of some perforate corals. Understanding partial survival and dead coral skeleton may hold additional hints at maintaining and protecting coral reef ecosystems.

CHAPTER 1:

Partial survival: remembering how colonial corals improve reef recovery

Kathryn A. Furby, Gareth J. Williams, Stuart A. Sandin

Colonial organisms compartmentalize death. Mortality occurs at the scale of the colonial unit, in many instances affecting only a subset of the entire colony without compromising basic colony function. Such 'partial mortality' of colonies (Hughes and Jackson 1980) can be offset by creation of new colonial units; among clonal taxa, such 'replacement' units are created through budding or asexual reproduction (Jackson et al. 1985). The paired process of compartmentalized death with modular replacement suggests that some colonial organism may approach so-called 'modular immortality' (e.g., Watanabe et al. 2009). Few ecosystems can rival coral reefs in their foundational dependence on modular life and death.

Early studies of coral clonal growth and mortality involved experimental injury to colonies (in the lab and in the field), often focusing on patterns of regeneration (Stockard 1909, Cary 1914). Focal topics ranged from understanding the energetic demands of regrowth, quantifying rates of function of type of severity of injury, to exploring taxonomic specificity in manner of recovery. As evidence that the health of corals themselves was being compromised by local and global environmental changes, coral research moved into a "doom and gloom" period. Studies were focused more singularly on rates and patterns of coral mortality (e.g., 1980 Caribbean disturbance events, Hughes 1994; 1998 mass bleaching event, Goreau et al. 2000; Fig. 3A, B). Despite the paradigm for coral ecology suggesting that coral reef communities reflect a history of disturbance (Connell 1978, Hughes and Jackson 1985, Bythell et al. 1993), it is critical to note that they are also a reflection of a history of recovery (Darling et al. 2012, Hughes et al. 2012). To maintain focus on growth and recovery, we emphasize the foundational

importance of coral coloniality and regrowth, and thus we prefer consideration of the reverse of partial mortality: 'partial survival.'

Ecosystems are no longer being shaped by the same conditions under which they evolved. Coral reefs were historically shaped by acute disturbances such as storm frequency and intermittent coral bleaching events, however, they are now driven by chronic stressors such as overfishing or sedimentation. Humans are changing selective pressures that shape coral reef community organization (Williams et al. 2015). As humans begin to drive evolution, how will this change coral survival strategy?

This piece will explore: (1) how clonal growth is critical to scleractinian coral persistence for the past 200 million years, and (2) how the current literature has treated colonial partial survival and regrowth and why we need to advance the scientific discussion.

From dinoflagellates to dinosaurs: the evolution of coral reefs

The colonial form evolved in a wide variety of organisms throughout geological time, growing in pieces (modules) with sexual and/or asexual reproduction (Harper et al. 1986). During the evolution of colonialism, individual units either adhered to each other or differentiated into modules via asexual budding. The established consortium and subsequent cell-to-cell communication created a more complex organism (Niklas and Newman 2013). Colonialism occurs in phyla such as Poriferans, Bryozoans, Chordates (i.e., tunicates) and Cnidarians (i.e., corals) (Harper 1985, Coates and Jackson 1985).

The first reef-building, or scleractinian, corals appeared in the fossil record at the end of the Triassic Period (~250 mya), however they did not form modern reef structures until the Jurassic (~200 mya, Veron 1995). In an ecosystem with large marine dinosaurs, early corals were small, solitary polyps. At the start of the Jurassic period, two significant changes occurred: (1) corals showed possible evidence of symbiosis with unicellular algae (zooxanthellae), and (2) corals created colonial structure (Veron 1995). Colonial morphology and photosynthetic symbionts created a positive feedback loop; the colony built large structures and the symbionts gathered more energy for growth. Now corals could acquire the resources needed to create and maintain large body structures (Wulff 1985), ultimately leading to the formation of geologically significant coral reef structures (Coates and Jackson 1987). If coral polyps are the modular building blocks of the reef, coral colonies are the architecture and the infrastructure (Coates and Jackson 1986).

Safety in numbers: benefits to colonialism

Across taxa, colonial organisms benefit from increased persistence, rapid clonal reproduction, and shared resources, when compared to singular organisms (Coates and Jackson 1985, Harper 1985, Hughes 1989). A colonial organism's capacity for modular survival is greater than the sum of its parts. The colony can survive significant injury, even losing a large percentage of its units, without losing much bodily function (Highsmith et al. 1980). Large colonial corals live to die another day. They can crowd source their issues.

The modular organization of coral colonies spreads the risk of mortality among units (Hughes 1983). Despite suffering injuries, a large coral colony may still survive to reproduce in the next years. By translocating metabolites to perimeter polyps, corals can grow faster from damaged areas (regeneration) than from undamaged areas (standard growth) (Rosen 1986, Loya 1976, Hughes et al. 1992). This mechanism aids rapid regrowth of live tissue over existing skeleton, a key adaptation that allows corals to quickly regain size and shape. It also means that there is less time spent in contact with the turf algae overgrowing the dead skeleton. Regrowth recovery mechanisms are more efficient and stable than the higher mortality growth associated with new settlement (Hughes et al. 1992). In some cases, the loss of modules results in fragmentation, where the removed piece of the colony is able to survive and contine growth as a separate unit (Highsmith et al. 1980). Fragmentation is both a mechanism of survival and death, with survival sometimes creating additional reef substrate, as a piece of dislodged coral may grow onto turf covered substrate or even sand (Highsmith 1982). Unlike a coral recruit, an adult fragment may have the size and resources for competitive success on substrates not available for settlement.

One critical benefit of coloniality is the capacity to store and share resources, improving the fitness of individual units relative to solitary modules. Increasing stored resources acts like an insurance plan for changing environmental conditions (Edmunds and Davies 1986). Corals in particular benefit because they are benthic, sessile organisms, which must compete for available substrate and cannot move to track resources (Harper 1985, Tiffnay and Niklas 1985). As such, colony size often linked positively to overall survival and reproductive capacity (Harrison and Wallace 1990,

Lang and Chornesky 1990, Hughes et al. 1992, Baums et al. 2006, Muller et al. 2014). A larger colony can store more energy reserves and use to increase fitness. Further, there are positive feedbacks associated with coloniality. Larger colonies with increased surface area have greater access to water flow and the particulate matter in the water that the filter-feeding coral relies on for food (Ryaland and Warner 1986). The benefits of heterotrophy in scleractinian corals are many, including increased condition, growth and reproduction, as well as a likely increased ability to recover from warm water bleaching events (Ferrier-Pagès et al. 2003, Grottoli et al. 2006).

Long generation times and minimal senescence allow coral colonies to persist on reefs as reproductively contributing individuals. Coral colonies can grow and shrink in response to environmental factors, creating a lack of association between size and age (Hughes and Jackson 1980). Depending upon history, a young colony under favorable conditions can be much larger than an older colony that has suffered continued stress. Independent of age, the relative size of these hypothetical colonies will be linked closely to patterns of survival (Vermeij and Sandin 2008). However in some cases the relative age of a colony will interact with size. For example a large coral colony that suffers partial mortality has size advantage over a newly settled coral recruit (Barnes and Hughes 1999). This size advantage allows the coral to maintain its place on the reef, giving it a competitive advantage over a new recruit that must establish a place to grow. In addition, surviving remnant colonies allow the reef to maintain its original community composition, as new recruits may be a different composition and diversity as the original corals (Connell et al. 1997, Tamelander 2002). If the corals on a reef suffer partial

mortality, it loses coral cover but not diversity. If the corals on a reef suffer complete mortality, it loses cover and potentially diversity simultaneously.

Three's a crowd: disadvantages to colonialism

The integration of modules into a large colonial organism can also be a detriment. One common disadvantage to being a colony is reduced genetic variation. However, some colonial organisms can reproduce both clonally and sexually, thus avoiding the clonal reproductive issues of limited genetic variation (Hughes et al. 1992). In addition, increased colony size and increased surface area increases the statistical likelihood of exposure to stressors such as disease or predation (Hughes and Jackson 1980, Grober-Dunsmore et al. 2006). While colonial morphology allowed the corals to evolve into larger body forms, they cannot move (Veron 1995). Coral reef growth is limited by available light and suitable substrate (Connell et al. 2004). Once a coral has settled, its colonial growth is mostly limited by its surrounding habitat. Most colonial organisms experience a tradeoff: they can store large amounts of resources and built big structures, but they lose mobility (Harper et al. 1986). Since most corals cannot physically avoid injury or competition by rapid movement, most corals must use increased defenses to survive (Harper et al. 1986, Chadwick-Furman and Loya 1992). In addition to increased mucus production for protection (Chadwick 1988), corals have nematocyst cells coiled for strike, should a competing organism come too close (Sheppard 1979). The growth and maintenance of these defenses has metabolic costs (Chadwick 1988).

Cheating death: the ecology of regrowth

Regrowth has the potential to significantly accelerate population recovery (Hughes et al. 1987, Diaz-Pulido et al. 2009, Gilmour et al. 2013). Under favorable conditions, the surviving coral tissue may regrow over the skeleton after partial survival. This process is referred to as "regrowth," and in some cases, "resheeting" (Jordán-Dahlgren 1992). As the colonial corals are modular, they can suffer the loss of polyps and regrow from surviving units. This occurs at small intra-colony scales following injury (Williams 2013), as well as at a population scale to promote resilience following environmental stress. In order for regrowth to be used as a primary recovery mechanism, a number of (partial) adult colonies must survive (Fig 1). For example following bleaching-induced coral mortality in the Keppel Islands, *Acropora* coral colonies remained alive in the cryptic microhabitats at the base of its branches. They were overgrown by a competitive macroalgal community, disappearing from view. Once a seasonal decline in the algae occurred, the corals regrew over their dead skeleton, leading to complete population recovery (Diaz-Pulido et al. 2009). Analysis of cross sections of *Acropora* colonies revealed that although extended areas of the colonies had died, resheeting of the coral skeleton was the mechanism of rapid population recovery.

Survival and recovery of coral populations varies drastically depending on species (Bythell et al. 1993, Baird and Marshall 2002), biological traits and environment (Hughes et al. 2012, van Woesik et al. 2012). For example, severe, chronic disturbances, such as overfishing or sedimentation, can decimate a population, leaving minimal numbers of surviving colonies (Fig 2, Nyström et al. 2000, Graham et al. 2013). This is also known

as a press disturbance, or a disturbance that may start suddenly, but that reaches a constant level over time (Parkyn and Collier 2004). If during these events the colonies suffer complete mortality, the success of the genet relies on the survival of previously spawned recruits. Coral survival during these events is reliant on environmental history and species-susceptibility. In 1993, Bythell and colleagues tagged and tracked the success of several coral populations through chronic and pulse disturbances. Coral response and community diversity changes varied due to morphology, species, location (and the location's history of disturbance) (Bythell et al. 1993). In 1997, Connell, Hughes and Wallace published a study based on 30 years of data surveying Heron Reef. They argue that in addition to the species and history of disturbance, the kind of disturbance is also critical. If the disturbance is chronic and impacts the reef as a whole (e.g., cyclones that change tidal movements, or human impacts such as sewage outfall), the corals will recovery more slowly, if they recover at all (Connell et al. 1997).

In contrast, a pulse disturbance is similar to the disturbances with which corals have evolved over time (e.g., tropical cyclones, brief warm water periods, freshwater pulse events). Corals are able to use their evolutionary tool kit to survive these events. Fragmentation, partial survival and regrowth are all common in these cases. Unfortunately, human impacts may be pushing the ecosystems towards increased press disturbance, increasing whole colony mortality and eroding key resilience mechanisms such as the potential for coral regrowth (Fig 2).

Not cheating death: the ecology of how humans may ruin everything

Coral recruitment and herbivory facilitate coral community growth (Hughes et al. 2012, Graham et al 2013). However, partial survival and regrowth are additional advantages available to colonial organisms adapting to changing environmental conditions. As scientists, we may not be writing about regrowth and partial survival because we are not observing it. Coral reefs worldwide are vulnerable to the interactions of climate change and local stressors (Nyström et al. 2000), and as such, also vulnerable to human-induced selection. If human-induced environmental stresses trigger more complete mortality, the current literature could reflect that.

How could human impacts favor complete mortality? After partial mortality, conditions must be favorable for successive coral growth and regrowth. The coral needs to recolonize lost substrate. In Figure 2, we describe alternate scenarios for partial mortality, given different reef conditions. Regardless of the mortality size or cause, turf algae is an early, quick colonizer of the dead coral skeleton (Diaz-Pulido and McCook 2002). What happens to the substrate after this depends on corals' environmental context (Connell et al. 1997). A human impacted system has increased local stressors such as nutrient pollution from runoff and sedimentation from coastal development. Increased nutrients and sediments often give algae the competitive advantage over hard corals (Lapointe 1997). Impacted systems may also have low grazing pressure due to human overfishing of herbivores (Hughes et al. 1987). These concepts are all part of the ratcheting down of coral reefs: humans remove and degrade the grazing, calcifying players and indirectly promote the bioeroding, nonreef-building, or corallivore players through overfishing and pollution (Birkeland 2004). Thus under these conditions, partial mortality may lead to complete mortality. In a functionally intact system (a system

relatively un-impacted by humans), herbivore grazing and clear oligotrophic waters contribute to excellent growth conditions for reef-building organisms such as crustose coralline algae and hard corals (Williams et al. 2015, Smith et al. 2016). Thus the coral partially survives mortality to continue regrowth over its dead spaces (Fig 2). These conditions are favorable for recovery at a community scale (Connell et al. 1997). By promoting calcifiers and protecting the reefs against breakage, the corals and calcifying algae like crustose coralline algae (CCA) cement the three-dimensional structures in place (Smith et al. 2016).

The chronic disturbances mentioned above are common on reefs with human populations. The colonial mechanisms that corals use to respond to natural disturbances are no longer advantageous in the chronic stresses scenarios (Connell 1997). Anthropogenic impacts may be changing the environmental conditions of coral reefs too rapidly or too severely for the corals to survive using their current adaptations. Does our access to reefs skew our view of them?

However, it is a big world out there and many remote reefs escape direct human impacts. Recent studies in near-pristine reef systems reveal hope for coral reefs of the future, provided humans can stop bothering them. A recent study by Furby and colleagues (2017) found regrowth was an important factor in *Porites superfusa* growth after a selective bleaching even in the remote central Pacific. In 2014 George Roff and colleagues found *Porites sp.* regrowth allowed unprecedented recovery from the 1997/1998 El Niño bleaching event in French Polynesia. The authors remarked on the rapid resheeting from remnant, surviving patches of tissue. In 2013 James Gilmour and colleagues published a long term study of recovery. In this case there was evidence of

coral recovery during a recruitment lag. Upon further investigation, they indicating that regrowth of surviving adults may have played a critical role at Scott reef far off the coast of Australia. Other remote reefs show similar signs of promise (Sandin et al. 2008, Smith et al. 2016) and recent papers studying regrowth and recovery are increasing (Graham et al. 2015, Cinner et al. 2016). Removing local stressors and protecting healthy reefs with a dominance of calcifiers, will encourage colonial recovery mechanisms to maintain diversity and structure on reefs (Williams et al. 2010, Vroom et al. 2010, Jouffray et al. 2015, Smith et al. 2016). Unfortunately with increasing temperature anomalies and coral bleaching events, just reducing local stress may not be adequate for some reefs (van Hooidonk et al. 2016, Hughes et al. 2017).

Death faster than growth: challenges of studying recovery

Despite the fact that coral communities generally lose more tissue from partial mortality than complete mortality, most research to date has focused on whole colony mortality (Hughes et al. 1992). Partial mortality and regrowth are more subtle processes than complete mortality and recruitment. The biggest challenge to studying these processes is scale. Complete mortality and recruitment occur at the population level, but partial mortality and regrowth by definition occur within a colony. Thus we have to alter the scope of research to capture these changes, some of which are often slow and subtle. Recruitment and whole mortality can also be recorded in a single time point, based on tiny corals or large dead skeletons. Regrowth by definition requires a time component to prove the same coral has regrown.

Thus methods for studying these different processes are obviously different. Analyzing regrowth as a recovery mechanism requires studying corals at a small and large scale simultaneously. Spatial and temporal coverage at a colony scale is needed to capture ecological variability. This can be challenging, since it generally requires tagging of individual colonies, which can be logistically complicated and time-intensive; thus replication is often low, making inferences about whole population dynamics difficult (Connell 1997, Bruckner and Hill 2009, Glassom and Chadwick 2006).

In comparison studying recruitment and mortality is logistically simpler. Coral cover, a standard metric of study, is a measure of the total corals evident on the substrate. These surveys work adequately for big changes at a population level, like recruits and whole dead corals. However, monitoring coral cover may be missing the dynamic mechanisms on the reef that are essential to understanding communities (Porter et al. 1982, Burt et al. 2008, Bruckner and Hill 2009). Within-colony changes like regrowth have high potential for preserving diversity and maintaining reef structure. However, broad surveys overlook fine-scale dynamics and risk building skewed models of reef health.

Modeling studies are able to address the limitations field work by creating reconstructions of coral reefs in digital space to examine (Mumby et al. 2007, Sandin and McNamara 2012, van Woesik et al. 2012). We need to emphasize additional studies at fine-scale interactions, in order to parameterize models. With the advent of new, large-scale photographic techniques, modern study of regrowth may become relatively easy (Lirman et al. 2007). Using photographic mosaics and detailed *in situ* analysis, it may be

possible to begin collecting data relevant for answering questions about the changing recovery mechanisms on coral reefs.

Prepare for the worst, expect the best: managing reefs for recovery

Despite the documented persistence of corals through the fossil record, it appears modern reefs are changing at an unprecedented rate (e.g., Hoegh-Guldberg 1999, Pandolfi et al. 2003). Understanding coral death is important, but coral decline has been well-documented for the past 30 years. Coral recovery is the ultimate goal, but it is a slower and subtler process. Unfortunately, the time scale for coral reef death is much faster than the speed of recovery. If we can understand the conditions under which corals regrow, we can use management to intelligently limit local stressors and set coral reefs up for success (e.g., Bellwood et al. 2006, Diaz-Pulido et al. 2009). By doing this, we continue to safeguard coral reefs and promote resistance.

We need to understand when and why partial survival occurs in order to promote it. To give corals the advantage over fleshy algae in the fight to recolonize lost space on the reef, we need to reduce nutrient inputs, manage herbivore fisheries and most importantly reduced carbon dioxide emissions (e.g., Jackson et al. 2001, Pandolfi et al. 2003, Hughes et al. 2017). Local, chronic stressors compound global ones, and thus to promote survival, we need to reduce those human impacts (e.g., Knowlton 2001, Nystrom et al. 2000, Graham et al. 2013). Terry Hughes and colleagues recent report on global stressors indicates coral reefs are suffering from frequent massive coral bleaching

events at an unprecendented scale, and immediate reduction of greenhouse gases is critical (Hughes et al. 2017).

If we want to utilize the evolutionary advantages of colonial corals, management strategies should be designed around maximizing tissue survival and promoting regrowth. Recent studies have shown promising effect of surviving remnant corals (Diaz-Pulido et al. 2009, Gilmour et al. 2013, Roff et al. 2014). These corals live in remote places where there may be a tradeoff between reproductive isolation and reduced human impacts (Gilmour et al. 2013). More information is needed to solidify the link between regrowth recovery and remote reefs. Understanding patterns of regrowth could be a key process in maintaining existing coral communities. For example, if regrowth is the primary recovery mechanism, management needs to focus on protecting that reef from local impacts. However if recruitment and connectivity of neighboring reefs is primarily seeding recovery, then we want to protect networks of reefs (Edwards et al. 2010). Although ultimately because of climate change and severe coral bleaching events, current coral communities have little chance of survival. Relying on recruitment effort alone risks changing communities in response to anthropogenic pressure and risks all isolated reef systems. We need high taxonomic resolution and an understanding of species-specific switches following acute and chronic stress, in order to create science-based solutions to global and local coral issues.

Conclusions

For much of coral research, we have applied a glass half empty approach to studying coral death. Coral colonies have evolved with a complex survival kit for natural stresses, such as intermittent hurricanes and short-lived sea surface temperature increases. Corals colonies are capable of partial death, leaving other parts of the coral alive. The partial survival modularity maintains the diversity of the original coral community.

Human impact may be selecting for a loss of resilience. We know how to kill corals, and we know how to record their death. However, if we do not completely understand how to regrow corals, how will we be able to predict and promote their survival? Therefore we need to promote reef structure resistance and rapid regrowth.

Recent technology has made it easier to study the fate of surviving coral colonies, even after partial mortality. Understanding partial survival and subsequent regrowth can fundamentally change our view of what a healthy or dead coral is. Is the coral cup half empty or half alive? Focusing on coral recovery over coral destruction is not enough to prevent their demise. How close are we to having aquariums resemble museums of extinct organisms? Reducing carbon emissions significantly and minimizing local impacts is our road map for preserving corals reefs into the future.

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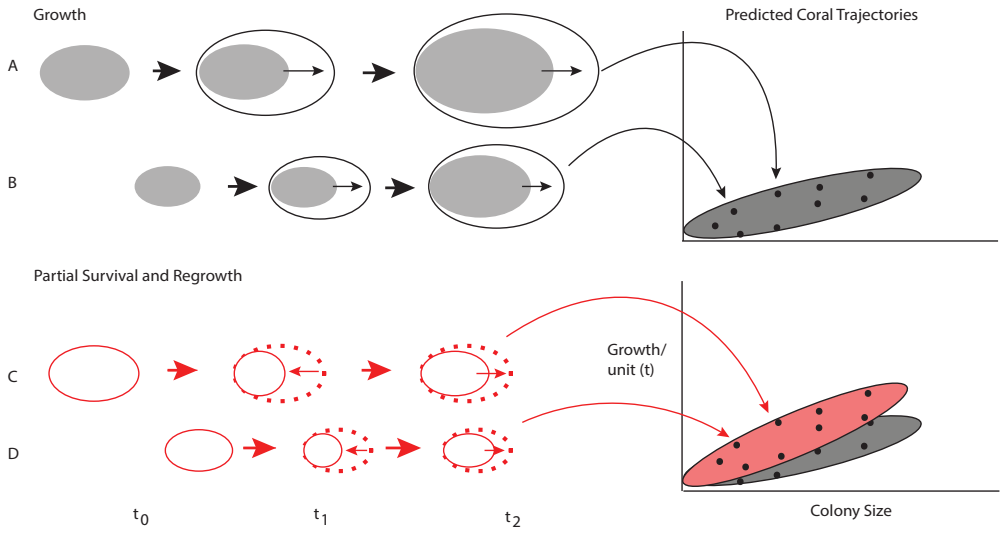

Figure 1-1. Standard coral growth compared with coral survival and regrowth after tissue loss. White indicates initial coral state. Grey illustrates ideal growth scenarios under linear life history and growth. Red indicates growth and death scenarios under coral partial mortality and regrowth scenarios. A) and B) show two theoretical paths for coral linear growth. C) and D) show two theoretical paths for coral partial survival and regrowth. Regrowth allows the coral to regain the structure and space on the reef more rapidly than standard coral growth and recruitment. Regrowth can accelerate the theoretical recovery and coral growth.

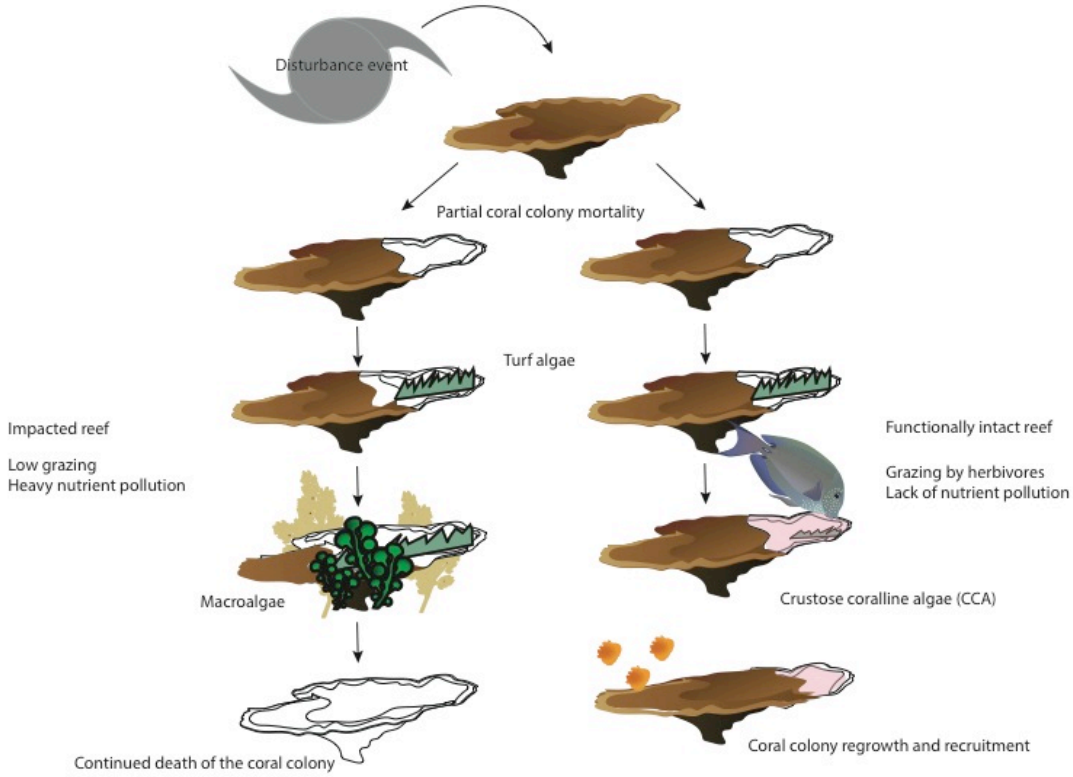

Figure 1-2. Contrasting scenarios of ecological succession on a coral reef following an acute disturbance event. The scenario on the left represents the succession of newly available substrate resulting from partial mortality of a coral colony on an impacted reef; this scenario leads to continued death of the coral colony. In contrast, in the scenario on the right, the same partial mortality on a functionally intact coral reef results in the substrate returning to coral via either colony regrowth or recruitment.
Images from the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

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CHAPTER 2:

Porites superfusa mortality and recovery from a bleaching event at Palmyra Atoll, USA

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Porites superfusa mortality and recovery from a bleaching event at Palmyra Atoll, USA

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ABSTRACT

Background. The demography of a coral colony is not a binary trajectory of life and death. Based on the flexibility afforded by colonial organization, most reef-building corals employ a variety of dynamic survival strategies, including growth and shrinkage. The demographic flexibility affects coral size, shape and reproductive output, among other factors. It is thus critical to quantify the relative importance of key dynamics of recruitment, mortality, growth and shrinkage in changing the overall cover of coral on a reef.

Methods. Using fixed photographic quadrats, we tracked the patterns of change in the cover of one common central Pacific coral, *Porites superfusa*, before and after the 2009 ENSO event.

Results. Coral colonies suffered both whole and partial colony mortality, although larger colonies were more likely to survive. In subsequent years, recruitment of new colonies and regrowth of surviving colonies both contributed to the modest recovery of *P. superfusa*.

Discussion. This study is unique in its quantitative comparisons of coral recruitment versus regrowth during periods of areal expansion. Our data suggest that recovery is not limited simply to the long pathway of settlement, recruitment and early growth of new colonies but is accelerated by means of regrowth of already established colonies having suffered partial mortality.

Subjects Ecology, Marine Biology

Keywords Climate change, Coral bleaching, Population dynamics, ENSO, Coral regrowth, Coral recovery

INTRODUCTION

Understanding patterns of mortality and recovery among reef-building corals is foundational to the development of accurate predictions of coral population trajectories (*Baird & Marshall, 2002*). Recruitment has been recognized as important for long-term recovery after major and frequent disturbances, especially for coral populations that have suffered widespread, whole-colony mortality (*Dollar & Tribble, 1993*). However, many disturbance events are less extreme, leading to a combination of partial and complete mortality. We define partial mortality of colonial corals as the response to a stress event that leads to tissue loss, but with the survival of some colony area. For colonies suffering from partial mortality, regeneration of tissue following stress is a critical mechanism in

assuring individual colony survival (*Chadwick & Loya, 1990*). As such regrowth of corals has been suggested as an important mechanism in coral population recovery (*Diaz-Pulido et al., 2009*; *Gilmour et al., 2013*). In this context, analyzing recovery characteristics can help project population resilience.

Given the colonial nature of most reef-building corals, the definition of an 'individual' presents real challenges (*Hughes, Ayre & Connell, 1992*). Coral ecological literature often refers to an individual as a colony. Interactions and population dynamics are sometimes best described at the colony scale, including patterns of size-specific mortality, competition for space on the benthos, and vulnerability to storm damage (e.g., *Hughes & Jackson, 1980*; *McCook, Jompa & DiazPulido, 2001*; *Baird & Marshall, 2002*; *Madin et al., 2014*). In contrast the physiological definition of an individual is the polyp (*Harper, 1985*). In particular both asexual (fission/ budding) and sexual (spawning/ brooding) modes of reproduction occur at the scale of the polyp. When tracking demographic responses to environmental change or disturbance, it is critical to account for dynamics occurring at both the scale of the colony and the scale of the polyp.

Corals are capable of four known mechanisms of loss and recovery: complete-colony mortality, partial colony mortality (tissue loss), recruitment, and partial-colony growth (sometimes referred to as regrowth) (Fig. 1). A few recent studies have documented the relative importance of recruitment and regrowth as mechanisms of coral population recovery following a disturbance (*Diaz-Pulido et al., 2009*; *Gilmour et al., 2013*; *Roff et al., 2014*), however, the colonial mechanisms of coral recovery have not been regularly quantified.

Due to its remote location and lack of local anthropogenic impacts, Palmyra Atoll is an ideal location for studying demographic rates of corals in response to global change (*Knowlton & Jackson, 2008*; *Sandin et al., 2008*; *Williams et al., 2011*). Palmyra is a US National Wildlife Refuge and is part of the Pacific Remote Island Areas National Marine Monuments and is protected from fishing and other impacts. In 2009, Palmyra Atoll experienced a mild bleaching event associated with an El Nino Southern Oscillation (ENSO) event (*Williams et al., 2010*). During the 2009 ENSO event, sea surface temperatures reached 1.5 °C above the maximum long-term monthly temperatures, and the anomaly continued for four months (*Williams et al., 2014*). October 2009 through March 2010 had over 4 DHW (degree heating weeks) with November through March over 8 DHW (NOAA Coral Reef Watch).

This study examines patterns of change in an encrusting coral, *Porites superfusa,* on Palmyra Atoll during and after the thermal stress event. Specifically, our objectives were to address two complementary questions: (1) Does *P. superfusa* cover change through time from 2009 to 2012 on Palmyra Atoll? (2) What are the relative rates of colony survivorship (recruitment/ mortality) versus colony growth (growth/shrinkage) during and after a bleaching event?

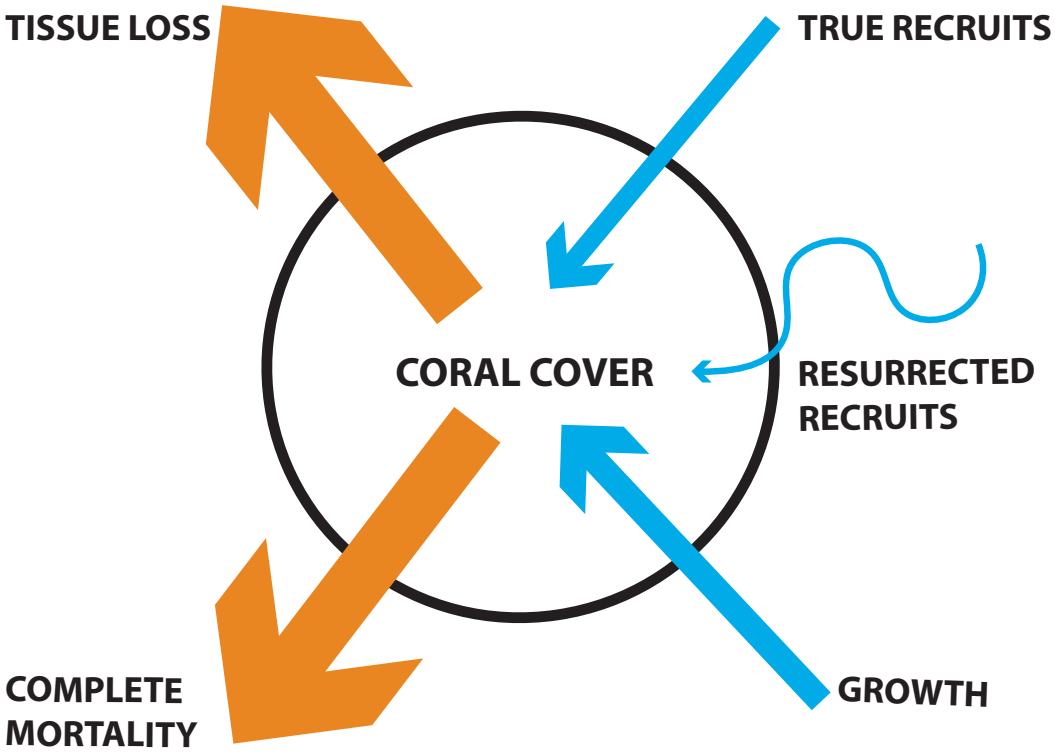

Figure 2-1 Conceptual diagram of coral demographics contributing to total coral cover for a popula-tion. Blue (right side) arrows denote contribution to increasing coral cover (e.g., True recruits, 'Resur-rected' recruits, and Growth). Orange (left side) arrows denote contribution to decreasing coral cover (e.g., Partial mortality and Complete mortality). 'Resurrected recruits' is a new term, an operationally defined form of regrowth (see Methods for detailed description). The thickness of arrows indicates the mechanism's relative contribution, as found in this study.

MATERIALS & METHODS

Study species

Porites superfusa is a small coral that is relatively ubiquitous on Palmyra Atoll, reaching almost 400 cm^2 in area with an overall mean colony size of 9.9 cm^2 (median = 4.4 cm^2). Our targeted design involved this species because it is one of the most numerous corals in the area, and its compact morphology allows study of multiple individuals within square meter photoquadrats (see below). In 2009, the *P. superfusa* population showed extensive evidence of bleaching with a notable reduction in cover in the permanent photoquadrats.

Surveys

Four forereef sites on Palmyra Atoll were selected, two sites on the north and two sites on the south shore, each approximately 2 km apart (Fig. 2). Sites were chosen to be representative of Palmyra's forereef habitats. These sites all had high initial densities (C_{2009}) of *Porites superfusa* colonies, and as they are evenly spaced across the island they should capture within-island differences in the forereef habitat. Sites were surveyed four times at approximately annual intervals (September 2009, July 2010, September 2011, and September 2012).

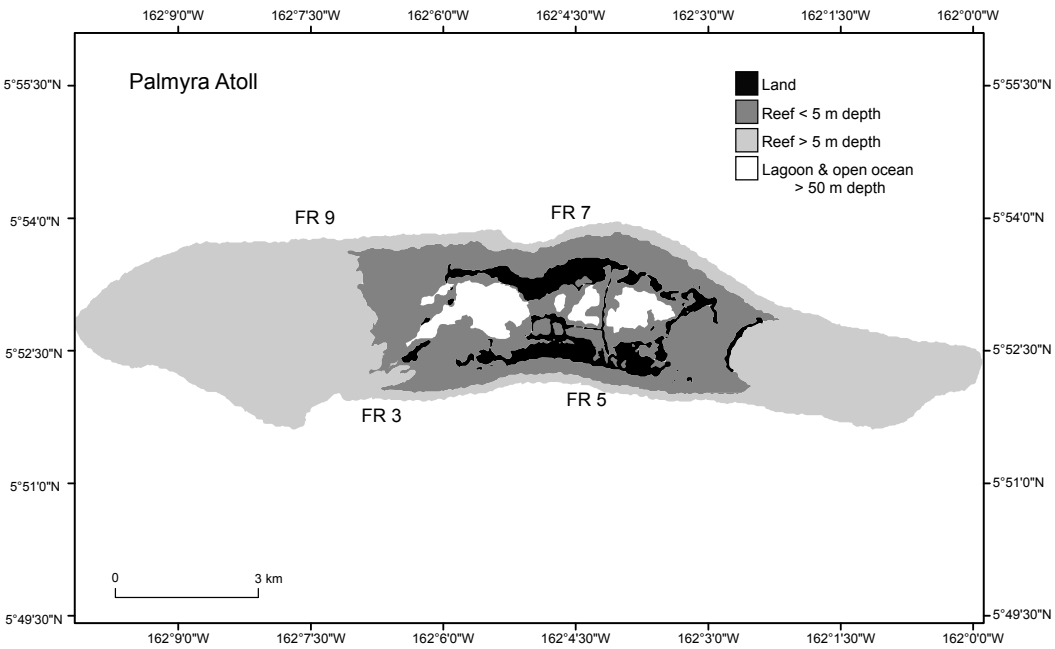

Figure 2-2 Study sites (FR3, FR5, FR7, FR9) around Palmyra Atoll. Palmyra is located in the remote cen-tral Pacific. The black area shows the atoll land area and the gray and white areas denote different depth strata of reef and open ocean.

At each site, one 50 m transect was permanently marked, parallel to shore and at a depth of 10 m. In 2009, ten permanent photoquadrats were established at each site, positioned every 5 m along the transects. The corners of plots were marked with stainless steel eyebolts held in place with marine epoxy (US Fish and Wildlife Special Use Permit 12533-16006). Each photoquadrat was imaged with a Canon G12 camera attached to a PVC frame (0.54 m^2) by SCUBA divers (*Sandin et al., 2008*). These marked plots were revisited and re-surveyed, enabling the tracking of individual colony fates through time. In sum, 40 0.54 m^2 plots were surveyed annually for a total of four time points each.

Image analyses

Within each photograph, colonies of *P. superfusa* ranged in area from 0.2 cm^2 to 440 cm^2. Due to the limitations of the photographic resolution, the tracking of the smallest colonies was not possible and thus we focused our study on colonies exceeding 1 cm^2. Photographs were analyzed using ImageJ to calculate the size (estimated as 2-dimensional area when viewed from the top down) and survivorship of each *P. superfusa* colony within the images (Fig. 3, *Abramoff, Magalhaes & Ram, 2004*). Each colony was tagged digitally and tracked through time (similar to methods of *Hughes & Jackson, 1985*). Fates of the colonies were placed into one of five categories—complete mortality, partial mortality, true recruits, growth, and 'resurrected' recruits. Complete mortality was defined as the death of the entire visible coral, with its previous location overgrown by other organisms (Fig. 3B-1). Partial mortality (i.e., injury, shrinkage) was recorded when colonies lost tissue (Fig. 3B-2). True recruitment (or settlement) indicated a new coral recruit had claimed

substrate in an area previously without *P. superfusa* (Fig. 3B-3). Growth occurred when a previously present colony created additional tissue (Fig. 3B-4). 'Resurrected' recruits were defined as apparent recruits that appear in a location where a colony had been recorded as suffering mortality in previous time points (Figs. 3C-5 and 1). Because of the limitations of the photographic census, it was impossible to determine whether such recruits were new recruits (new colony settling at the same location) or regrowth from microscopic areas of cryptic remnant tissue. Individual colony cover and total *P. superfusa* cover were calculated per quadrat. Data are reported in two formats: colony-specific fates and total live *P. superfusa* cover summed within quadrats tracked through time.

Data analyses

Analyses assessed patterns of variation in starting live *P. superfusa* cover across sites, thus determining whether changes through time were similar among years. One-sample *t*-tests were used to calculate differences of *P. superfusa* cover among sites in 2009 and to determine differences between absolute change and proportional change in coral cover between time points. Absolute change was the difference in coral surface area (cm^2) per quadrat (e.g., cover in 2010—cover in 2009, or C_{2010}-C_{2009}). Proportional change was the relative difference in coral cover between time points compared to the original coral cover in 2009 (e.g., $[C_{2010}\text{-}C_{2009}]/C_{2009}$). Tukey's post-hoc tests were used to determine potential differences among sites.

Because the same individual colony can appear in multiple years, analyses assessed the possible effect of independence of colonies through time. ANCOVA was used to determine interaction effects of year and site. Because 109 colonies were repeatedly assessed, data were randomly sampled using each colony only once. A binomial logistic regression was used to determine the effect of colony size on survivorship across years. Analyses were performed using R version 3.1.2 (R Development Core Team, http://www.r-project.org).

RESULTS

The 2009 temperature rise impacted approximately 75% of the *Porites superfusa* population at Palmyra Atoll with bleaching and mortality observed island-wide. The initial size of individual colonies was greater on average than in subsequent years. The number of colonies present in 2009 was also greater than in later time points. True recruit numbers in 2010 (after the bleaching event of 2009) were lower than in 2011 and 2012 (Table S1).

All sites surveyed exhibited similar patterns of decline in *P. superfusa* from 2009 to 2010, followed by fluctuations of growth in 2011 and 2012 (Figs. 4 and 5). From 2009 to 2010, *P. superfusa* mortality was ubiquitous across sites, and the growth for this period was the lowest observed during the study (Fig. 4). Mortality was divided into two categories: complete and partial mortality. These mechanisms contributed similarly to the reduction in cover observed (Fig. 4, partial mortality: −101.8[56.79] cm^2 per quadrat, mean [SE], complete mortality: −105.7 [39.10] cm^2 per quadrat, mean [SE]). Recruitment and growth were minimal initially, revealing no appreciable difference among growth mechanisms (Fig. 4, Recruit: 4.99 [1.31] cm^2, Growth: 4.56 [0.69] cm^2 per quadrat). Across the study duration, the highest rates of growth were observed from 2010 to 2011. Resurrected recruits (i.e.,

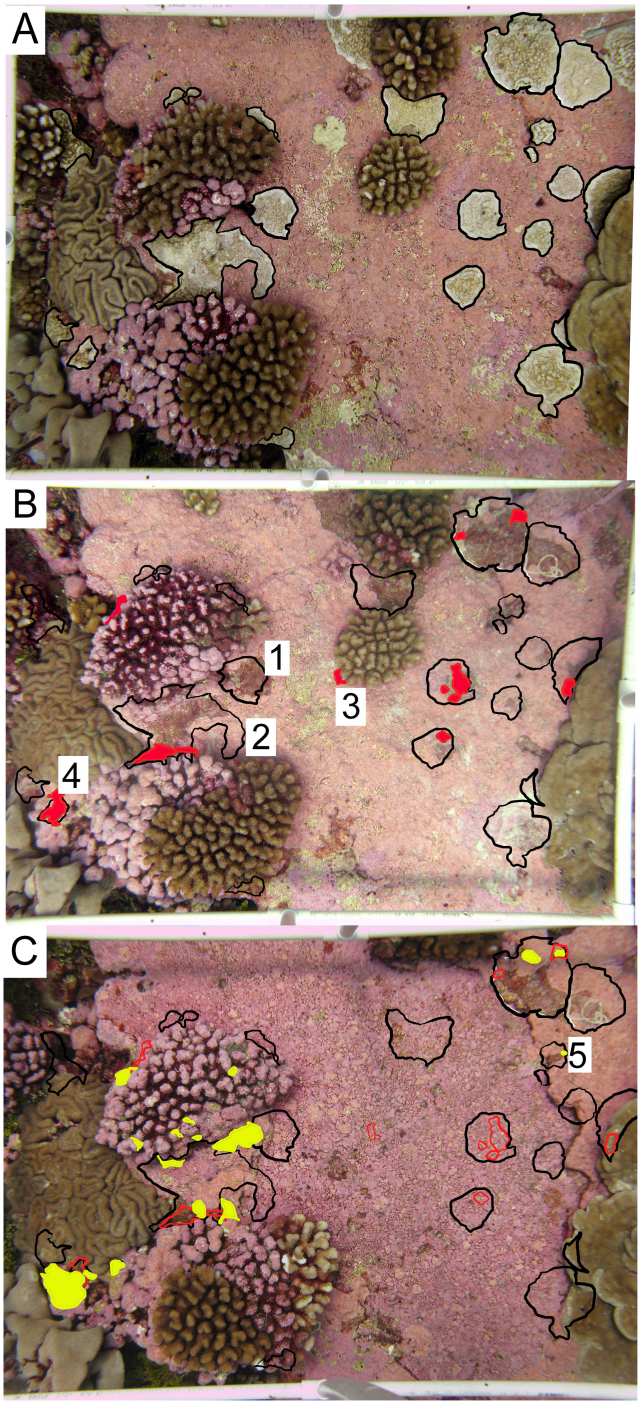

Figure 2-3 Representative permanent photoquadrat sequence from one site (FR9). (A) September 2009 (C_{2009}) with living *Porites superfusa* outlined in black. (B) July 2010 (C_{2010}) with previous 2009 colony area outlined in black, and living coral highlighted in red. (C) September 2011 (C_{2011}) with previous 2009 colony area outlined in black, 2010 colony area outlined in red, and living coral highlighted in yellow. Colonies that show examples of different fates are labeled with numbers: (1) complete mortality, (2) par-tial mortality, (3) true recruitment, (4) growth, and (5) resurrected recruits. Quadrats are 0.6 m^2.

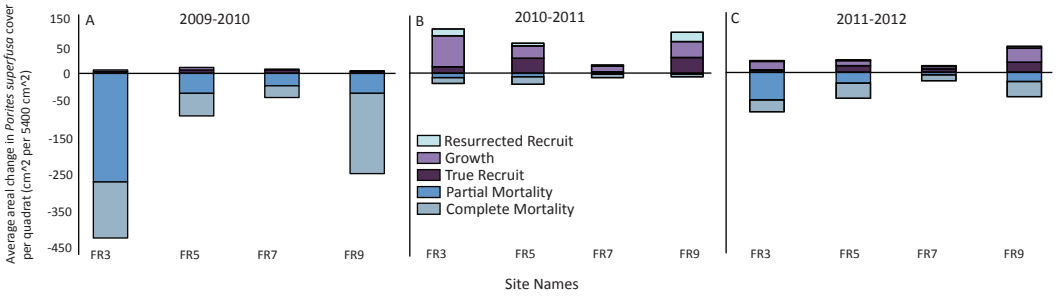

Figure 2-4 Patterns of gain and loss for *Porites superfusa* during- and post-bleaching event. (A) 2009 to 2010 change in cover, (B) 2010 to 2011 change in cover, (C) 2011 to 2012 change in cover. 'Resurrected' recruits do no exist within the first time transition, as no data exists prior to 2009.

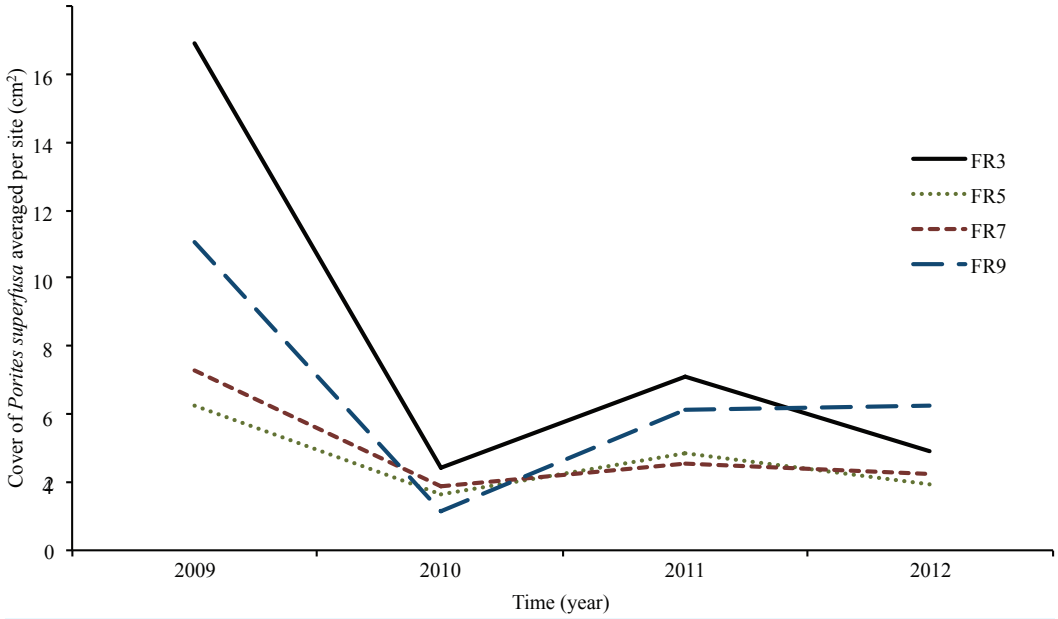

Figure 2-5 *Porites superfusa* cover from 2009 to 2012, averaged by site through time, at yearly surveys. ENSO event occurs in 2009. Sites (denoted by different lines) follow similar patterns through time. Cover decline is followed by variations in recovery and further decline. Figure 2 map shows site locations around island.

apparent recruits that appear in a location where a colony had been recorded as suffering mortality in previous time points) made up a third of total "recruitment" (12.45 [4.45] cm^2). Recruitment contributed less than colonial growth to the overall gain in cover (Fig. 4, Recruit: 24.06 [8.77] cm^2, Growth: 39.84[13.19] cm^2). From 2011 to 2012, resurrected recruits played a decreased role (2.702 [0.49] cm^2) in population growth. True recruitment and growth contributed similarly to overall growth (Recruit: 14.29 [18.37] cm^2, Growth: 18.37 [6.45] cm^2).

In 2011, 83 of over 400 colonies (21%) reappeared (after being overgrown by turf and crustose coralline algae in 2010) and began to re-colonize the substrate 10 months later (resurrected recruits, Table S1). Out of 1,150 colonies tracked, approximately 100

fragmented into apparent daughter clones (due to partial mortality) and 79 fused. Of the 100 colonies that fragmented, 52 fused back with a remnant section of the original colony within 2 years.

This study found that *P. superfusa* colonies gained upwards of 80 cm^2 of new tissue area per year and lost up to 170 cm^2 of tissue area per year, with a mean tissue change of $|4.75[0.232]\ cm^2|$ per colony. During the time period that included the bleaching event from 2009 to 2010, *P. superfusa* declined an average of 8.3 cm^2 per quadrat (total quadrat size, 540 cm^2). From 2010 to 2011, the corals increased an average of 1.7 cm^2 per quadrat (Welch two sample t-test, $t = -3.74$, $p = 0.028$) and from 2011 to 2012 corals declined an average of 0.9 cm^2.

Average change in area, when normalized to initial coral size in 2009, was largely negative (Figs. 4 and 5) and inversely related to size. Smaller corals gained more proportional area relative to larger colonies. The year with the most negative growth was 2009–2010, with the following years slightly less negative (Fig. 5). The overall change in *P. superfusa* cover from 2009 to 2010 (C_{2010}-C_{2009}) was negative (one sample t-test on C_{2010}-C_{2009}, $p < 0.001$) with no clear differences among sites (Tukey post-hoc). The change in cover from 2010 to 2011 (C_{2011}-C_{2010}) was minimal growth (one sample t-test on C_{2011}-C_{2010}, $p < 0.001$), followed by a slightly negative change from 2011 to 2012 (one sample t-test, $p = 0.09$, Fig. 5). There was no statistical artifact associated with including repeatedly measured colonies, i.e., colonies with multiple transition data through time, in the ANCOVA analysis, as the results from bootstrapped subsampled data were comparable to those from the entire dataset (in only one iteration of the random resampling was a significant site effect was noted).

We recorded a significant relationship between initial size of a coral colony in 2009 and survival across time points (Fig. 6). Larger corals had the greatest declines in area, but they were more likely to survive across time points. The size of the colony in 2009 predicted survival in 2012, with larger colonies showing a higher probability of survival (binary logistic regression, $p < 0.05$, for all time points). Figure 6 depicts the frequency of colony size classes and their survival from 2009 to 2012. The pattern is bimodal, with an increase in frequencies in the smaller corals, followed by a sharp decrease and then a slight increasing pattern of size. The size frequency between the two groups (survivors and non-survivors) was similar, however the largest size classes all survived to the end of the study, while the smaller size classes were more abundant across all years (Fig. 6). The average size of colonies varied from approximately 5 cm^2 to 14 cm^2.

DISCUSSION

The goal of this study was to quantify the population dynamics of a common encrusting coral during and after an ENSO event on a remote reef in the central Pacific. The effects of the ENSO warm-water event on the population of *Porites superfusa* on the forereef on Palmyra Atoll were dramatic and widespread. The ENSO event was associated with temperatures up to 30 °C at the sites surveyed in 2009 (Maximum degree heating weeks 16, *Williams et al., 2010*), and in our results *P. superfusa* suffered high mortality rates for over a year after the bleaching event.

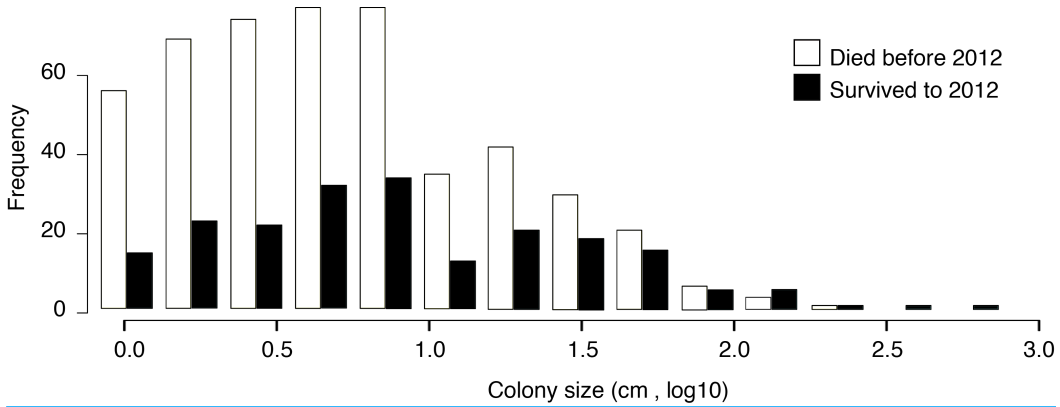

Figure 2-6 Paired histogram of initial colony size (in 2009) and survivorship (to 2012). Larger coral colonies were more likely to survive from 2009 to 2012. White denotes coral colonies that died before the 2012 survey. Black denotes coral colonies that survived to 2012.

The fates of colonies tracked through time revealed that the mechanisms of growth and death changed in surprising ways. After the bleaching event in 2009, corals suffered widespread mortality. Although, clonal growth (colony expansion) was recorded in a subset of individuals, complete and partial mortality were sufficiently large so as to overcome the signal of minimal growth (Table S1). Regrowth (from colony expansion and resurrected recruits) was an important driver of recovery and contributed to over 50% of the increase in cover of *P. superfusa*. Growth of corals in cryptic habitats, such as crevices or areas shaded by other corals, may be responsible for some of this survival and growth. In 2010, after the mortality event, colony growth increased. This is consistent with other documented case studies, which suggest that corals may increase growth rates to heal after injury (e.g., *Kramarsky-Winter & Loya, 2000*. However, the recorded increase in growth contradicts the idea that bleaching may decrease regeneration abilities of some corals (*Meesters & Bak, 1993*).

Resurrected recruits played a surprising role in the coral's population growth (over 12% of total growth). The resurrected recruits could be a type of regrowth of cryptic tissue, beyond the observational scope of this study. Some of the regrowth appeared to be emerging out from underneath coral species that were shading the underlying benthos, such as *Pocillopora meandrina,* and this survival could indicate that partial mortality was induced from UV stress in exposed portions of the benthos (*Baird & Marshall, 2002*). However, in many cases, the resurrected recruits appeared on a flat reef surface, emerging from benthic areas apparently covered in turf or crustose coralline algae. Unfortunately, the resolution of the photographs did not allow us to determine without question how the corals may have resurrected, for example, from a surviving piece of tissue in a small crevice or otherwise obscured from view. Alternatively, it is possible that these patterns represent true recruitment of larvae onto the exact same location as an adult colony had formerly occupied. Nonetheless, these observations hint at the modularity of coral colonies, and the spatial consistency among 'new' recruits is intriguing and warrants further investigation.

In certain cases the partial mortality of *P. superfusa* resulted in fragmentation (100 colonies) or clone fission (79 colonies). Fragmented coral colonies living in close proximity (groups of daughter clones) have a potentially higher rate of survival, as they are not as easily eliminated from disease or competition (*Highsmith, 1982*). However, fragmentation in this study was relatively low (<10%), and thus it is unclear if the "fused" colonies (<10%) were more likely to survive. While recruitment is important for some coral recovery, within-colony expansion and regrowth were of comparable quantitative importance regarding *P. superfusa* growth at Palmyra Atoll. Some *Porites* spp. have been found to have a limited capacity for recruitment (*Potts et al., 1985*), which may make regrowth a specifically useful recovery mechanism for this genus. A recent study by Roff and colleagues (*2014*) found that regrowth was an important contributor to the population-scale recovery of massive *Porites*. The ability of corals to regrow from remnant polyps may prove vital to recovery as climate change continues. If the regrowth of smalll fragments of remnant tissue can bring colonies back from apparent mortality, then colonies that appear dead in traditional coral surveys may actually have a chance at survival. As a result, traditional coral surveys may overemphasize mortality.

Coral colony size was an important predictor of the coral's ultimate fate (Fig. 6). Interestingly, *P. superfusa* seemed to have size-dependent growth and death. Growth decreased with increasing size, suggesting that this species may have determinate growth. This growth pattern could be due to high levels of partial mortality. In addition, as is consistent with the literature, smaller colonies of *P. superfusa* experienced higher rates of overall change, including mortality and recovery (*Hughes & Jackson, 1985*). Large colonies are more likely to suffer partial mortality, which may be part of the declining growth with size phenomenon (*Hughes & Jackson, 1985*). Probabilistically, larger colonies are more likely to suffer injury due to a larger surface area, and this may be a factor in the decline of overall growth. Smaller colonies tend to experience damage in a more binary way: either resisting disturbance entirely or dying completely, suffering less incidence of partial mortality (*Connell, 1973*; *Hughes & Jackson, 1985*).

While this study documents the potential for coral regrowth following anomalous temperature events, it is important to note that recovery did not exceed mortality over the course of this study. The decline in *P. superfusa* cover from 2009 to 2010 occurred more rapidly than the increase in coral cover from 2010 to 2011 and the decline from 2011 to 2012. Full population recovery is a slow process, and the longer-term trajectory of *P. superfusa* on Palmyra remains to be seen.

Understanding the effects of large-scale phenomena on community dynamics in relatively pristine reefs provides critical benchmarks for coral demography (Edmunds, 2002). Further studies on this topic will help quantify the importance of fragmented or remnant corals for reef recovery processes. Examination of other species at these sites may provide important comparisons among morphologies and between species with different life history strategies, which combined may help infer the likelihood of community level

recovery. The causes of bleaching, mortality, resistance, and recovery are clearly complex and species-specific. Thus, additional studies from remote Pacific island coral communities are important for understanding the capacity of these systems for recovery via different mechanisms; such studies will help to directly improve the policies for protection of reefs worldwide.

CONCLUSIONS

This study documented a bleaching event and subsequent change in areal cover of a common coral on a remote central Pacific atoll. Coral populations can change via four different dynamic processes: complete mortality, partial mortality, growth, and true larval recruitment. This study suggests a fifth dynamic, 'resurrected' recruits, as a mechanism contributing to coral recovery. Additional research is needed to determine the source of this coral growth (e.g., exploitation of cryptic habitats, regrowth of remnant tissue).

With increasing climate pressures, many coral reefs have experienced rapid decline. In such cases, tissue loss due to mortality is faster and more obvious than tissue gain due to growth. Coral growth is often slow and subtle, making it a difficult research and management target for short time scales. Despite the challenges associated with quantification, regrowth is a critical process in coral recovery, and it is vital we understand the mechanisms controlling it. The implications from this study of colony-specific patterns of decline and recovery should be scaled up to examine the role of regrowth and the future of remote Pacific islands' reef recovery.

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Author Contributions

- Kathryn Anne Furby conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Jennifer Ellen Smith and Stuart Adrian Sandin conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

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The following information was supplied regarding data availability:

The raw data has been supplied as a Supplementary File.

Supplemental Information

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CHAPTER 3:

Exploring the possibility of an intraskeletal circulatory system in a perforate table coral, *Acropora cytherea*

Kathryn A. Furby, Nicholas Holland

Abstract

In their survival toolkit, corals have a variety of available responses to ecological damage. Scleractinian corals are capable of suffering partial mortality, surviving, and then growing back over its dead skeleton, i.e. resheeting. In perforate coral the skeleton is permeated with ramifying canals allowing a reserve of tissue, zooxanthellae and circulation of resources. Regrowth of coral tissue has largely been observed through surface spreading of coral polyps, but the role of tissue transport through canals to facilitate or accelerate regrowth is less investigated. In this study, a perforate, scleractinian coral's (*Acropora cytherea*) intraskeletal canal network was studied by scanning electron microscopy (SEM) and light microscopy. In healthy specimens only those canals < 0.5 cm of the colony surface were lined with living coral tissue (e.g., polyp body wall extensions). Each extension is three-layers comprising an ectoderm of calicoblasts facing the skeleton, an endoderm of uniflagellated cells (containing zooxanthellae) facing the gastrovascular cavity, and a very thin intervening mesoglea. No living coral tissue is detectable lining the canals in the shallow region of the skeleton, if partial mortality has occurred and turf algae has overgrown the surface. Instead the canals there are packed with sediment. Endolithic fungi and algae were present in both the living and dead coral skeletons. However, the canals deeper in the skeleton of living and dead corals were mostly free of debris and endoliths. This morphological pattern of empty pore space suggests that gastrovascular fluids have the potential to circulate beneath the superficially damaged areas. In *Acropora cytherea*, such a deep circulation might play a role in the marked capacity for this species to repair damaged areas of the colony (the so-called "phoenix effect").

Introduction

Hard corals (Scleractinia) build the foundation of tropical coral reef ecosystems. The capacity of scleractinians to build prodigious reef structures is enhanced by their coloniality and clonality (Veron 1995). Beyond sexual reproduction, scleractinians can grow through asexual reproduction and clonal spread to expand colony borders. Post-disturbance, colonies can lose tissue but survive in remnant areas (Hughes and Jackson 1980). If disturbances abate, lost tissue can be replaced through clonal regrowth. The mechanisms of partial survival and subsequent regrowth are incompletely understood. Coral have two separate skeletal morphologies that affect their growth and survival: imperforate and perforate skeleton. Imperforate species are characterized by a polyp base that is embedded in a dense calcareous skeleton. When small spaces are present there, they are isolated, neither interconnected nor lined by living tissue (Northdurft and Webb 2007, Kelley 2009; diagrammed in Fig. 1a). Perforate corals (Fig. 1b) are characterized by a network of intraskeletal canals, at least some of which are lined by living body wall tissues ramifying from the polyps (Gladfelter 1983). These internal structures of the coral, including zooxanthellae, are able to act as reserves in times of thermal stress (Edmunds et al. 2012). In comparison to imperforate corals, perforate corals comprise considerably fewer species and are distributed polyphyletically in the Scleractinia (predominantly in clades III and VI as recognized by Budd et al. 2010).

If one ignores morphological variation due to environmental factors (e.g., light intensity, Muco et al. 2000), there are two common growth forms of perforate coral colonies: branched/ encrusting or compact/ massive. For one branching, perforate species, *Acropora cervicornis,* Gladfelter (1983) described living coral tissue lining all parts of the canals ramifying in the skeleton and measured the direction and rate of the flagellum-driven fluid flow. In perforate species such as *Montipora capitata*, with a colonial organization approximately intermediate between branched and compact, living tissue is also present throughout the canal system (Santos et al. 2009). By contrast, in massive corals in the perforate genera *Porites* and *Acropora*, living tissue lines the canals in the shallow region of the skeleton, but not the canals deeper in the skeleton, as diagrammed in Fig. 1b (Barnes and Lough 1992, Le Campion-Alsumard et al. 1995b, Edmunds et al. 2012). In compact/massive perforate corals of the genus *Porites*, horizontal skeletal partitions (dissepiments) coincide with the boundary between the shallow and deep regions of skeleton, but this skeletal feature is absent in massive table corals of the genus *Acropora* (Wallace et al. 2007).

The present study is focused on the canals in a perforate coral, *Acropora cytherea*. The more superficial tissues of the polyps (tentacles, septal filaments, etc.) and zooxanthellae are not covered because they have previously been well described for hard corals (Fautin and Mariscal 1991, Brown and Bythell 2005, Wallace et al. 2007, Sudek et al. 2012, Cordie and Budd 2016, Edmunds et al. 2012). Detailed information on the structure and possible circulatory function of the shallow and deep intraskeletal spaces of perforate corals is lacking. We focus on the structure of these spaces, considering their potential role in restoring damaged areas (Jokiel et al. 1993, Santos et al. 2009, Yost et al.

2016). The ability to recover from stress via regrowth of cryptic animal tissue has been termed the "phoenix effect" and has been studied in other species (Krupp et al. 1993, Roff et al. 2014). The aim of the present study is to describe the detailed structure of the canal system and its living tissues in *A. cytherea*, a table coral species. Information on the endolithic organisms (chiefly intraskeletal) in both living and dead coral is included. The results raise questions about the chemical composition and possible cellular content of the circulating fluid and its possible relation to attainment of large colony size and relatively rapid repair of damage in *A. cytherea*.

Materials and methods

Colonies of the table coral, *Acropora cytherea* Dana (1846), were studied at Palmyra Atoll, Northern Line Islands, Central Pacific (05^{o} 52' N 162^{o} 05' W), a remote island in the central Pacific known for limited human impacts (Sandin et al. 2008) in June, 2016. The corals were at the northwest corner of the atoll about 500 m from shore on the reef terrace at a depth of about 3 m. A healthy colony of this table coral (Fig. 2a), seen from a distance, appears as a relatively smooth plate. However, a closer view shows that the edges of the colony comprise short, upwardly-projecting branches (Fig. 2b). Toward the center of the colony the branched structure transitions to the relatively flat surface already mentioned (Fig. 2c). In all, the relatively flat central region of nine colonies, each approximately 3 m in diameter, was sampled by a SCUBA diver coring with a hand-held pneumatic drill (Fig. 3a). Each core was 2.5 cm in diameter (Fig. 3b)

and extended from the surface of the colony to level about 2.5 cm beneath (Fig. 3c). Nine cores were taken from healthy regions of each coral, and six cores were taken in regions of the coral colony that were overgrown with turf algae (three 5 cm and three 30 cm from the interface between the overgrown region and the healthy coral). The cores were transferred to seawater-filled plastic bags and maintained at field temperature for approximately 1 h. They were fixed in a solution of 2% glutaraldehyde in dilute (77%) sea water for SEM, a solution of 10% formalin for light microscopy. The fixing solution was isotonic with full-strength sea water (1015 milliosmoles/l) as determined by freezing point osmometer.

Cores were stored in their respective fixing solutions at 20^{o} C for six weeks before further processing. Flat surfaces of the cores (perpendicular to the upper edge of the colony) were obtained by slicing with an IsoMet low speed saw (Buehler, Lake Bluff, IL). An EDTA decalcification fluid was prepared from a 10% (w/v) solution of EDTA that had been titrated to pH 7.5 by addition of solid NaOH. Some samples were partially decalcified (overnight) while others were completely decalcified (4 d) at room temperature (22 ^{o} C). For SEM processing, fixed specimens were washed for three changes of distilled water for 2 min each. Washed specimens were dehydrated in an ethanol series, transferred through two 15-min changes of hexamethyldisilazane and air dried. Dried specimens were mounted on stubs via double-stick tape and grounded further with a foundation of silver paint, sputter coated with iridium, and examined in a Hitachi S4800 SEM.

For plastic sectioning for light microscopy, uncalcified, fixed specimens were washed in three changes of distilled water for two minutes each, dehydrated in ethanol

series, and transferred to accelerated Spurr's resin, which was polymerized overnight in an oven at 70°C. The polymerized block contained Spurr-embedded regions of soft tissue and regions of calcium carbonate skeleton where the Spurr had not penetrated. Several sections were cut from the block with a glass knife to expose part of the skeleton, and then the block was soaked in the EDTA decalcifying solution for 48 hours, washed in three 1-hour changes of distilled water, and dried for 40 hours in a 70°C oven. This procedure etched the calcium carbonate from the block, which was then paced for 6 hours at room temperature in accelerated Spurr to permit the liquid resin to run into the etched portions of the block by capillarity. Subsequently, the entire block was heated to 70°C overnight to polymerize the resin in the former skeletal regions of the specimen. An LKB untramicrotome III with glass knives was used to cut 3-micrometer thick sections, which were subsequently stained with azure 0.5% azure A in 0.1% sodium borate.

Results

Morphology of a healthy region of Acropora cytherea

Thin slices of healthy cores revealed an intraskeletal canal system lined with living tissue near the coral surface. Figure 4a is a side view of the sawed surface of one healthy coral core sample. At the top are the oral regions of the polyps, while the rest of the specimen comprises calcified skeleton characterized by openings leading to the ramifying internal spaces interconnecting the polyps. Some areas of skeleton are green, indicatively of the presence of endolithic green algae (considered below). The same

specimen was partially decalcified overnight in EDTA to etch away several hundred microns of calcium carbonate from the sawed surface of the skeleton (Fig. 4b). This procedure emphasizes that the skeleton is divisible into a shallow and a deep region (as diagrammed in Fig. 1b). The spaces in the shallow region of the skeleton are lined by coral tissue, while those in the deep region are not. The interface between these two regions (indicated in Fig. 4b by the arrow) is roughly 4 to 6 mm beneath the surface of the colony. Observations on nine different colonies of *A. cytherea* showed this interface consistently located at a similar depth. The specimen in Figure 4(a,b) is shown in Figure 4c after complete decalcification in EDTA for four days. This procedure accentuates differences between the shallow region, where the internal spaces lined by coral body walls from the deep region where the spaces were unlined by such tissues. In addition, the spaces in the skeleton of the deep region are no longer recognizable. Instead, the zone is occupied by a dense mass of endoliths (described further below).

The exposed surface of the shallow region of the skeleton penetrated by pores represent gastrovascular cavities lined by living body wall tissues (Fig. 4d). These living tissues are not firmly attached to the underlying skeleton, and, in several regions of the micrograph, have been artifactually torn away during processing. The arrow indicates a region where the body wall tissue remains in place. Figure 5a shows the endodermal side of a sheet of body wall at the top and a view of the skeletal surface at the bottom. In this region of skeleton, the surface topography is relatively flat (i.e., not organized into domed bundles of crystal fibers, as defined in Tambutté et al. 2007), indicating that the skeleton is not actively being deposited by the adjacent calicoblastic ectoderm. The endodermal cells face a gastrovascular space in the shallow region of a healthy specimen (Fig. 5b).

The endoderm is a squamous (relatively flat) epithelium. Each cell bears a single cilium that arises from a circlet of microvilli (Fig. 5c). The cilium is about 5 mm long and tapers slightly over the apical 0.5 m.

The gastrovascular spaces are lined with body wall tissues in the shallow regions of the healthy colony, however, endoliths were only sparsely distributed. It is noteworthy that none of them was detected occupying the gastrovascular spaces in shallow regions of the healthy corals. In a light micrographic cross section of a region of body wall tissue, the lining of a gastrovascular space in the shallow region of a healthy coral colony is clearly visible (Fig. 5d). From top to bottom, important features of Figure 5d are: gastrovascular cavity; endoderm cells (twin arrow) with scattered zooxanthellae (asterisk); a mesoglea (too thin to be visible in this section); a relatively thin calicoblastic ectoderm (single arrow); an artefactual space (twin asterisk) where the soft tissue is separated from the surface of the underlying skeleton; and a region of skeleton (decalcified in this preparation), in which a few endoliths (tandem arrows) are embedded just beneath its surface. The thinly dispersed endoliths will be discussed further below.

In the deep region of the skeleton of healthy colony of *A. cytherea*, the internal spaces are no longer lined with coral tissue. Undecalcified SEM preparations of the deep region (Fig. 5e,f) show that the internal spaces contain widely scattered endoliths (arrowed in Fig. 5e,f). Endoliths are also visible on the surface of the broken skeleton (for example, at the lower right in Fig. 5e), but it not clear whether they were originally located in internal spaces or simply exposed by the cracking open of the skeleton. It is important to note that SEM shows no evidence of endoliths present in the broken skeleton (Fig. 5 e,f), although they can be demonstrated in abundance there by staining a broken

piece of skeleton overnight in 0.5% aqueous alcian blue (Spicer 1963) and then treating it with EDTA overnight to reveal the stained endoliths in the decalcified region (5f, inset). Complete decalcification (Fig. 4c) liberates the abundant intraskeletal endoliths, permitting them to expand into a fibrous mass that obscures the former boundaries between the internal spaces and the skeletal regions. SEM reveals that the fibrous mass comprises a tangle of threads ranging in diameter from about 1 to 10 mm (Fig. 5g). Evidently these represent a mixture of organisms (like algae, bacteria, or fungi), many of which cannot be assigned even to broad taxonomic categories by morphological criteria. Only a few of the endoliths isolated from the mass had distinctive identifying features—like the lateral branches on the strand of fungus shown in Fig. 5h. Previous literature on endolithic bacteria, fungi and algae associated with coral skeletons is summarized in Table 1.

In healthy regions of *A. cytherea* colonies, neither SEM light nor hyperspectral microscopy revealed what might have been freely-circulating coral cells (caused by histological preservation to adhere to the endodermal layer lining the canals in the shallow region of the skeleton). It is likely that such cells in the gastrovascular canals are at least relatively uncommon if not altogether absent. This contrasts with their considerable abundance of free endoderm cells freely circulating in the gastrovascular spaces in some octocorals (Gateño et al. 1998). In addition, there was no evidence of possible coral cells adhering to the skeleton bounding the spaces in the deep region of the skeleton in healthy (or overgrown) regions of the *A. cytherea* colonies. There was no evidence of living coral or zooxanthellae fluorescence in the deep regions.

Morphology of overgrown Acropora cytherea *skeleton near healthy coral tissue*

The cores in this treatment were adjacent to living tissue (approx. 5cm), but appeared completely dead, overgrown with a comples turf community at the surface. The spaces in the shallow region of the skeleton appear packed with sediment, a feature that is also evident after partial decalcification (Fig. 6b). In the deep region, complete dissolution of the skeleton released abundant endoliths that expanded into an extensive mat, obscuring the spaces (Fig. 6c). The SEM survey view of the shallow region of an overgrown region of the coral colony reveals that most spaces in this region of the skeleton are filled with sediment (Fig. 6d). Many of the spaces contained abundant masses of endoliths (Fig. 6e). The skeleton in the shallow region was bored with a few conspicuous galleries (Fig. 6f), apparently excavated by larger endoliths.

In the deep region of these samples, the skeletal spaces were largely empty except where a few scattered endoliths adhered to their walls. These endoliths were unidentifiable fine fibers (Fig. 7a single arrow), fungi with sporangia (Fig. 7c), or bacteria (Fig. 7d). It appears many of the endoliths in deep skeletal spaces were growing inside the skeleton itself, and thus not inside the pore spaces (Fig 1b Deep Region, Fig. 7b).

Morphology of overgrown Acropora cytherea *skeleton distant from healthy coral tissue*

To account for possible successional changes, samples were also taken in a third treatment, within the same coral table, but farther from visible living tissue. The overgrown coral 30 cm from the healthy coral tissue had a combination of turf and crustose coralline algal cover (additional investigation of this in the following Chapter 4).

The interior generally resembled the overgrown coral samples within 5 cm of healthy coral tissue. Side views of sawed preparations (similar to those shown in Fig. 6a-c) showed sediment-packed spaces in the shallow region, but largely empty spaces in the deep region of the coral. Moreover, decalcification of the skeleton liberated an extensive mat of endoliths (data not shown). The sediment-packed spaces in the shallow region included a tiny (100 um), unidentifiable, possible carapace (Fig. 8 a,b). In an undecalcified specimen, a few of the spaces in the shallow region were filled with a mat of finely fibrous endoliths (Fig. 8c) occasionally with a fungal sporangium (Fig. 8d) among them. Several of these filamentous endoliths were inconspicuously segmented at roughly 10 mm intervals (Fig. 8e). Elsewhere, adhering to the edge of some of the skeletal spaces in the shallow region were conspicuous algal filaments, probably *Ostreobium* (Fig. 8f). Bacteria were sparsely distributed on the surfaces of these algae (Fig. 8f, arrow and 8g). The hyperspectral imaging and fluorescence reveal a more complex signature of fluorescence and a similar decline in complexity and reduction of emissions from turf algae and endolithic organisms in the deep regions. The fluorescence is similar to the wavelengths recorded in the dead coral proximal to living tissue, however, we observed anecdotally more diverse wavelengths, which may reflect a diversity of surface organisms. The deep skeletal region of the long overgrown part of the coral colony was characterized by spaces strikingly free of formed contents, except for a few widely scattered, fibrous endoliths (Fig. 9, arrowed).

Discussion

Acropora cytherea has tissue-lined gastrovascular cavities that are limited to approximately the top 4 mm. The dead portions of the large perforate table corals have a succession of endolithic invaders that are similarly limited in depth. The deep portions of the living and dead coral have been cleared of most living organisms and detritus.

Natural history background of Acropora cytherea *at Palmyra Atoll*

Acropora cytherea are large charismatic table corals which characterize the reef terrace at Palmyra Atoll. *Acropora*'s rapid growth rate and its ability to survive fragmentation allow it to suffer and recover in a dynamic way (Lang and Chornesky 1990, Williams et al. 2016). Palmyra experiences high wave energy that is inversely correlated to *Acropora* coral cover; however *Acropora* is still the second most abundant coral on the reef (Williams et al. 2016). Table corals on Palmyra are found flipped over and regrowing, breaking into pieces or breaking of the reef and getting swept away entirely (Kate Furby, pers. obsv). Colonies of the table *Acropora* persisted after a disease outbreak that left many of them partial survivors (Williams et al. 2011).

Circulation in the shallow and deep skeletal canal system in healthy perforate corals

Examination of the healthy perforate coral *Acropora cytherea* revealed a limit to the depth of living tissue within the skeletal pores. Sedimentation and endolithic invasion of the dead corals adhered to a similar depth limitation. We consider in this study the possibility that the ramifying spaces deep in the skeleton of massive perforate corals, even where not lined by living tissue, might permit circulation of fluids and cells beneath

both healthy and damaged regions of the colony. It is very likely that beating of the endodermal flagella circulates gastrovascular fluid in the canals in the shallow region of the colony and might conceivably also create pressure gradient forces inducing flow in the deeper canals as well. In branching colonies of one perforate coral, *Acropora cervicornis*, Gladfelter (1983), described how the beating of endodermal flagella drives the flow of gastrovascular fluid through the tissue-lined spaces pervading the skeleton. She quantified both the pattern and the flow rate for this circulation. In contrast, there have been no comparable studies for any massive perforate coral with a table-like or boulder-like colonial organization. Even so, in such corals (like *A. cytherea* studied here), it reasonable to assume that gastrovascular fluid can also circulate through the tissue-lined spaces of the shallow region of the skeleton. Such a flow would presumably be driven by the beating of the abundant endodermal flagella there. It is less certain if and how flow in the shallow region influences the fluid in the canals in the deep region of the skeleton. However, due to the interconnection of the shallow and deep canals, it is quite possible that flagella-driven pressure gradient forces in the former can induce flows in the latter even in the absence of any flagellated endoderm. The endoliths located extraskeletally would not be expected to impede the flow of gastrovascular fluid appreciably because they were virtually absent in the shallow region and encountered heterogeneously in the deep skeletal regions of the healthy coral.

The gastrovascular fluid circulating in the shallow and deep regions of the skeleton presumably distributes dissolved or particulate products of digestion. Gladfelter (1983), studying a perforate coral with a branching morphology (*Acropora cervicornis*) found small amounts of particulate matter, presumably products of digestion, in the

gastrovascular fluid in the intraskeletal canal system. She also reported that the canal fluid contained some free zooxanthellae. This contrasts with the zooxanthellae in the gastrovascular fluid in the stolons of some octocorals, in which the symbionts are encapsulated within coral cells and transported (Gateño et al. 1998, Parrin et al. 2012, Harmata et al. 2013). Zooxanthellae have also been shown to have refuge in deeper tissues of perforate corals due to shading with depth and migrate vertically in coral tissue (Toyoshima 2003, Edmunds et al. 2012, Lecointe et al. 2016). Using Gladfelter's (1983) methods, it would be interesting to test the fluid within the deep, apparently empty pore spaces to test for changes in chemistry of seawater or the presence of gastrovascular fluid. The empty spaces hold potential for passively transporting nutrients or cells.

Lack of obstruction of the deep skeletal canal system in overgrown regions

One unexpected finding of the present study is that in overgrown regions of *Acropora cytherea* only the canal system in the shallow region of the colony was clogged with sediment and organisms like algae and fungi (Fig. 6d). The granular sediments appearing out of the skeletal pores may be evidence of polyp defenses against fungi detection (Fig. 8a, Le Campion-Alsumard et al. 1995b). While the surface of this coral was dead when sampled, the calcified granules might be evidence of the previously-alive polyps calcifying small granules to deter fungi. Additionally they could be evidence of microborers expelling sediment (Chazottes et al. 1995). By contrast, the canal system in the deep region was relatively free of such obstructions (Fig. 9). Endoliths are limited by light and nutrients, which may explain the lack of frequent biology in the deep areas of the skeleton (Chazottes et al. 1995). As corals are known for resheeting to recover (Roff

et al. 2014), it would be interesting to examine deep cores through the table, searching for layers of sediment-filled pores. Evidence of past partial mortality and resheeting should be visible in the record, even for table corals.

The spaces in the deep region of an overgrown sample contained more extraskeletal endoliths than the deep spaces of a healthy coral, but probably not enough to impede circulation markedly. Consequently, it is possible that gastrovascular fluid with products of digestion, coral cells, or zooxanthellae could continue to circulate in deeper regions of the skeleton even beneath overgrown parts of the coral colony. The persistence of such circulation might influence the rate of recovery of damaged regions of *A. cytherea*, as discussed in the following section.

Cellular basis of repair of damaged parts of coral colonies

For hard corals generally, including imperforate as well as perforate, several mechanisms have been proposed to account for the repair of damaged parts of colonies. One explanation for damage repair in corals is that conspecific planula larvae preferentially land on damaged parts of the colony to provide new cells for initiating the repair (Jordán-Dahlgren 1992). However, this scenario may not be universally beneficial, given evidence that planula landing near conspecific corals can suffer reduced survival relative to those settling near heterospecifics (Marhaver et al. 2013). The remaining schemes that have been proposed to account for repair of injury depend on the recruitment of coral cells from nearby healthy regions of the colony. For imperforate corals, van de Water et al. 2015, Rodriguez-Villalobos et al. 2016) proposed that such cells are located right on the surface of the colony and grow centripetally into the

wounded area. A similar mechanism was suggested for a perforate coral by Le Campion-Alsumard et al. (1995a) and Denis et al. (2011). A similar kind of superficial overgrowth has been proposed for branching species of the perforate genus *Acropora* (Riegl and Pillar 2001, Diaz-Pulido et al. 2009). During this process (called "re-sheeting"), tissues originating from small living fragments of broken branches can overgrow neighboring dead branches to restore large areas of healthy colony relatively quickly. No counterpart of re-sheeting has been proposed for the more massive perforate corals. One disadvantage of resheeting is that it might suffer degradation of structural integrity due to bioeroders (Grober-Dunsmore et al. 2006).

A contrasting scheme for wound repair in massive perforate corals proposes that new tissue for regeneration arises from cells surviving deep beneath the damaged surface region and growing upwards into the lesion (Davies 1991; Meesters and Bak 1993; Work and Aeby 2010). This mechanism gives perforate corals a theoretical advantage in recovery. The implication is that "stem-like" cells in the deep region of the skeleton are translocated to the damaged surface and seed the growth of new coral tissue there. Within the phylum Cnidaria much work has been done on hydrozoan stem cells, although they have been relatively little studied in corals (Gold and Jacobs 2013, Barfield et al. 2016). This type of regrowth may provide more stability and support than resheeting regrowth because it has the opportunity to heal internal structural damage from bioeroding. As already mentioned, the present study found no coral cells associated with the spaces in the deep region of the skeleton in either healthy or overgrown regions of *A. cytherea* colonies, but that is not sufficient to rule out a small number of coral cells reserved in the spaces in the deep region of the skeleton or transported there in the

gastrovascular fluid. It would be interesting to investigate this by studying cell proliferation and migration tissues associated with the intraskeletal canals of perforate corals, but to date such methods have been applied only to an imperforate coral (Lecointe et al. 2016). The open "empty" pores in the deep perforate corals presumably have a fluid inside, but the composition (whether seawater-like or gastrovascular fluid-like or other) has yet to be determined.

Convergent evolution of deep circulation in skeletons of diverse cnidarians

Several kinds of cnidarians are characterized by well-developed calcium carbonate skeletons riddled with a network of gastrovascular canals. Within the Hydrozoa, the milleporids and stylasterids evolved this feature independently from each other and from perforate species of hard corals (Cairns 2011, Puce et al. 2011). Similarly within the hard corals, as already mentioned, a perforate skeleton has also evolved several different lines of descent (predominantly in clades III and VI in the phylogeny of Budd et al. 2010). For the hard corals, it is interesting to consider what selective pressures might have influenced evolution of a circulatory system for gastrovascular fluid independently in several clades. The perforate condition tends to be correlated with large size and a facility for regenerating damaged parts of the colony. Before one could propose that such correlations reflect causal relationships, a suite of questions needs to be addressed. For instance, is there actually a flow of gastrovascular fluid through the intraskeletal canals in the deep regions of the colony, and, if such flows exist, what are their their trajectories and rates? Moreover, what is the chemical composition (including possible products of the innate immune system) of the fluid in the canals, and is there any

transport of coral cells or zooxanthellae or both? Finally, it would be interesting to determine whether there are any commonalities in the functions of the intraskeletal circulatory systems among polyphyletic groups of perforate hard corals and the similarly polyphyletic milleporids and stylasterids.

Conclusion

This study examined the internal cavities of living and dead *Acropora cytherea*. Corals in varying stages of life and death were investigated using SEM and light microscopy. Coral cells and endoliths were categorized within the coral skeleton framework. Algae, fungi, bacteria and other endolithic and epilithic organisms were found boring into the skeleton and utilizing the shallow, perforate skeletal spaces. Due to light, nutrient or other unknown limitations, deep sections of the skeleton in both living and dead coral were found almost uniformly empty, suggesting a deep inner desert. The benefits of a subterranean canal system could include movement of cells and resources. Additional research is needed to determine the extent of perforate coral recovery capabilities on a cellular level.

Acknowledgements

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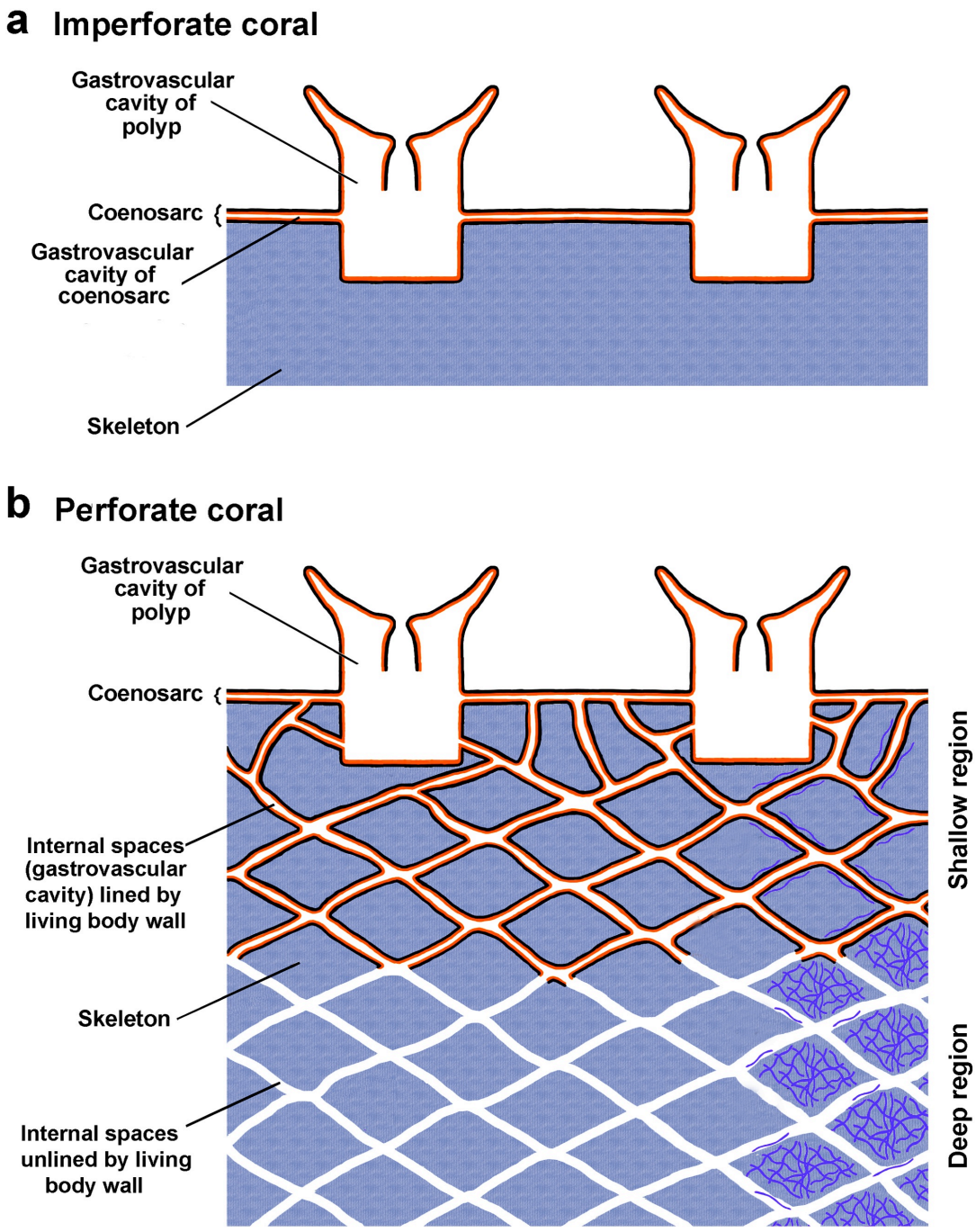

Figure 3-1. Diagram comparing **a** imperforate and **b** perforate corals. The fine strands toward the right side of b indicate the endoliths (mostly located within the skeleton of the deep region of the colony).

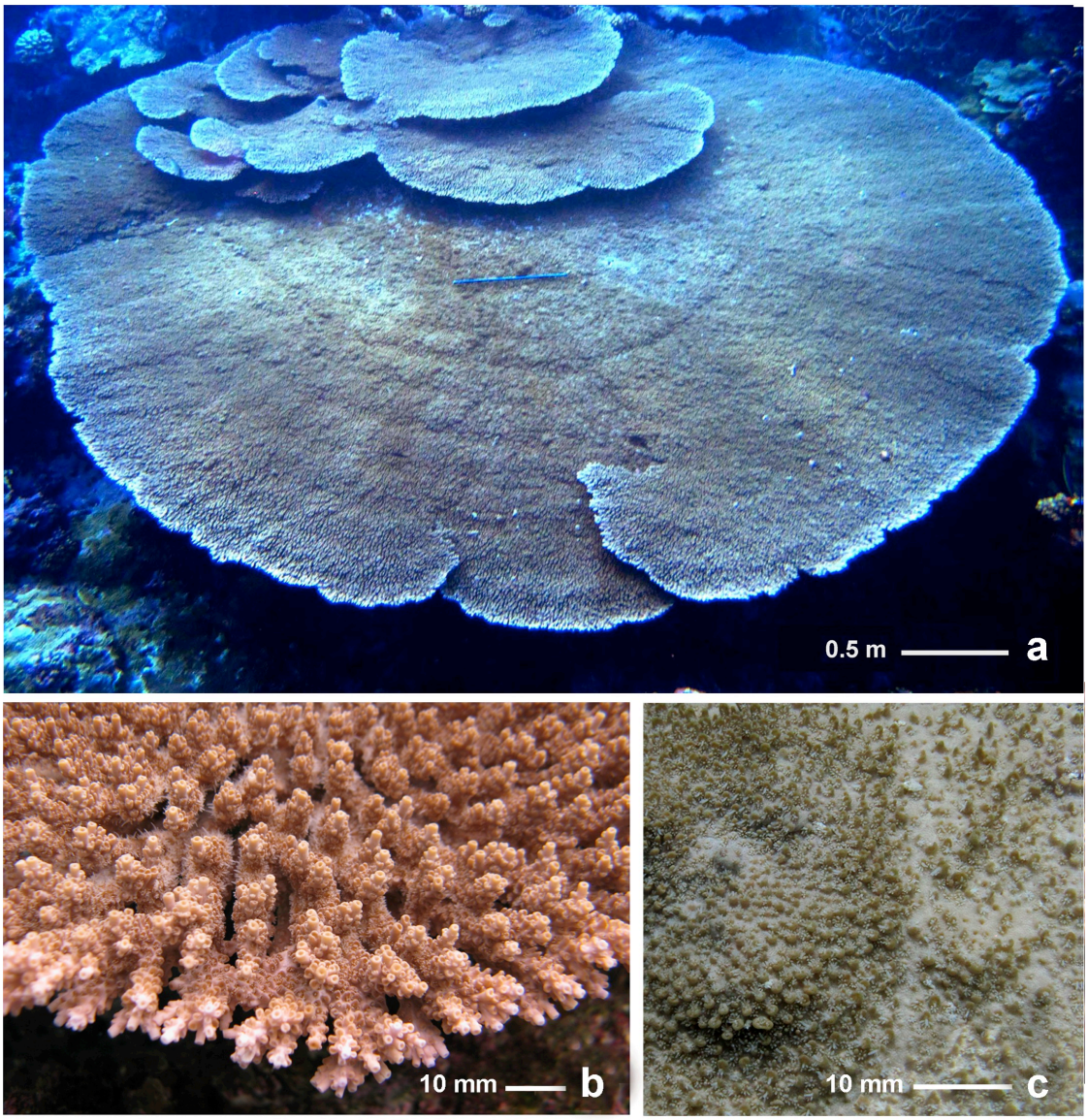

Figure 3-2. *Acropora cytherea* morphology **a** A healthy massive colony comprising several overlapping foliose plates, each of which appears relatively smooth surfaced from a distance. **b** Edges of each plate comprise short, upwardly-projecting branches, whereas, the center **c** is relatively flat.

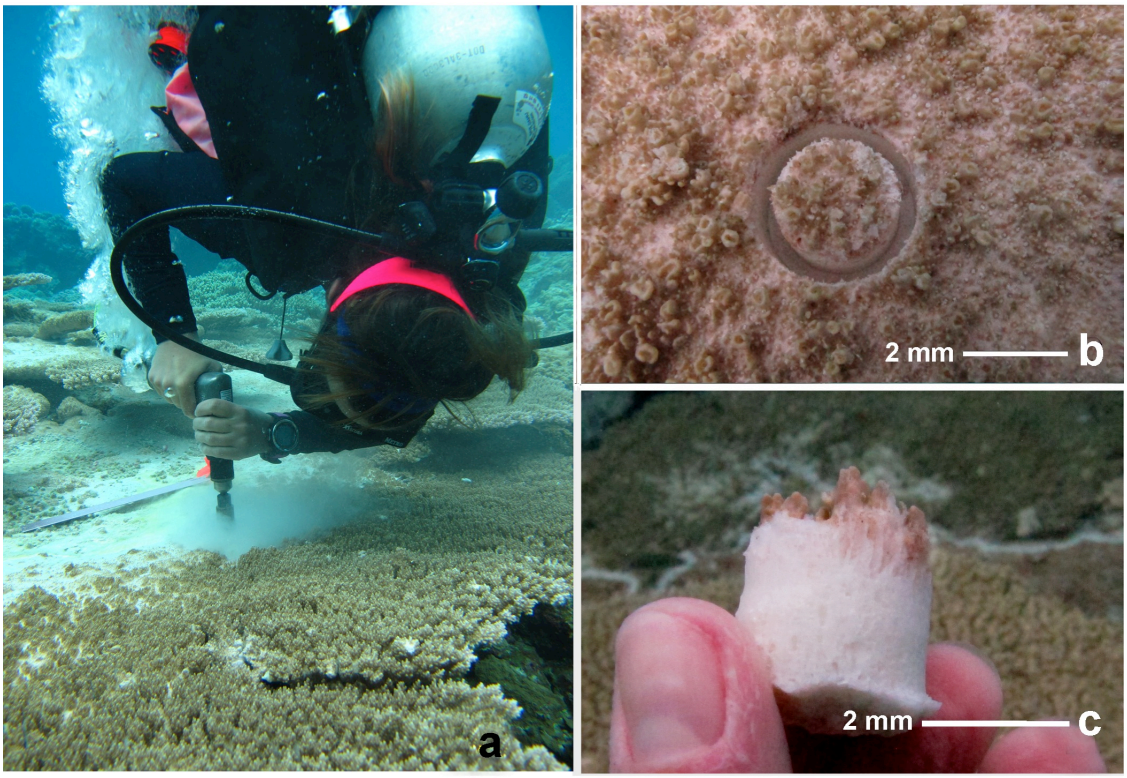

Figure 3-3. *Acropora cytherea* sampling. **a** Extracting a core sample. **b** Surface view of a colony right after the start of coring. **c** Side view of completed core sample.

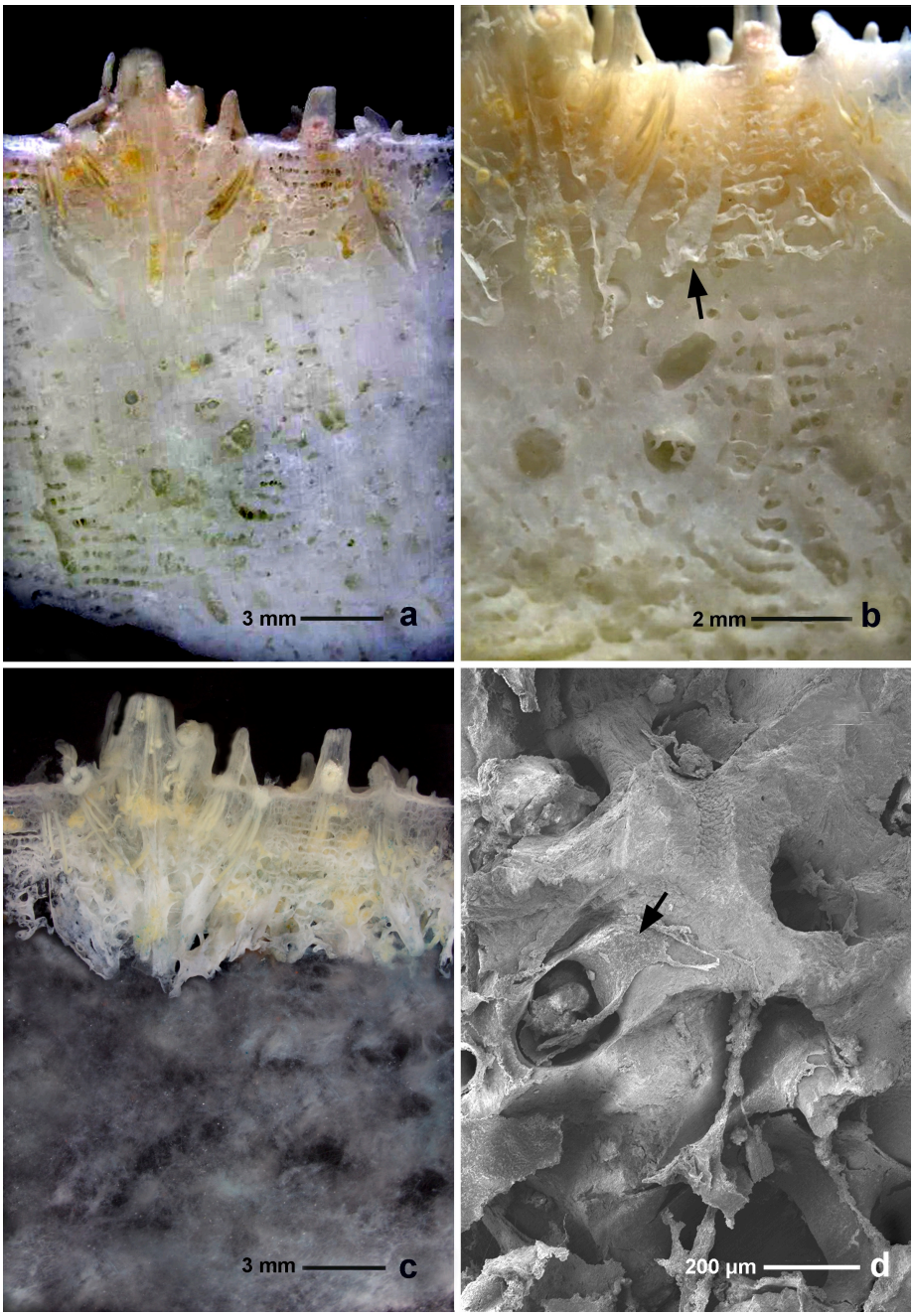

Figure 3-4. *Acropora cytherea* healthy. **a** Side view of undecalcified core sample from the healthy part of the colony. **b** Side view after an overnight decalcification to visualize the penetration of coral body wall tissue (arrow) lining the spaces in the shallow region of the skeleton. **c** Side view of a after 4 days of decalcification. In the shallow region of the skeleton, coral body wall tissue outlines the gastrovascular spaces ramifying within the skeleton; in contrast, in the deep region of the skeleton, the removal of the skeleton has freed masses of fibrillar endolithic organisms that have spread out into a fibrous mat, obscuring the spaces formerly visible there. **d** SEM in the shallow region of the skeleton from a healthy part of the colony; body wall tissue (arrow) is still visible lining a space.

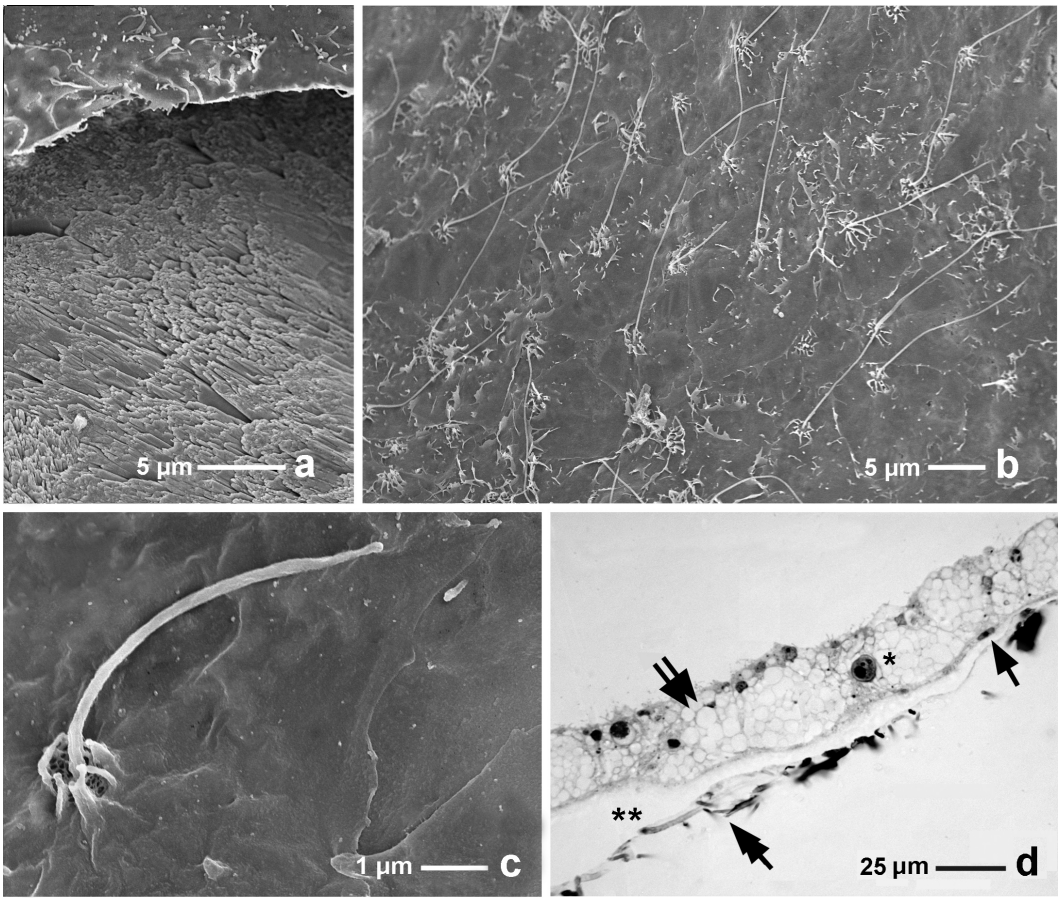

Figure 3-5. *Acropora cytherea.* Healthy portion of colony. **a** SEM enlargement of the shallow region of the skeleton showing the endodermal side of body wall tissue (top) and the skeletal surface (bottom) where the former has been torn away. **b** Ciliated cells comprising the endodermal side of the body wall tissue lining a gastrovascular space in the shallow region of the skeleton. **c** Detail of one of the ciliated cells in b. **d** Cross section of the body wall in the shallow region of the skeleton. The space at the top is the gastrovascular cavity, which is lined by body wall comprising ectoderm cells (twin arrows) some containing zooxanthellae (single asterisk), a mesogloeal layer (too thin to be visible at this magnification), and a thin layer of calicoblastic ectoderm (single arrow). The body wall is separated from the decalcified skeleton (bottom right) by an artifactually widened space (**); endolithic organisms (tandem arrows) are embedded in the skeleton in close proximity to the body wall tissue.

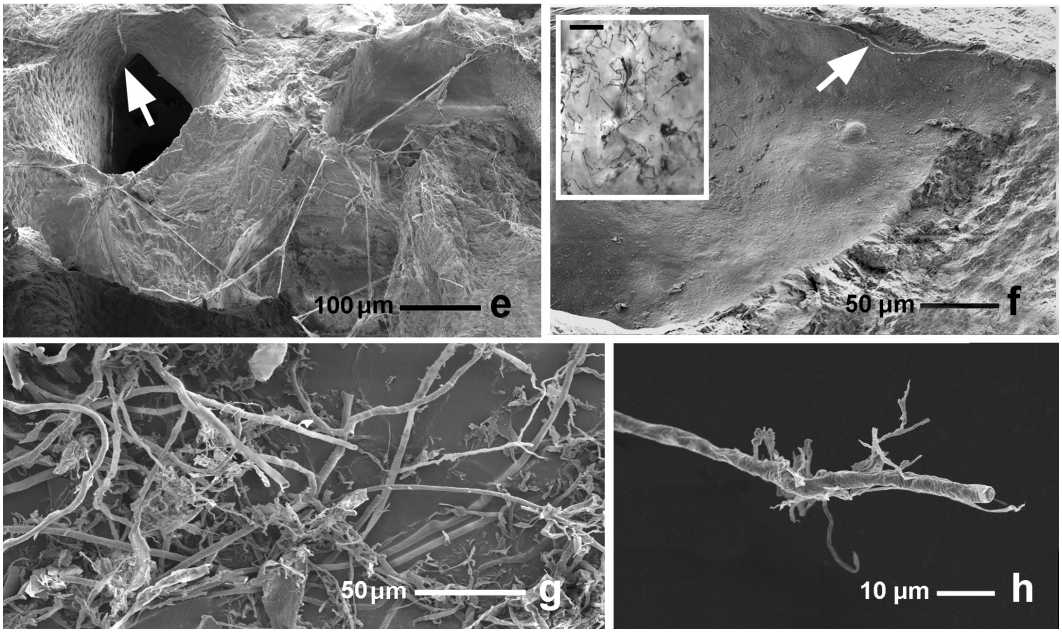

Figure 3-5. *Acropora cytherea.* Healthy portion of colony. **e,f** SEM views of the deep region of the skeleton, showing the absence of body wall tissue lining the spaces and a few endoliths (arrow); inset in 5f shows alcian blue-stained endoliths after partial etching of the calcareous skeleton (scale line is 200 mm). **g** A tangled mat of endoliths in the deep region of the skeleton after their liberation by decalcification. **h** Part of a single endolith from the aforementioned mat; the side branches off of the main thallus identify it as a fungus.

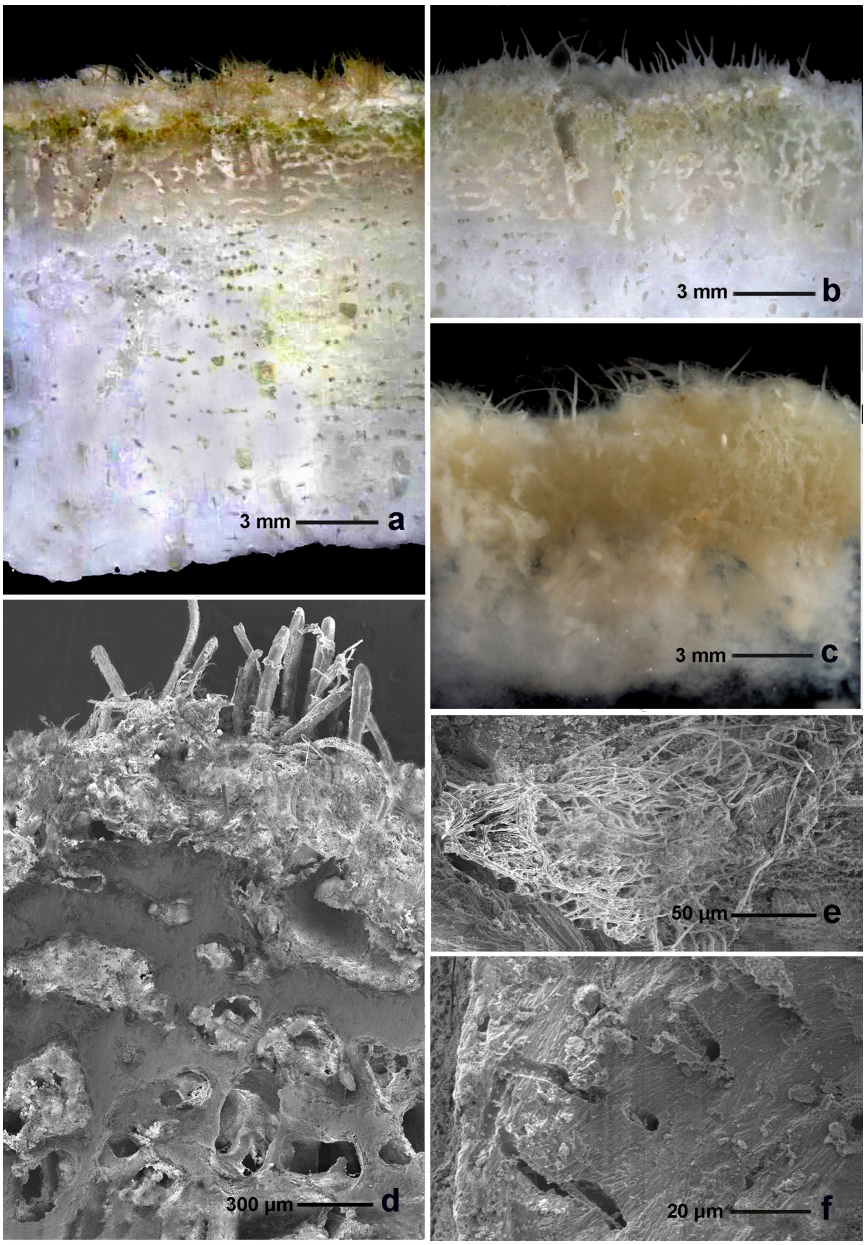

Figure 3-6. Overgrown portion of *Acropora cytherea* colony 5 cm from the interface with healthy portion of the colony. **a** Side view of a cut, undecalcified core sample from the dead, overgrown coral skeleton. **b** Side view of a after partial decalcification showing that the spaces in the shallow region of the skeleton ae filled with sediment. **c** Side view of a after 4 days of decalcification. In the deep region of the skeleton, the removal of the skeleton has freed masses of fibrillar endolithic organisms that have spread out into a fibrous mat, obscuring the spaces formerly visible there. **d** SEM overview of the shallow region of the overgrown portion of the colony showing most of the spaces in the skeleton are filled with sediment. **e** An exceptional instance of a mass of endoliths free in a space in the shallow region of the overgrown portion. **f** Relatively large endolith borings in the shallow region of the skeleton in the overgrown portion.

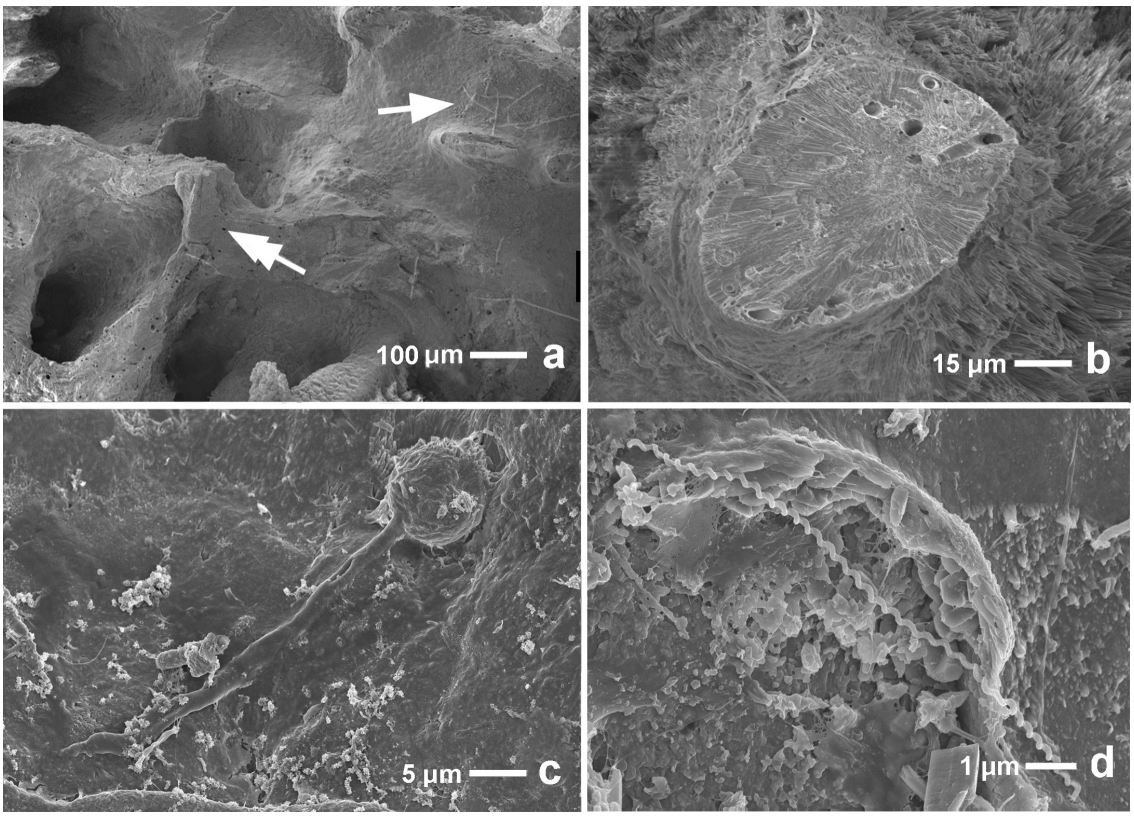

Figure 3-7. Overgrown portion of *Acropora cytherea* colony 5 cm from the interface with the healthy portion of the colony; deep region of undecalcified skeleton. **a** The spaces deep in the skeleton are free from sediment and contain only a few endoliths (single arrow). The broken edges of the skeleton show some openings of relatively large endolith bore holes (tandem arrow). **b** Higher power SEM of the relatively large endolith borings. **c** A fungal sporangium in a space in the deep region of the skeleton. **d** A spiral bacterium associated with the likely remains of an opened sporangium in a space in the deep region of the skeleton.

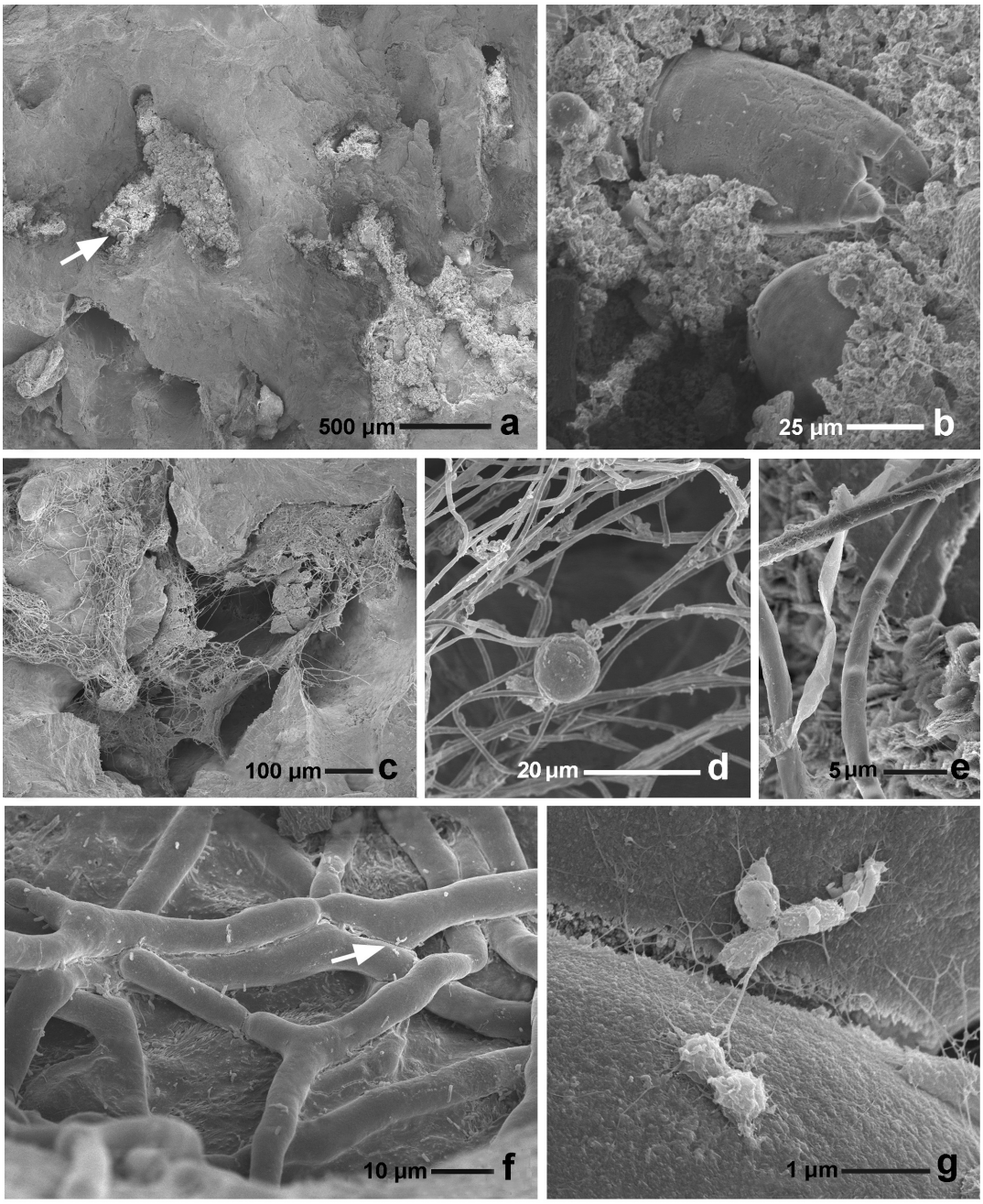

Figure 3-8. Overgrown portion of *Acropora cytherea* colony 30 cm from interface with healthy portion of colony; shallow region of undecalcified skeleton. **a** Sediment-filled spaces, one of which includes a possible carapace (arrowed). **b** Enlargement of a possible crustacean carapace. **c** A mass of fibrillar endoliths in a space. **d** Shallow region of an undecalcified specimen showing endoliths, including a fungal sporangium, free in a space in the shallow region. **e** Enlargement of several of the fibrous endoliths in previous figure showing faint segmental cross walls. **f** Ostreobium on the skeletal surface bounding a space; a cluster of bacteria is arrowed. **g** Enlargment of the bacteria in preceding figure.

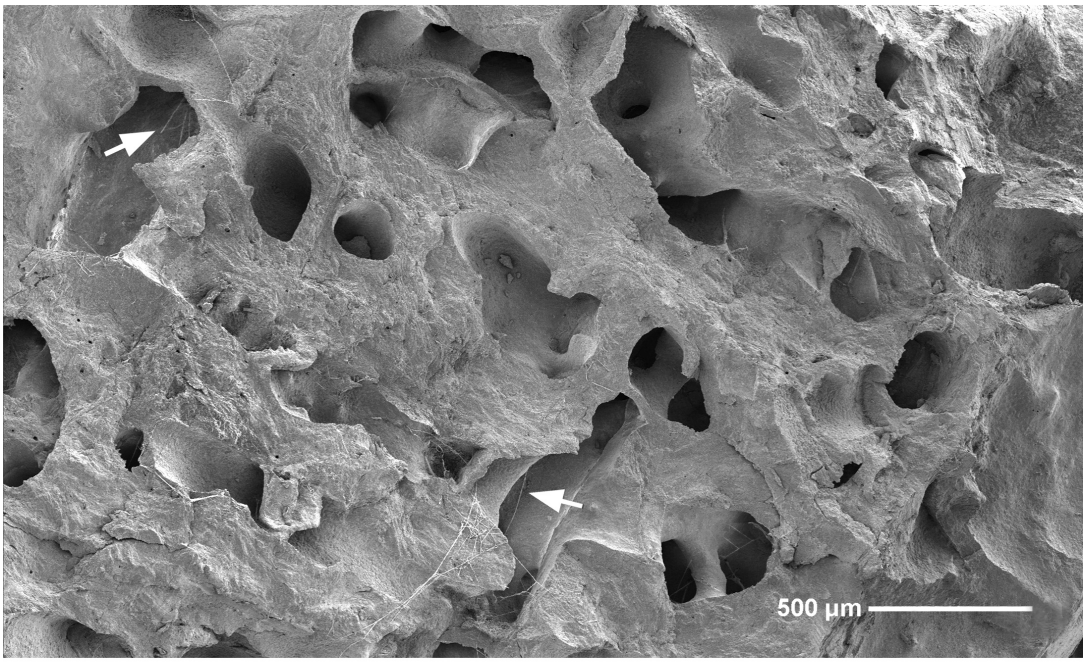

Figure 3-9. Overgrown portion of *Acropora cytherea* colony 30 cm from interface with healthy portion of the colony. Deep region of the colony showing skeletal spaces largely empty, except for some sparsely distributed fibrous endoliths (arrows).

Table 3-1. Endolithic bacteria, fungi and algae associated with coral skeletons

"Healthy" corals

Species (and Locality)	Endoliths	Reference
Six species (Enewetak)	Chlorophyta[1]	Highsmith (1981)
18 species (Pacific & Caribbean)	17 identified fungi Rhodophyta, Cyanobacteria[2]	Kendrick et al. (1982) Laborel and Le Campion-Alsumard (1979)
Porites lobata (Moorea)	Chlorophyta[1]	Le Campion-Alsumard et al. (1995a)
Porites lobata (Moorea)	Chlorophyta[1], Fungi	Le Campion-Alsumard et al. (1995b)
Pocilopora eydouxi (Johnston)	Fungi	Bentis et al. 2000
Acropora cytherea (Johnston)	Chlorophyta[1], Fungi	
Acropora humulis (Johnston)	Fungi	
Montipora studeri (Johnston)	Chlorophyta[1], Fungi	
Porites lutea (Moorea)	Chlorophyta[1], Fungi*	Golubic et al. 2005
Acropora hyacynthus (Samoa)	Fungi (11 OTUs)	Amend et al. (2012)
Orbicells faveolata (Mexico)	Chlorophyta[1], Fungi, Cyanobacteria[5-7]	Gutiérrez-Isaza et al. (2015)
Isopora palifera (Taiwan)	Green sulfur bacteria	Yang et al. (2016)
Many species	Great diversity; especially clades of green algae	Marcelino and Verbruggen (2016)**

"Sick", "overgrown", or "Dead" corals

Species (and Locality)	Kinds of endoliths	Reference
Six *Porites* spp. (Indonesia)	Chlorophyta[1], Fungi	Bak and Laane (1987)
Porites lobata (Moorea)	Chlorophyta[1,3], Cyanobacteria[2,4]	Le Campion-Alsumard et al. (1995a)
Porites lobata (Australia)	Chlorophyta[1], Fungi[2], Cyanobacteria[2]	Tribollet (2008)
Orbicella faveolata (Mexico)	As in healthy coral + 12 more	Gutiérrez-Isaza et al. (2015)

[1] *Ostreobium*
[2] *Plectonema*
[3] *Phaeophila*
[4] *Mastigocoleus*
[5] *Leptolyngbya*
[6] *Dalmatella*
[7] *Jaaginema*

* Fungi penetrating into pore space, but not clear whether in deep or shallow region of the skeleton

**Fungi may be underrepresented in this study because the primers used may detect fungal rRNAs less effectively than the internal transcribed spacer primer used by Schoch et al. (2012)

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CHAPTER 4:

Corals and their neighbors in partial mortality:

Multi-marker barcoding and micro-ecological exploration of living and dead coral

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Abstract

Corals host a diverse community of symbionts in addition to zooxanthellae, including endolithic algae, fungi, and cryptic invertebrates. Endolithic and epilithic organisms in living and dead corals are typically examined separately. However, colonial corals frequently suffer partial mortality, resulting in the live coral living next to regions of "dead" skeleton. Connected by skeleton, these communities of cryptic organisms live within close proximity, interacting with each other and the coral host. This study is novel in its combination of two investigation techniques: microscopy and meta-barcoding. Coral cores were collected across a gradient of living and dead coral skeleton in *Acropora cytherea* tables on Palmyra Atoll, USA. Cores were analyzed using compound and dissecting scopes for diversity of algal communities. Additionally, coral cores were sequenced using ITS and 18S markers. This study is a spatial analysis of the coral holobiont and algal succession across three treatments: (1) living coral, (2) recently dead coral (near living tissue border), (3) long dead coral (far from living tissues). Microscopy and genomic techniques correlated, showing similar genera of algae. Meta-barcoding indicated a succession of organisms living in the different coral skeleton treatments. Overlapping genera of fungi in living and dead coral indicate that the coral host may be more connected to its apparently dead skeletal areas than previously known. Further study on this concept may provide insight into coral resilience after partial mortality events.

Introduction

The coral holobiont is greater than the sum of its parts. A coral is an animal (Cnidaria) living with an alga (zooxanthellae) and a diverse microbial (bacteria, archaea, and virus) and endolithic (fungi, algae, and invertebrates) community. All of these organisms together can be termed the coral holobiont. Garnering energy from many of these sources, the coral secretes a calcium carbonate skeleton, which in turn forms the main architecture of coral reefs. Additionally, the phages in coral mucus can add immunity to the host against bacterial pathogens (Barr et al. 2013). The coral's overall health and survival relies on its symbiotic relationships (Reshef et al. 2006). For example, the energy transferred from the zooxanthellae is thought to give coral the resources needed to build large skeletons (Veron 1995).

The coral animal, in general, is a colonial, modular organism, capable of suffering death in some regions, while surviving in other regions (see Chapter 1, 2). Once the coral tissue dies, the skeletal surface is colonized by algae and other microorganisms almost immediately (Diaz-Pulido and McCook 2002). The order of colonizers is generally consistent, with light bushy turf settling in the first month, followed by crustose coralline algae (CCA, > 15 mo), and/or fleshy macroalgae (>20 mo), depending on grazing pressure (Diaz-Pulido and McCook 2002, Diaz-Pulido and McCook 2004). The turf communities can fluctuate seasonally, but they can also increase in species richness over time (Diaz-Pulido and McCook 2002). For example, *Phaeophila sp.* is an early stage turf colonizer, and the large chlorophyte usually declines after a month of coral mortality (Grange et al 2005).

Death of a coral triggers a complex reaction in the endolithic and epilithic communities (Le Campion-Alsumard et al. 1995a). The two most common epilithic organisms on dead coral are turf algae (a consortium of organisms) and crustose coralline algae (CCA). The competitive interactions between algae and coral are known to be potentially toxic to the coral host (McCook et al. 2001, Diaz-Pulido and McCook 2004, Smith et al. 2006), and turf algae can have detrimental effects on coral growth and metabolism (Barott et al. 2009, Hauri et al. 2010). Positively, CCA can attract coral larval settlement and can show no signs of competition or aggression when bordering coral (Heyward and Negri 1999, Barott et al. 2009). While these studies would indicate a propensity for coral cells to survive underneath CCA, CCA is a calcifying, possibly opaque substrate. Turf algae may be more porous, allowing cells to survive more easily underneath. Several recent studies have found coral cells surviving underneath algae, even surviving to eventually overgrow it (Riegl and Pillar 2001, Diaz-Pulido et al. 2009, Roff et al. 2014).

Endolith communities are shaped by the surface colonizers, coral or otherwise, and how they shade the interior of the coral skeleton. Only endoliths with low-light adaptations, like *Ostreobium spp.,* can survive underneath more opaque organisms like corals and CCA (Tribollet 2007). Dead corals can host higher densities of endoliths, as the surface is more porous and less defended for colonizing; and the turf and macroalgae block less light than zooxanthellae, allowing increased internal algal growth (Tribollet 2007). Endolithic fungi are not light limited, but nutrient limited (Golubic et al. 2005). Thus fungi are endolithic saprotrophs that consume the orgnanic matrix within the coral skeleton (Raghukumar and Ravindran 2012). Endolithic fungi can occupy two distinct

ecological niches in coral skeleton; they can actively bore through calcium carbonate (euendolithic) or spaces previously excavated by other endoliths (cryptoendolithic) (Golubic et al 2005).

Endolithic organisms living with coral have complex relationships with their host. Endolithic algae can translocate photoassimilates to their coral host. However, if the coral bleaches, the algae in the skeleton receive more light and more photosynthetically active radiation (PAR). This can cause an endolithic algal bloom, which can provide nutrition to the host coral (Fine and Loya 2002). Corals then have the ability to survive a bleaching period longer until they recover zooxanthellae. Corals that harbor high density populations of phototrophic endoliths are known to have higher survival rates following bleaching events (Fine and Loya 2002).

As a method, genomic markers can widen our understanding of the complex coral holobiont system (Marcelino and Verbruggen 2016). DNA and rDNA meta-barcoding permit more accurate detection and identification of the micro- and cryptic organisms, which are chronically undersampled with traditional microscopy (Leray and Knowlton 2017). This study combines ecological and molecular methods to examine coral epiliths and endoliths. Our study species was *Acropora cytherea,* a common table coral on the western shallow reef terrace of Palmyra Atoll, USA. Our objective was to examine living and dead coral and the community of organisms that inhabit them. This is an ecological approach to studying the community changes of coral-algal succession with molecular markers. Using these two complementary approaches, we comprehensively identified species growing in and on top of these corals and skeletons than has been possible with either method alone.

Methods

Sample collection

Samples were collected at Palmyra Atoll, Northern Line Islands, US territories, in summer 2014. *Acropora cytherea* growing in a large (> 10 m diameter) table morphology several centimeters thick were sampled. Coral cores (2.5cm diameter, 2.5cm depth) were collected by scuba divers using a pneumatic drill attached to a scuba cylinder. 12 cores were collected in three locations on the coral across a gradient of living and dead: living coral that was < 5 cm from living tissue, and dead coral that was > 30 cm from living tissue. See Chapter 3 for detailed collection methods and experimental sampling design. All methods were collected from the same depth to avoid community differences due to ambient light levels. Samples were stored in RNAlater for 2 years.

Microscopy for algal identification

Turf algae on each plug were examined under dissecting and compound microscopes. The entire plug was placed under the dissecting scope. Images of the plug were taken at mag 0.7x and analyzed for overall percent cover of turf algae, CCA, and calcium carbonate / biofilm per plug as in Tebbett et al. (2017). Turf density was compared across plug location on the coral (n = 4 coral tables, 3 treatments or cores per

coral). Turf height was collected at five ad hoc locations equally spaced around the plug and averaged by plug per Harris et al. (2015). Turf height was compared across plug location on the coral (n = 4 plugs per location).

For each plug all turf algae were scraped off the surface (5 cm^2) of the plug using a razor blade, superficially < 1mm depth. Removed turf and calcium carbonate were decalcified with 5% HCl, stained with aniline blue (Price and Scott 1992), spread on a slide and then examined under a compound microscope (mag 100x). Turf algae were identified to genus or otherwise lowest taxonomic level.

DNA extraction

Coral cores were split horizontally in two segments. Only the top 1 cm had enough genetic material to process; the lower 1.5 cm contained insufficient genetic material for detection as assessed via PCR (polymerase chain reaction) and gel electrophoresis. Two out of three markers were successfully sequenced, and the other (tufA) was removed because it needed further optimization beyond the scope of the study. To gain a broad look at coral epiliths and endoliths, we used two metabarcoding markers: the 18S rDNA, a DNA barcode commonly used for eukaryotes, and ITS (nuclear ribosomal Internal Transcribed Spaces), a DNA barcode recommended for fungi (Schoh et al. 2012). Our DNA extraction protocol was adapted from Marcelino and Verbruggen 2016, with a few alterations described here. Coral cores were homogenized with liquid nitrogen in a mortar and pestle. RNA was extracted from approximately 250 mg of homogenized tissue and skeleton with Trizol and modified phenol/chloroform

extraction. RNA was converted into cDNA using the SuperScript III First-strand synthesis system (Invitrogen). RNA was cleaned using an ethanol precipitation. Quantity and quality of RNA and cDNA post-synthesis was assessed via Nanodrop 1000 and Qubit.

Amplification of markers,library prep, and Illumina MiSeq sequencing

ITS and 18S primers were used to amplify cDNA for sequencing. PCR cycling parameters were 98°C for 30 seconds, followed by 25 cycles of 98°C for 10 seconds, 50°C for 20 seconds, 72°C for 30 seconds, 72°C for 2 minutes, and holding at 4°C. Samples were combined from triplicate PCR amplification reactions. ITS resulted in several bands of different sizes per sample, showing signs of non-specificity. A gel excision method was used to target particular region of ITS. Barcoded amplicons were cleaned, pooled in equimolar concentrations, and multiplexed on a single run of 2×300 bp sequencing on Illumina's MiSeq platform by the UC San Diego Institute for Genomic Medicine.

Pre-processing of Illumina reads

For the 18S rDNA dataset, reads were trimmed with Trimmomatic version 0.33 (Bolger et al. 2014), merged using FLASH version 1.2.11 (Magoc and Salzberg 2011), and filtered to a minimum average quality score of q20 using scripts in Qiime version 1.9.1 (Caporaso et al. 2010a). Primers were removed with Cutadapt version 1.9.1 (Martin

et al. 2011), and trimmed sequences were then checked for chimeras against the Ribosomal Database Project gold database (training database v9; http://microbiomeutil.sourceforge.net) using vsearch version 1.1.1 (Rognes et al. 2016), retaining reads of at least 400bp length. Sequences were clustered into OTUs of 98% similarity using UCLUST (Edgar 2010) through the open-reference workflow in Qiime. This included removal of singletons, as well as PyNast (Caporaso et al. 2010b) alignment and taxonomic assignment using the SILVA v111 Database (Quast et al. 2012).

The ITS dataset was processed similarly, with the following differences: minimum length for quality-trimmed reads was set at 100 bp; chimera checking and taxonomic assignment was performed using qiime-formatted versions of the UNITE database v7.1 for ITS sequences (Abarenkov et al. 2016); PyNast alignment was skipped, due to the highly variable length of the ITS region. Final output of the preprocessing pipeline for the ITS and 18S marker datasets resulted in 761,023, 940,669, and 725,232 sequences respectively.

Results

Microscopy visual snapshots of dead coral skeleton

The dead coral skeleton cores that were taken from near (< 5 cm) living tissue appear to be “recently” dead as compared to the cores taken > 30 cm from living tissue,

based on the visible algal communities living on the surface of both core types. Cores taken < 5 cm from living tissue were characterized by mostly early stage turf and macroalgae (Fig. 1a, Diaz-Pulido and McCook 2002). The surfaces of the cores had an average of 58% turf algal cover [standard deviation, ± 52%], 3% crustose coralline algal cover (CCA) [± 3%], and 40% calcium carbonate/ biofilm cover [± 53%]. Turf height averaged 0.52mm [± 0.50 mm]. One sample with all bare skeleton and biofilm had a consistent turf height of < 0.1 mm. Surfaces of coral with turf algae varies too widely at small spatial scales to quantify statistically at the scale of our study (Harris et al. 2015). *Smithsoniella sp.* was found frequently in the recently dead coral skeleton.

The dead coral skeleton cores that were taken far from living tissue (> 30 cm) appeared less recently dead, as they were found with late stage turf algae, including complex cylinder red algae, and CCA (Fig. 1b, Diaz-Pulido and McCook 2002). The surfaces of the cores had 65% turf [± 33%], 34 % CCA [± 31%], and 2% biofilm [± 3%]. Turf height averaged 1.2 mm [± 0.60 mm].

Both treatments of dead coral skeleton types included *Gelidiella sp./ Gelidium sp.*, a turf-forming red algae with corticated, cylindrical branches, which is a common player in coral reef turf communities (Fig. 2a, b) (Jones et al. 2006). One sample's turf height measurements consisted of solely the red algae growing out of CCA. Another common turf alga in the early stage treatment was *Cladophora sp.* (Fig. 2c). Some epiliths overlapped both dead coral treatments, including *Polysiphonia*, rhodophytes (Fig. 2d,e), and *Ulva sp.* (Fig. 2f).

Multi-marker meta-barcoding

320 OTUs were found in the 18S rDNA survey, 43 previously known. The dominant signal in the live coral samples was the Dinophyceae Gymnodiniphycidae family (71% ± 5.1%), however, when using the 18S and SILVA database, this indicates the presence of zooxanthellae, *Symbiodinium*. All other signals found in the living coral treatment were < 15%. Other reads included mostly fungi: Thraustochytrium and Sordariomycetes. Some small indicators of cryptic red algae were present, including *Cryptonemia, Polysiphonia,* and *Phaeophila spp.* Rhodymeniophycidae, a broad red algae family, is the dominant signal in the "early stage" dead coral cores (12% ± 7.2%). Other principle organisms included Demospongiae (24%); however only in one early stage sample, CCA, polychaetes, and fungi. In the late stage coral skeleton, *Hydrolithon sp.*, CCA is the primary signal (25% ± 6.3%). Demospongiae is found in one sample only, however not the same coral as the one sponge signal in early stage coral. A group of diatoms, Bacillariophyceae, were found exclusively in recently dead coral. They are known to be both epiphytic and suspended on coral reefs (Glynn 1973, Lobban et al. 2012). The long dead coral samples were characterized by sponges, polychaetes (Eunice) and crustaceans. Other organisms discovered in the analysis at either a low signal or a low sample size included: *Spincula sp.* (worm) and *Dodecaceria sp.* (polychaete).

For the 18S rDNA sequencing, a principal component analysis (PCA) reveals that live coral samples cluster separately from dead corals (Fig. 4). However, our low sample size and low statistical power resulted in a bootstrap analysis which yielded no significant differences among samples (n=4). There was also no significant difference between the two treatments of dead coral, although this could be due to experimental design and turf community heterogeneity. The only Cnidarian the 18S rDNA identified on was the

Macrodactyla sp. anemone (found only in one late stage dead coral sample). The living coral itself, *Acropora cytherea* or other species, was not picked up in 18S dataset; however, we are completing ongoing work on CO1 markers (305 OTUs), which we expect might clarify this discrepancy.

56 OTUs were found with the ITS survey, only 4 of them previously known. The ITS barcode, specialized for fungi, found a small diversity of fungi across all treatments with no discernable differences in treatments. The majority of signal had low levels of matching with known sequences, with the categorization limited to "Fungi, Other". The primary fungi found were the class of Sordariomycetes, in both living and dead coral samples.

A SIMPROF cluster analysis using Euclidian distance was created using the *clustsig* package in R (R core team 2013). Live and dead coral samples were significantly dissimilar (Fig. 3). No difference between the two dead coral treatments (<5cm vs. >30cm from living tissue border) was discerned. A PCA was created to visualize the data differences. Samples clustered according to treatment (live, dead). 24 % of variance is explained by the first variable and 18% by the second. Live coral samples cluster around the Dinophyceae class of dinoflagellates and several fungi (Fig. 4).

Discussion

Epilithic and endolithic organisms in Acropora cytherea

We combined molecular meta-barcoding with traditional microscopy and ecological micro-surveys to understand the endolithic and epilithic communities living with coral table colonies of *Acropora cytherea.* These complementary approaches revealed cryptic biodiversity associated with living and dead coral, including an apparent successional gradient of epiliths and endoliths from older to more recent mortality, and extending to communities adjacent to living coral tissue.

Metabarcoding studies frequently rely on a single (or at most, a couple) standard markers, such as 18S, 16S and CO1. Unfortunately many potentially important organisms that are not amplified by these methods remain undiscovered or otherwise ignored (Marcelino and Verbruggen 2016). Our multi-marker meta-barcoding not only revealed some of this hidden biodiversity, but also identified a strong zooxanthellate signal in living coral samples, serving as a powerful positive control for the methodology. Furthermore, the red alga *Cryptonemia sp*. was found solely in the living coral, suggesting that it might be capable of growing underneath living coral, within the skeleton. This paper addresses the endoliths in living and dead coral, as well as epiliths on dead coral in varying distances from the living coral tissue.

We recorded a succession of epilithic organisms on dead coral skeleton close and far from living tissue. The data suggest that our coral cores occurred across a temporal gradient of mortality: from recently dead (close to living tissue) and long dead (far from living tissue). Turf algae and CCA were clearly visible in varying densities and communities on the surface of the skeleton. The recently dead corals had early stage turfs, such as *Cladophora sp.*

Our algal results partially corroborated Marcelino and Verburggen (2016), who reported *Phaeophilia sp.* in living and dead coral skeleton. *Labyrinthula sp.,* a marine slime mold (heterokont), was found in most of the recently dead skeleton. This genus is known for its pathogenic impacts on seagrass and has been associated with pink line disease in *Porites lutea* (Ravindran et al 2001, Peters 2015). Since this fungus is found in recently dead corals, but not in living or long dead specimens, it is possible that it is an opportunistic or causative pathogen associated with the coral's demise. One sample was apparently so recently (< 1 mo) dead that it was not yet colonized by turf. This sample was anecdotally in a white band intervening between the living/dying coral border.

The table morphology *of Acropora cytherea* colonies are often considered beneficial for competition initially. However, as the coral regrows live tissue over its partially dead skeleton now overgrown with epiliths, it encounters the competitive challenges of an encrusting coral (Swierts and Vermeij 2016). Turf algae can outcompete corals via abrasion, allelopathy, and overgrowth (Diaz-Pulido and McCook 2002) or indirectly via elevated microbial activity (Smith et al. 2006). As a result, interaction between turf algae and coral tissue can contribute to a coral's failure to recover from tissue loss.

Expansions to the study using the CO1 molecular marker will further address additional eukaryotic epi- and endoliths in living and dead coral skeleton, as well as molecular aspects of the host coral itself.

Benefits and detriments to having neighbors and roommates

Acropora cytherea grow in large tables on the reef terrace of Palmyra Atoll. *A. cytherea* has a history of disease, partial mortality, survival, and regrowth at Palmyra Atoll (see Chapters 2, 3). The living tissue surface area on the coral grows and shrinks in response to bleaching and disease (Williams et al 2011). This coral is known for "resheeting," a process where the living coral regrows over its own dead skeleton (Jordán-Dahlgren 1992, Grober-Dunsmore 2006). This mechanism promotes fast growth and much reduces the need to create new calcium carbonate (Jordán-Dahlgren 1992). The process of the large table *A. cytherea* resheeting, is similar to encrusting coral, in that the coral is essentially using an encrusting growth strategy to regrow over its original, dead, table body form (Swierts and Vermeij 2016). The coral may encounter similar stresses as encrusting corals (e.g., competing on the same physical plane as turf algae, rather than branching up over the algal canopy). How could the community of endoliths change the story of coral recovery?

Coral endoliths can be affect their hosts positively, negatively, or neutrally (Golubic et al. 2005). Endolithic algae and fungi are capable of contributing resources to the coral host or attacking it (Domart-Coulon et al 2004, Tribollet 2007, Le Campion-Alsumard et al 1995b). In 2002 Fine and Loya found that endolithic algae could promote coral recovery by translocating metabolites to the coral host. As the overlying zooxanthellate coral tissue can absorb 96% of photosynthetically active radiation (PAR), the endoliths inhabit a low light environment (Schlicter et al. 1997). However, during bleaching the loss of zooxanthellae allows more light to reach the algae, creating a bloom. The increase in PAR penetration increases the biomass and chlorophyll concentration in endolithic algae, and the coral is able to utilize the photoassimilates from

its endoliths (Fine and Loya 2002). This may permit longer survivorship of the bleached coral.

This study could not investigate a broad spectrum of endolithic photosynthesizers via barcoding because to include the endolithic cyanobacteria, we would have needed to use 16S barcode. However, we likely observed low signals of the green alga *Obstreobium sp*. in several corals across treatments, classified as "chlorophyta" (also identified from microscopy in previous Chapter 3). 18S captures a broad spectrum of organisms but euendolithic species, like *Ostreobium*, may be partially protected from extraction (Marcelino and Verbruggen 2016). Our study is limited in this identification, however, we assume Ostreobium is in our samples across treatments based on that preliminary data.

Endolithic fungi are usually considered parasitic and even pathogenic. A well-known fungal disease on gorgonians (seafans) is caused by *Aspergillus sydowii* (Alker et al. 2001), and one sample of dead coral skeleton in our study yielded a low signal from *Aspergillus sp.*. Fungi are reported to attack the coral-associated algae (Raghukumar and Ravindran 2012). Fungi have also been reported as pathogens of endolithic algae, phytoplankton and coralline algae (Kohlmeyer and Kohlmeyer 1979, Littler and Littler 1998). Raghukumar and Ravindran (2012) noted the literature gap in fungal pathogens on coral reefs and recommended improved molecular markers to further investigate the relationships. Paradoxically, few studies reveal fungi supporting cell growth (Le Campion-Alsumard et al 1995b, Domart-Coulon et al 2004, Tribollet 2007). Domart-Coulon and colleagues (2004) discovered that fungi could extend the survival of coral

cells in culture, specifically in cells involved in skeletonogenesis. This indicated that the fungi is might somehow serve as a short-term defense of the coral host.

The epilithic and endolithic communities invading dead coral skeleton during partial mortality events affect the coral's recovery mechanisms. Understanding how living and dead portions of corals are connected and influenced by their algal and fungal communities may become increasingly important in future studies. The key to coral regrowth should not remain buried in their skeletons. With new technologies and meta-barcoding techniques, we may be able to identify individual strands of cyanobacteria and fungi. These "individual" organisms may act as suppliers or modifiers of nutrients orchestrating coral survival or failure.

An ecological and molecular approach to investigating coral epilitihic and endolithic organisms

Additional multi-marker interdisciplinary approaches are needed to understand the living and dead coral communities and their contributions to coral reef ecosystems (Marcelino and Verbruggen 2016). Disparity between microscopy and barcoding most likely has to do with sample size, and variability on a micro scale. This study is unique in its design and combination of molecular and ecological methods.

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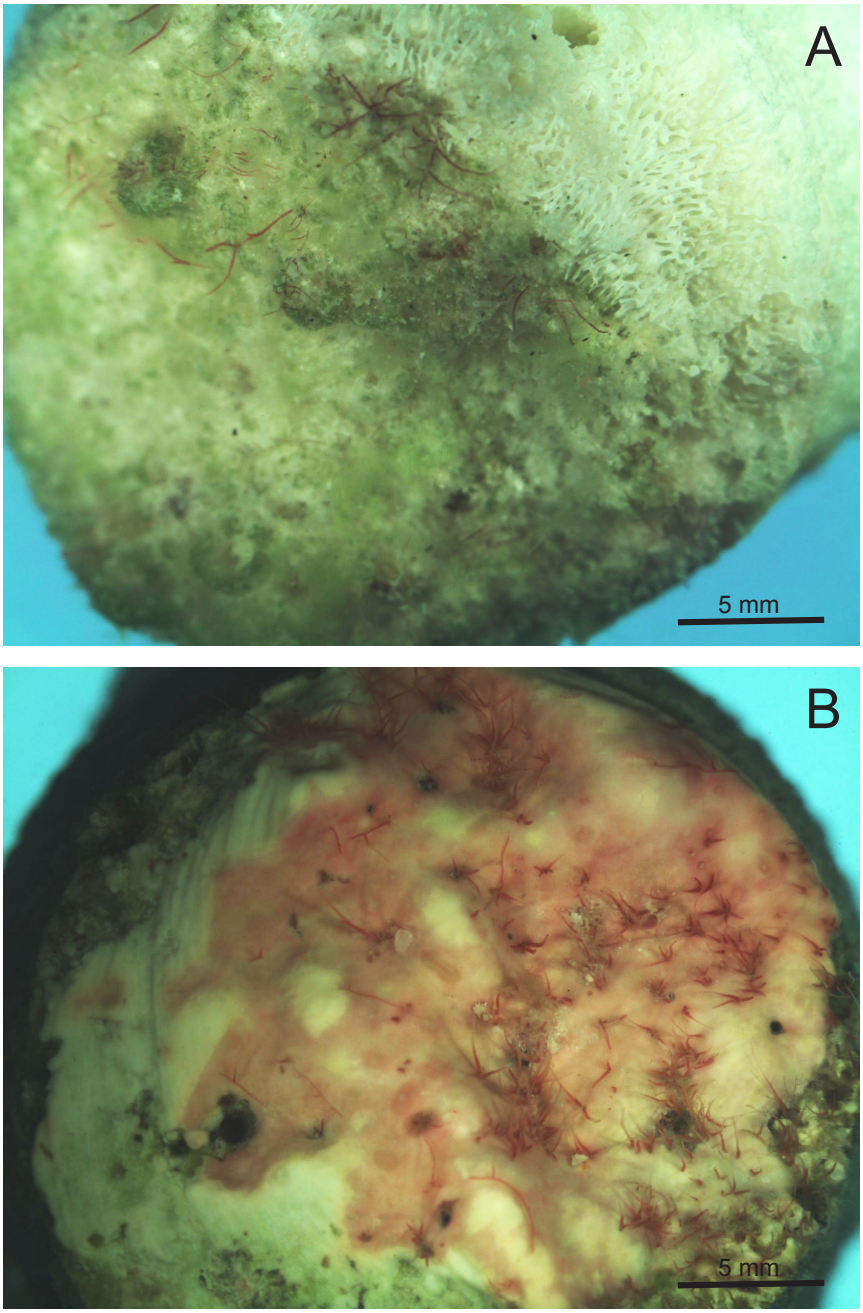

Figure 4-1. Overview of dead *Acropora cytherea* coral sample treatments. A) Surface of "recently" dead coral core with early successional turf algae and epiliths. B) Surface of "long" dead coral core with crustose coralline algae (CCA) complex cylinder red algae.

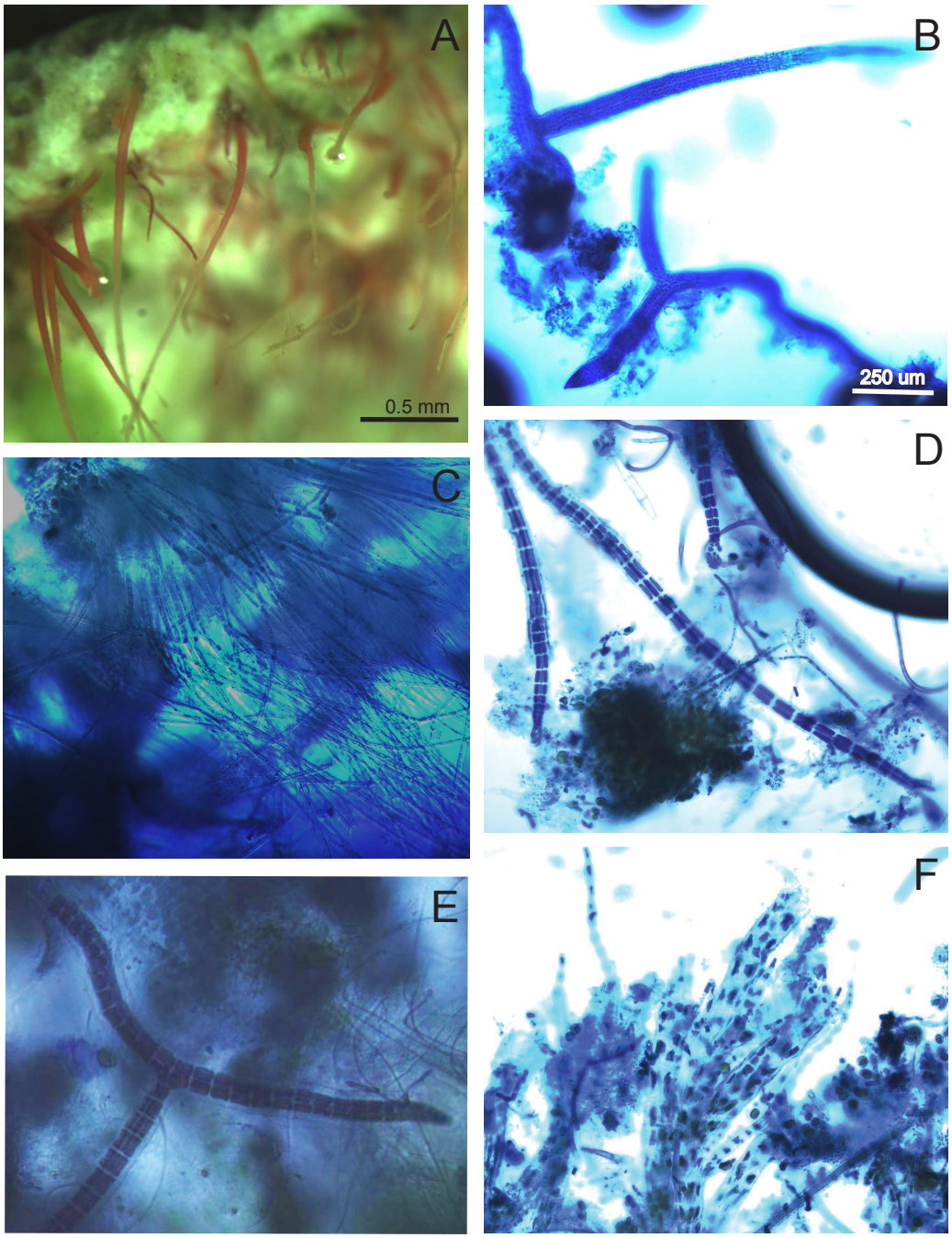

Figure 4-2. Detailed images of dead coral surface and turf algae under (A) dissecting (2.5x) and (B-F) compound (100x) microscopes. A) Detailed photograph of *Gelidiella sp./ Gelidium sp.* growing on recently dead coral (2.5x). B) *Gelidiella sp* from recently dead coral C) *Cladophora sp.* from recently dead coral. D) *Polysiphonia sp.* from recently dead coral. E) *Polysiphonia sp.* in long dead coral. F) *Ulva sp.* from long dead coral. Scale bar for dissecting microscope in (A) and compound microscope in (B-D).

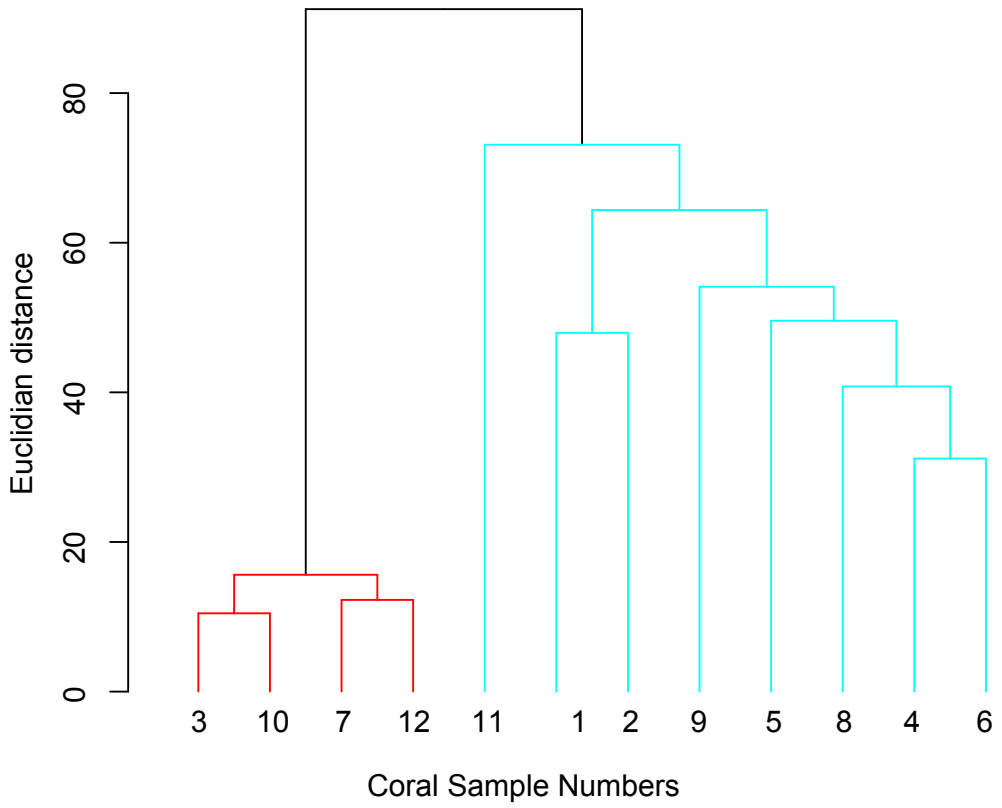

Figure 4-3. SIMPROF's. Euclidian distance of dissimilarity. Red lines indicate relationships of living coral samples, and blue lines indicate relationships of dead coral samples. Live coral (red) samples different from dead coral samples (blue).

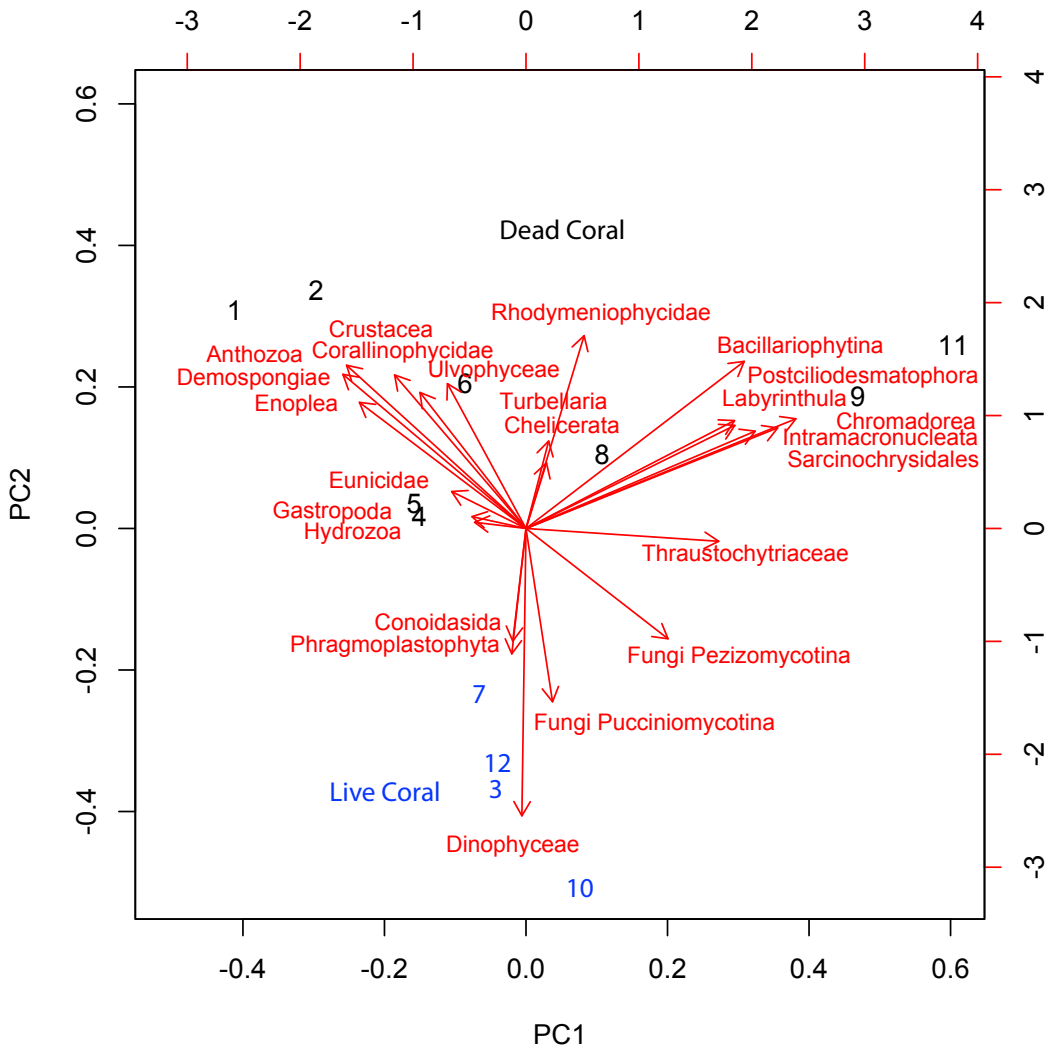

Figure 4-4. Ordination plot based on principal components analysis (PCA) of coral endoliths, showing different treatments (Live and dead coral skeleton). Red arrows denote endoliths found in samples. Live coral is blue and numbered 3, 7, 10, 12). Dead coral is black and numbered by sample. Dead near living, numbered 2, 8, 9, 11 and dead far from living, numbered 1, 4, 5, 6. First and second axes of the diagram account for 24 and 18% of variance respectively.

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CONCLUSION

Corals can survive death. Modular, colonial corals can suffer injury, lose tissue to disease, bleaching, predation, or breakage, and partially survive. This dissertation asks what is the limit of coral survival, and where does any subsequent coral recovery originate. My answer is in four chapters: (1) an evolutionary perspective on colonialism and partial survival, (2) an observational ecology study on the biological source of survival during a bleaching event, (3) cellular mechanisms of coral recovery and endolithic interactions in coral skeleton, and (4) a genomic and microscopic inventory of life inside living and dead coral. The main conclusion of this dissertation is that coral regrowth is a critical mechanism for recovery but is not limited to the boundaries of the coral animal.

Based on the literature and preliminary data from Chapter 2, I thought there could be living coral cells hidden inside dead coral skeleton: endolithic, stem-like cells that could contribute to rapid regrowth under the right conditions. In a world of declining coral reefs, there are plenty of dead corals to search.

Evidence for the zombie coral hypothesis

Understanding the relative contribution of coral regrowth to the spatiotemporal dynamics of coral benthic communities is critical for predicting reef resilience. Coral mortality can be rapid and punctuated, however, recovery of the same system is typically gradual and sustained. The difference makes death easier to recognize and record than the subtle

changes associated with growth. Chapter 1 reviews the literature of coral regrowth and recovery, describing the evolutionary advantages and morphological possibilities of coral regrowth. Although the literature pertaining to coral reef recovery highlights recruitment and biological connectivity as key contributing dynamics, regrowth of surviving corals following partial mortality is an equally important dynamic (Gilmour et al. 2013).

Chapter 2 describes a selective bleaching event that disproportionately affects a small encrusting coral, *Porites superfusa,* in a remote reef system. This coral species is very common in the central Pacific. It suffered widespread mortality at Palmrya Atoll in 2009, and our study documents its steep decline, survival and subsequent growth. Three main findings of the study were (1) coral bleaching mortality caused a significant decline in cover in a year, whereas the following years of growth and decline were not significant, leaving the coral population still much lower than baseline; (2) larger corals were more likely to survive; (3) coral regrowth was an important mechanism of recovery. The study also identified "resurrected recruits" or coral growth that appeared to come out of a previously dead *P superfusa*, either cryptic habitat or remnant fragments. Cryptic tissues are coral cells that survive in recessed or shaded areas of the reef, often not visible by standard coral surveys. Coral recovery from stress events by using regrowth of cryptic tissues has been well-documented (e.g., Kramarsky-Winter and Loya 2000, Riegl and Pillar 2001, Lirman et al. 2002).

In the classic ecological literature, the tradeoff between reproduction, growth and survival is known as Cole's Paradox. Regrowth represents energy put into growth and survival, as opposed to recruitment. *Acropora spp.* have shown great potential for regrowth as a population recovery mechanism (Bak 1983, Diaz-Pulido et al. 2009). They

are fast-growing, branching and/or plating corals, growing up to 100mm per year (Gladfelter et al. 1978). *Porites superfusa,* along with others in the Poritidae, Acroporidae, and Siderastreidae families, is a perforate coral. Perforate coral species comprise approximately half of the known coral species (van Woesik et al. 2013). Perforate corals are typically fast-growing species. Their internal aragonite crystal structures can be lined with gastrointestinal tissue (Gladfelter 1982, 1983). The remainder of the thesis (Chapters 3 and 4) investigated the architecture and inhabitants of living and dead *Acropora cytherea.* The skeletal structure of *Acropora* theoretically lends itself to regrowth, as tiny hollow sphericles and vesicles have been found within the branching matrix, and this could provide ample cryptic habitat for surviving coral tissue (Isa 1986). Mechanisms for this are relatively unknown, as regrowth at a cellular level appears unknown in the coral literature.

Regeneration at the cellular level is well described in the hydrozoan literature. Cnidarians like *Hydra* have interstitial (stem) cells, which are capable of "wandering" (Künzel et al. 2010). They have also been found to regenerate after disassociation and reassembly (Gaillot 2002). The studies on *Hydra* and other Cnidarian regeneration and immortality begs study on the limit of coral survival from a single cell.

Dissertation conclusions not supporting cryptic cells

Using scanning electron (SEM) and light microscopy, Chapter 3 visually investigates the potential for coral survival at a cellular level. Healthy *Acropora cytherea* housed living cells within the perforate spaces, but that life is limited to a shallow depth of

approximately 5 mm. No interstitial cells were observed living in the healthy or dead coral samples. The genomic inventory of the same corals revealed no detectable DNA or RNA below 1 cm into the skeleton (Chapter 4). Our sample size for these chapters is low enough to suggest that further study should be done to rule out differences in sampling season, individual coral colony, island location, and species. The year we sampled, summer of 2016, was a warm water anomaly, and it is possible that the coral bleaching that was occurring could have influenced our results. No visibly bleaching corals were sampled, but they could have been stressed nonetheless. Conducting studies in aquaria where parameters could be controlled, and fresh samples taken continuously could greatly enhance confidence in the observed results.

Endolithic life after death

Endolithic life is limited by light and nutrient availability, however the deep canals of the perforate *Acropora cytherea* provide a host of possibilities. Cleaned out by bacteria and other saphrophytes, the empty canals are presumed to hold seawater or gastrovascular fluid of some kind. Studies have been done on the chemistry and flow of fluids in perforate coral systems when they are lined with tissue (Gladfelter 1983). However without the tissue and cilia lined walls, what fills the deep, dead coral spaces?

Where Chapter 3 failed to find interstitial coral cells or signs of dormant, remnant coral life with dead portions of skeleton, we did find a host of promising endolithic organisms. *Ostreobium*, a common cyanobacteria, and unidentified fungi were growing throughout living and dead coral skeleton. Chapter 4 failed to find coral at all (although

ongoing CO1 barcode investigations may change that result before publication). However, Chapter 4 also inventories a longer and more diverse list of algae, fungi, nematodes, polychaetes and crustaceans which inhabit living and dead corals. The role of the endolith in coral recovery has yet to be explored. A few studies have looked at how algae and fungi are capable of translocating resources to the corals, however those are limited (e.g., Fine and Loya 2002). As coral (and human) microbiomes are known to affect their immunities (Barr et al 2013), perhaps the endolithic community is capable of conferring similar effects (Reshef et al. 2006).

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