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Histological observations in the Hawaiian reef coral, *Porites compressa*, affected by *Porites* bleaching with tissue loss

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ABSTRACT

The scleractinian finger coral *Porites compressa* is affected by the coral disease *Porites* bleaching with tissue loss (PBTL). This disease initially manifests as bleaching of the coenenchyme (tissue between polyps) while the polyps remain brown with eventual tissue loss and subsequent algal overgrowth of the bare skeleton. Histopathological investigation showed a loss of symbiont and melanin-containing granular cells which was more pronounced in the coenenchyme than the polyps. Cell counts confirmed a 65% reduction in symbiont density. Tissue loss was due to tissue fragmentation and necrosis in affected areas. In addition, a reduction in putative bacterial aggregate densities was found in diseased samples but no potential pathogens were observed.

1. Introduction

In recent decades, coral diseases have increased in prevalence and geographical extent worldwide, threatening the health and survival of coral reefs (Harvell et al., 2004; Sokolow, 2009). However, descriptions of most coral diseases have been based on field surveys, and many diseases lack systematic morphological descriptions at both the gross and cellular levels (Work et al., 2008). Confounding the presence of lesions with causation of disease without appropriate laboratory confirmation has led to considerable confusion in the literature (Richardson, 1998; Work and Aeby, 2011). The use of a standardized nomenclature that provides a systematic morphological description of coral disease lesions at the gross and cellular levels allows uncoupling of the description of the lesion from the inference of causation and comparisons across geographical areas (Work and Aeby, 2006, 2011; Work and Rameyer, 2005). Systematic descriptions of lesions at the gross and cellular levels provide the initial step in the development of case definitions and may assist in identifying possible pathogens (Work and Rameyer, 2005; Work et al., 2008).

Porites compressa is one of the main framework building corals in Hawaii. In Kaneohe Bay, Oahu, this species is affected by *Porites* bleaching with tissue loss (PBTL) that manifests as diffuse areas of white discoloration (secondary to translucence and visibility of skeleton through tissue) of the coenenchyme with pigmented polyps that are often retracted (Fig. 1). The lesion may be located in the center or on the periphery of a colony or may be colony-wide (on smaller colonies). In most cases, PBTL results in partial tissue loss with subsequent algal colonization of the dead skeleton (Sudek et al., 2012).

Coral bleaching is defined as a de-pigmentation of the coral's tissues due to a disruption of the symbiosis between the endosymbiotic dinoflagellates (*Symbiodinium* spp.) and the coral host; it is typically characterized by the loss of the symbiotic dinoflagellates (Glynn, 1996; Glynn and D'Croz, 1990; Hoegh-Guldberg and Smith, 1989). Environmental stimuli such as high or low seawater temperatures (Glynn, 1996; Hoegh-Guldberg and Fine, 2004), high light or UV radiation (Drollet et al., 1995; Glynn, 1996), or bacterial infections (Kushmaro et al., 2001) can trigger this process. PBTL does not appear to be a response to elevated sea surface temperatures as it occurs only in isolated colonies at times when water temperatures are well within the thermal threshold of this species. Given the uncertain causes of this disease, we set out to characterize PBTL at the cellular level. Specifically, we measured tissue thickness and *Symbiodinium* cell densities and described changes at the cellular level. This study presents the first histological information on PBTL, so providing a foundation for a case definition of this disease.

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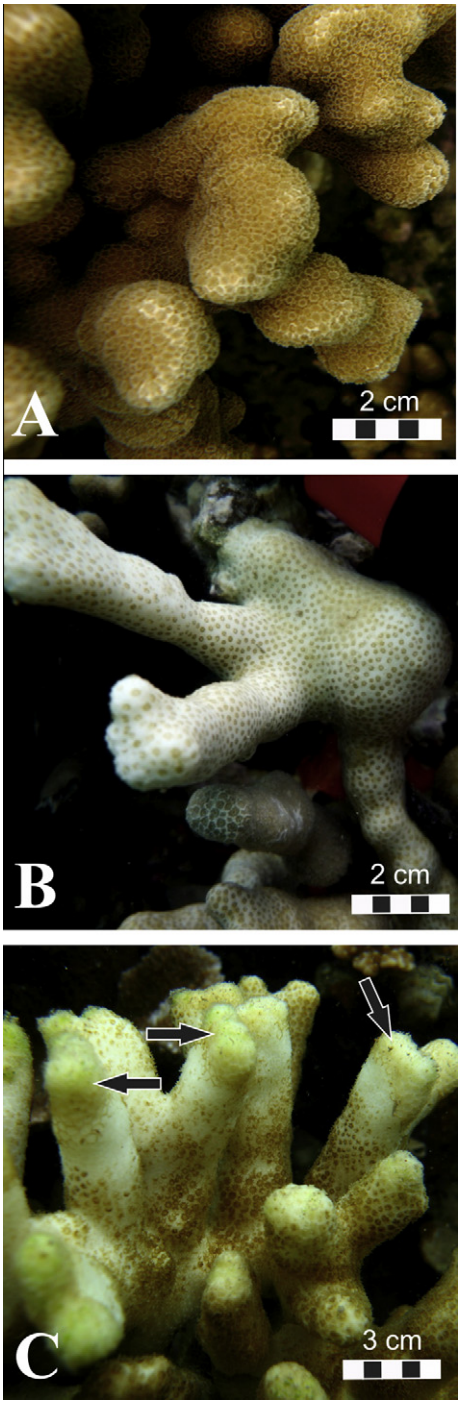

Fig. 1. (A) Healthy *Porites compressa*. Note regular brown coloration. (B) *P. compressa* affected by PBTL (early stage). Note white discoloration of coenenchyme and pigmented polyps but no signs of tissue loss. (C) *P. compressa* affected by PBTL (progressive stage). Note beginning of tissue sloughing on the tips of the coral branches (arrowhead).

2. Methods

2.1. Sample collection

Branches (2–3 cm^2) of *P. compressa* (36 fragments with signs of PBTL and 27 fragments from healthy control corals) were collected from the reef crest around Coconut Island, Kaneohe Bay, Oahu, Hawaii (21°26.000′N, 157°47.000′W) at a depth of 0.5–2 m in June 2010 and June 2011. The samples were fixed in 20% zinc-formaldehyde solution (1 part Z-Fix concentrate (Z-Fix® Anatech, Battle Creek, MI, USA) in four parts filtered seawater) immediately after collection.

2.2. Sample preparation

Samples were decalcified in 2.5% HCl buffered with 0.1% EDTA, rinsed and stored in 70% ethanol until further processing. After decalcification, all samples collected in 2010 ($n = 53$) were cut in half with a razor blade (tip to bottom) and laid open; measurements of tissue thickness were taken from the tip and the sides of the coral fragment using a Kincrome© Digital Vernier Caliper. A 2 cm diameter core was then removed from one half of all control and diseased fragments with a cork borer placed at a distance of 1 cm from the tip of the fragment. The core was homogenized with 1 ml of 0.2 μm-filtered seawater in a tissue homogenizer and algal cells were counted on a haemocytometer (Improved Neubauer, Boeco Ltd., Germany), with eight replicate counts per core. Cell densities were standardized to tissue volume of the core ($\pi r^2 \times$ thickness).

The other half of the decalcified coral fragment was trimmed and embedded in paraffin. Wax blocks were sectioned at a thickness of 6 μm using a rotary microtome, and the resulting sections stained with hematoxylin and eosin (H&E). Sections were examined at the microscopic level and lesions classified according to the presence of: (1) necrosis characterized by cytoplasmic hypereosinophilia or fragmentation coupled with nuclear karyorrhexis, karyolysis or pyknosis; (2) tissue fragmentation characterized by loss of epidermis and exposure of the basal body wall and mesenterial filaments; (3) changes in *Symbiodinium* and melanin-containing granular cell densities and/or morphology; and (4) presence or absence of associated organisms.

We also observed putative bacterial aggregates (Peters, 1997) in the tissues of *P. compressa* in both healthy and diseased samples (Fig. 2A). All putative bacterial aggregates were enumerated in a standardized 1712 × 1289 μm area of coral tissue approximately 1 cm below the branch tip.

3. Data analyses

The data for coral thickness, *Symbiodinium* cell densities and bacterial aggregate counts were checked for normality and equal variance. For coral thickness, these assumptions were met and a two-sample *t*-test was used to determine differences in tissue thickness between fragments affected by PBTL and control corals. Data for *Symbiodinium* cell and bacterial aggregate counts did not meet assumptions of normality and equal variance, so a non-parametric Mann–Whitney *U* test was used for comparisons.

4. Results

Branches affected by PBTL had significantly thinner tissue on the tip ($df = 51$, $t = 6.887$, $p < 0.001$) and sides ($df = 51$, $t = 2.322$, $p = 0.024$) than healthy controls (Table 1). Corals affected by PBTL also showed a significant decrease in *Symbiodinium* cell density (Mann–Whitney $U = 25$, $n = 53$, $p < 0.001$) and in the abundance of putative bacterial aggregates (Mann–Whitney $U = 148.5$, $n = 53$, $p < 0.001$) (Table 1). Of the healthy coral samples, 77% had putative bacterial aggregates in the examined tissue section versus 26% of PBTL-affected samples. Putative aggregates were round to oblong and ranged from approximately 170–1520 μm^2 in PBTL-affected samples and 175–1914 μm^2 in healthy samples.

Histological examination showed reductions in both *Symbiodinium* and melanin-containing granular cell densities that were more pronounced in the gastrodermis of the coenenchyme than

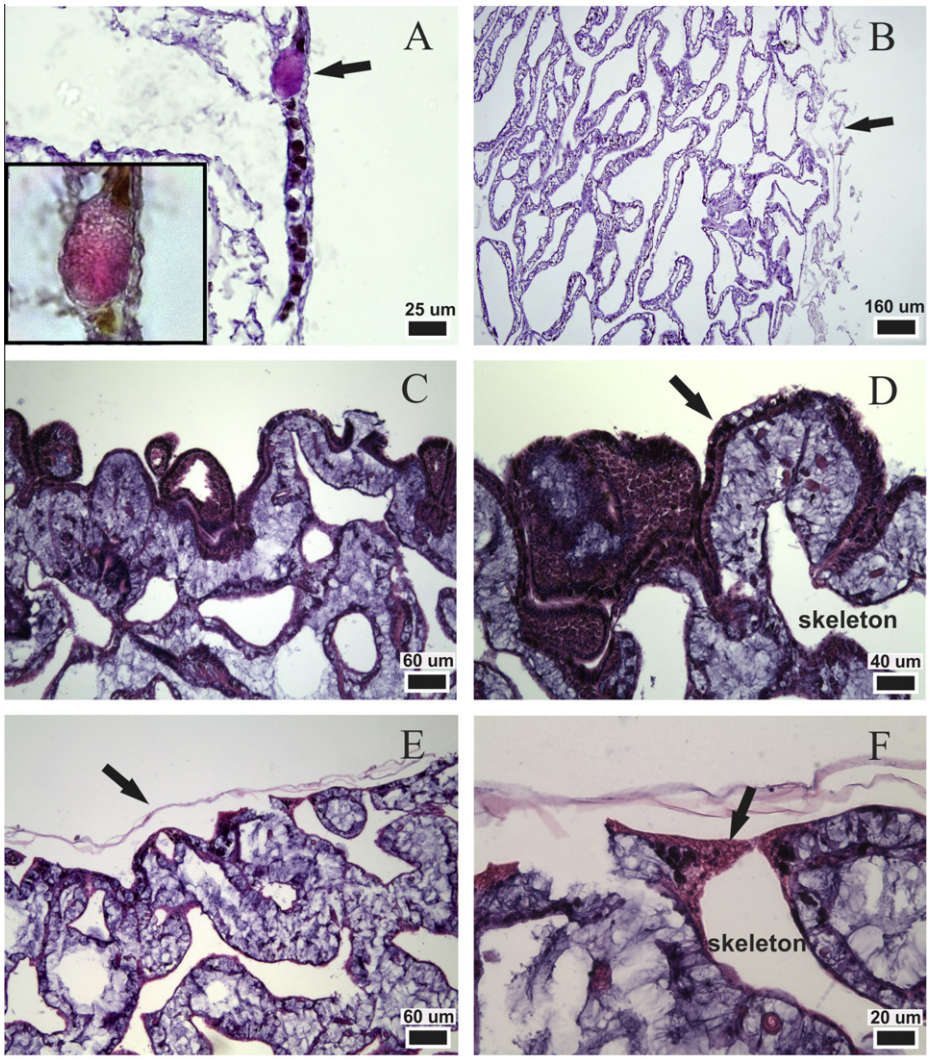

Fig. 2. (A) *P. compressa* with PBTL. Note putative bacterial aggregate in epidermis (arrowhead). Inset shows a close-up of the bacterial aggregate. (B) *P. compressa* with PBTL. Note ablation of the epidermis (arrowhead). (C and D) Normal *P. compressa*. Note regular columnar epidermis (arrowhead). (E) *P. compressa* with PBTL. Note attenuation and ablation of epidermis with overlaying hyaline membrane (arrowhead). (F) Close-up of E. Note hyaline membrane overlaying epidermis that manifests as cytoplasmic hypereosinophilia and karyorrhexis (arrowhead).

Table 1
Tissue thickness from the tip and side of the branches (mm), *Symbiodinium* cell densities (cells/cm^3) and putative bacterial aggregates (number/mm^2) in healthy control branches of *Porites compressa* and branches affected by PBTL. Values are mean ± SE and range. All pair-wise comparisons between healthy and PBTL-affected colonies were statistically significant ($p < 0.05$).

	Healthy	Range (min–max)	PBTL	Range (min–max)
Tissue thickness: tip (mm)	6.08 ± 0.18	4.69–8.21	4.38 ± 0.17	2.16–6.27
Tissue thickness: side (mm)	2.46 ± 0.07	1.84–3.19	2.21 ± 0.08	1.28–3.18
Symbiodinium density (cells/cm^3)	$1.4 \times 10^6 \pm 74533.1$	4.7×10^5–2.1×10^6	$4.8 \times 10^5 \pm 37027.8$	1.5×10^5–1.3×10^6
Putative bacterial aggregates (number/mm^2)	0.74 ± 0.14	0–2.3	0.19 ± 0.07	0–1.8

in the polyps. Ablation of the epidermis associated with algal overgrowth (Fig. 2B) was observed in 39% of samples affected by PBTL. Necrosis manifested as cytoplasmic hypereosinophilia and karyorrhexis overlaid by a hyaline membrane (Fig. 2E and F) and was observed in 19% of samples examined. Tissue fragmentation (Fig. 3A–C) was observed in 11% of PBTL-affected samples, and in two of these samples, helminths were observed in the degrading tissue (Fig. 3E). Clumps of diatoms were also found on the epidermis of one diseased sample (Fig. 3F). No other microbial or metazoan organisms were seen associated with diseased tissue for all remaining samples.

5. Discussion

Corals affected by PBTL showed a significant loss of their symbiotic dinoflagellates (*Symbiodinium* spp.) and melanin-containing granular cells, mainly from the gastrodermis of the coenenchyme.

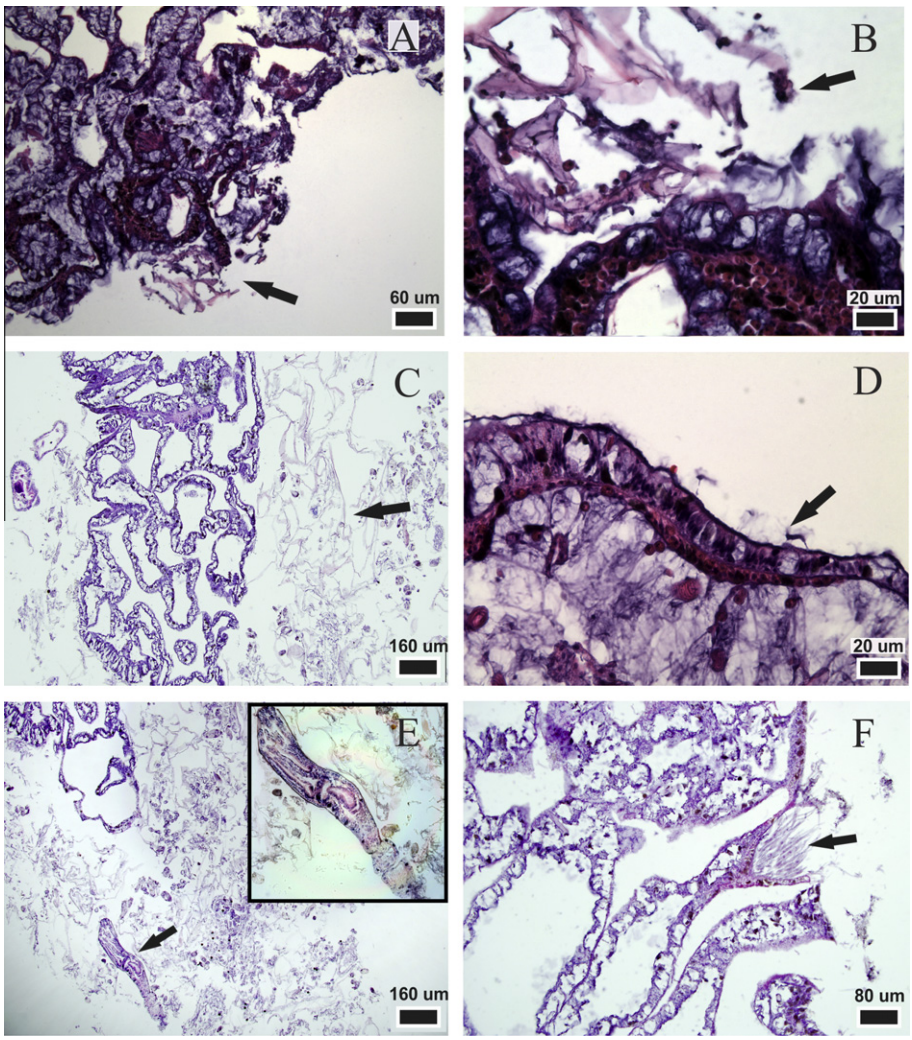

Fig. 3. (A) *P. compressa* with PBTL. Note tissue fragmentation and hyaline membranes effacing epidermis (arrowhead). (B) Close-up of A. Note cell debris (arrowhead) mixed with hyaline membrane. (C) *P. compressa* with PBTL. Note tissue fragmentation and cell debris (arrowhead). (D) Normal *P. compressa*. Note regular columnar epidermis with remnants of mucus (arrowhead). (E) *P. compressa* with PBTL. Note tissue fragmentation and helminths in the tissue debris (arrowhead). Inset shows a close-up of the helminths. (F) *P. compressa* with PBTL. Note clump of diatoms on epidermis (arrowhead).

This response was less pronounced in the polyps, thereby giving affected corals the typical "speckled" appearance (i.e. bleached coenenchyme and pigmented polyps). The extensive tissue loss of affected colonies observed in the field (Sudek et al., 2012) was found to result from tissue fragmentation and necrosis, leading to tissue mortality of affected areas.

PBTL-affected samples showed a 65% reduction in *Symbiodinium* cell density. This is similar to the loss seen in other corals that exhibit bleaching in response to disease. For example, bacterial bleaching in the reef coral *Pocillopora damicornis*, is characterized by a loss (>88%) and lysis of *Symbiodinium* cells due to an infection by *Vibrio* spp. (Ben-Haim et al., 2003). *Symbiodinium* cell loss (41–96.9%) is also seen in yellow-band disease (YBD), which affects *Montastraea* spp. from the Caribbean and starts as small blotches with reduced pigmentation, advancing over the colony and leaving dead skeleton behind (Cervino et al., 2001). PBTL, however, shows a very different and distinct bleaching pattern, with the coenenchyme bleaching first and the polyps remaining brown.

The loss of *Symbiodinium* cells may have contributed to the observed atrophy in affected samples (tissue thinning of 28% on the tip and 11% on the sides), which is indicative of a stressed coral colony. *Symbiodinium* cells can contribute over 90% of the coral's energy requirements through photosynthesis (Muscatine et al., 1984); a loss of *Symbiodinium* therefore leads to less energy being available for growth and other life processes such as reproduction and repair. Moreover, to counteract the prolonged loss of nutrition, corals may reabsorb their tissues (Szmant and Gassman, 1990). Atrophy can be observed in bleached corals (Glynn et al., 1985) and corals that are affected by sediment stress (Vargas-Angel et al., 2007).

Tissue fragmentation and necrosis were only observed in a few samples affected by PBTL, probably because the sampled coral colonies were at different stages of the disease. During sampling, branches in earlier stages of the disease (i.e. with intact tissue) were targeted and only a few could be collected with signs of tissue loss. Early stages of PBTL are mainly characterized by the loss of *Symbiodinium* cells, but it progresses to tissue fragmentation and necrosis in later stages. Cell death associated with tissue loss has been recorded in association with several coral diseases (McClanahan et al., 2003; Renegar et al., 2008; Williams et al., 2011; Work and Rameyer, 2005) and appears to be a common response to disease.

Ablation of the epidermis was associated with microalgal overgrowth, which may have contributed to tissue death. Other potentially opportunistic invaders, helminths and diatoms, were also observed in samples affected by PBTL. Helminths were found in

the tissue debris associated with tissue fragmentation that has also been reported in other coral diseases (Work and Aeby, 2011). Diatoms were observed on the epidermis of a diseased sample. Few studies have reported the occurrence of diatoms on the surface of corals (Johnston and Rohwer, 2007; Rublee et al., 1980) likely due to healthy coral's ability to protect themselves from settling organisms or sediment by mucus shedding (Brown and Bythell, 2005). Sorting out whether organisms such as helminths, diatoms or micro algae were primary invaders or sequelae to primary tissue loss will require longitudinal studies.

Using histology, bacterial aggregates have been observed in the tissues of corals (mostly *Acropora* spp.) affected by disease (Peters et al., 1983; Galloway et al., 2007), but no bacterial aggregates have been found in many other disease lesions (Ainsworth et al., 2007; Bythell et al., 2004). In this study, numerous clusters of putative bacterial aggregates were observed in both healthy and PBTL-affected corals but they were not associated with cell pathology, as seen in bacteria-induced diseases of vertebrates (Magi et al., 2009; Olsen et al., 2006), invertebrates (Johnson, 1976) and plants (Nelson and Dickey, 1970; Wallis and Truter, 1978). Indeed, a 74% reduction of putative bacterial aggregates was observed in corals affected by PBTL versus healthy colonies suggesting a disruption of the coral holobiont. If the symbiotic relationship between the coral and its associated microbial community is disrupted for any reason (for example changes in environmental factors), the whole balance of the holobiont could be compromised, ultimately contributing to a disease state (Vega-Thurber et al., 2009). Identifying how these bacteria interact with the coral host and their role in coral defense is a potentially fruitful avenue of investigation.

Given the lack of consistency between lesions and a particular etiological agent, we do not suspect that PBTL is caused by metazoans, bacteria or protozoans. However, smaller pathogens such as viruses, which are not easily detectable by light microscopy, cannot be ruled out and would necessitate ultra-structural examination of tissues. Other possible causes to consider are toxins and/or environmental triggers. Field studies have confirmed that PBTL can cause extensive tissue loss in affected areas of the colony (Sudek et al., 2012) so further research into the etiology of PBTL is underway to build a comprehensive case definition of this disease.

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