RESEARCH ARTICLE

Disease and cell death in white syndrome of Acroporid corals on the Great Barrier Reef

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Abstract White syndromes (WS) are among the most prevalent coral diseases, and are responsible for reef demise on the Great Barrier Reef. The disease manifests as a clear differentiation between tissue and exposed skeleton and results in rapid tissue loss. Fluorescence in situ hybridisation (FISH) was used in conjunction with histology and transmission electron microscopy (TEM) to investigate bacterial communities and cell death associated with WS. No evidence of bacterial communities or microbial association (using six bacterial probes, TEM and histopathology) was evident within the lesion or adjacent tissues, despite the presence of dense possible secondary invaders in the exposed skeletal regions. Despite widespread reference to necrosis in coral disease literature, there was no evidence of necrosis in any WS lesion or the adjacent tissues in this study. However, in situ end labelling, light microscopy and TEM of WS and healthy coral tissue sections showed evidence of extensive programmed cell death (PCD) exclusively in WS. This study provides the first evidence of intrinsic or PCD as a primary mechanism of cell death in WS, and may provide some explanation for the failure to isolate pathogens from over 80% of identified coral diseases, many of which show similar lesion patterns and WS characteristics.

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Introduction

Reports of disease-like syndromes in reef-building corals have increased since the first report by Antonius (1973). This increase has been attributed by many authors to anthropogenic changes to the coral environment (Harvell et al. 2002; Rosenberg and Ben-Haim 2002). To date, 18 different syndromes affecting corals have been described worldwide (Sutherland et al. 2004). Disease identification has predominantly occurred on reefs of the Florida Keys (Holden 1996), Red Sea (Kushmaro et al. 1996, 1997, 1998, 2001; Ben-Haim and Rosenberg 2002) and the Caribbean, where disease is now considered the major threat to future sustainability of reefs (Peters et al. 1983; Hughes 1994; Harvell et al. 1999; Green and Bruckner 2000; Aronson and Precht 2001). Despite several decades of research, a specific pathogen has only been identified for five diseases in corals and related cnidarian taxa: *Vibrio shiloi* has been identified as the causative agent in the bleaching of the Mediterranean *Oculina patagonica* (Kushmaro et al. 1998; Banin et al. 2001); *Vibrio coralliilyticus* is associated with tissue lysis of the Red Sea coral *Pocillopora damicornis* (Ben-Haim and Rosenberg 2002);

Aurantimonas coralicida as the possible causative agent of white plague type II (Denner et al. 2003); the fungus *Aspergillus sydowii* is the causative agent of mass mortalities of gorgonians in the Caribbean (Kim et al. 2000a, b); black band disease is believed to be caused by a mixed microbial consortium dominated by the cyanobacterium *Phormidium corallyticum* (originally *Oscillatoria submembranacaceae*) (Richardson et al. 1998a, b; Richardson and Kuta 2003). However, identifying the majority of disease causation has remained elusive particularly for many of the white diseases/syndromes (Sutherland et al. 2004).

Bythell et al. (2004) described white diseases/syndromes of coral as the most enigmatic coral diseases, despite the fact that these white diseases are widespread. The documentation of these white syndromes (WS) has been associated with a range of different diseases including white band (Aronson and Precht 2001), white plague (Richardson et al. 1998a, 2001) and "shutdown reaction" (Antonius 1981; Bythell et al. 2004). The white band or white area, after which these syndromes are named, is the result of rapidly sloughing coral tissue, characterised by distinct tissue loss patterns showing a clear lesion boundary between apparently healthy tissue and exposed skeleton (Antonius and Riegl 1997, 1998; Willis et al. 2004). Also, previous studies of most WS have noted a distinct lack of microbial communities associated specifically with disease lesions despite clear lesion boundaries and rapid progressions of tissue loss (Antonius 1977; Peters 1984; Richardson et al. 2001; Bythell et al. 2002).

White syndrome on the Great Barrier Reef (GBR) has been described as the most prevalent syndrome and occurs predominantly on tabular acroporids (Willis et al. 2004). The syndrome results in rapid tissue loss, with rates of loss varying from 1.0 to 124.6 cm^2 per day (tissue loss is not associated with damage or predation by predators such as the snail *Drupella* or the starfish *Acanthaster*) (Roff et al. 2006). Rapid tissue loss has previously been associated with the white disease "shut-down reaction", where tissue disintegration progresses across entire colonies and can be easily triggered after long periods of stress (Antonius 1977). In light of widespread difficulties experienced in pathogen identification in coral disease and the observed rapid lesion progression, we hypothesised, that patterns of tissue loss in WS may be intrinsic or a programmed cell death (PCD), triggered by a pathogen, physiological stress or combination of the two.

Cell death mechanisms have been widely described for higher organisms and have provided insight into many biological processes and diseases. Two mechanisms of cell death have been characterised across all Phyla; (1) necrotic cell death and (2) PCD or apoptosis.

Necrosis being described as an accidental or passive death of cells that have been exposed to extreme conditions (Syntichaki and Tavernarakis 2002). There are several distinct morphological characteristics of necrosis; these include cellular swelling, organelle distension, random DNA degradation, nuclear clumping, and extensive membrane endocytosis, and is most prominently distinguished by outstretched swelling of the cell to several times its normal size and the subsequent release of protoplasm (Böhm and Schild 2003; Cristea and Esposti 2004). Necrosis is considered only a relevant form of cell death in circumstances of gross injury, whereas PCD is described as the primary form of pathophysiological cell death (Raffray and Cohen 1997). PCD is a normal molecularly regulated mechanism occurring during many biological processes, and is biochemically and morphologically distinct from necrosis (Corcoran et al. 1994; Raffray and Cohen 1997). Cells undergoing PCD can be distinguished by distinct changes to cellular structure, including cell shrinkage, nuclear fragmentation and condensation (karyorrhexis), membrane blebbing (the formation of apoptotic bodies or undigested membrane bound cell fragments) and by caspase activation. Cells undergoing PCD have a gradual re-arrangement of the intracellular structures, without rupturing of the plasma membrane and without leaking of the intracellular contents, remaining intact during the condensation of the cell. PCD can occur in response to specific physiological or pathological stimuli and can be mediated in several ways during pathogenesis. PCD can be inappropriately or accidentally activated, as is seen in degenerative disease, viral disease, and toxin induced disease, or it can be rapidly induced as a basic mechanism to prevent or remove infection (Amersien 2002). While research into coral diseases has attempted to determine specific pathogens associated with various disease states, little is known about the resistance of corals to infectious disease or the mechanisms of cell death during disease (Sutherland et al. 2004; Richardson et al. 1998a; Mullen et al. 2004). PCD has been demonstrated in cnidarians associated with thermal stress (Dunn et al. 2004), but has yet to be investigated in the many coral disease-like syndromes. Understanding the mechanisms of coral disease resistance and cell death is of particular importance given possible effects of continued environmental stress and the potential for compromised disease resistance (Harvell et al. 2002; Mullen et al. 2004). There is also an imperative to understand the mechanisms underpinning disease-like syndromes given the rapid increase in the number of syndromes affecting corals in recent years, and evidence of resultant large-scale changes to reef ecosystems.

Materials and methods

Collection of WS in tabular acroporids and other corals

The study was performed at Heron Island and neighbouring Wistari Reef on the Central GBR (23.4417S, 151.9125E) Australia during 2004. Tabular acroporid (*Acropora cytherea, Acropora clathrata, and Acropora hyacinthus*) corals exhibiting signs of WS were collected from sites on Wistari Reef and Heron Island on SCUBA during March and July 2004, diseased ($n = 6$) and healthy tabular ($n = 6$) coral colonies were sampled, with three tissue fragments collected from each colony.

The collected tissue fragments were immediately fixed in 4% (w/v) paraformaldehyde in sterile phosphate buffered saline for up to 8 h. To ensure that tissues and microbial communities associated with the disease lesion remained intact during processing, the coral samples were subsequently enrobed with 1.5% (w/v) agarose (as described by Bythell et al. 2002) prior to decalcification with 20% (w/v) EDTA (St John et al. 2000) and standard processing for paraffin embedding and tissue sectioning. Replicate tissue samples from each colony ($n = 3$) were processed for sectioning. Serial tissue sections (4 μm) were collected onto Superforst Plus slides (Menzel, Brauschweig, Germany) and used for the staining techniques.

Acroporid corals with observed predation by the snail *Drupella* sp. feeding on the tissues were also collected from the reefs around Heron Island. In this case, *Drupella* sp. were removed from the colony and the region of the lesion caused by tissue predation was sampled, fixed and processed using the same protocol. This was conducted to evaluate potential bacterial differences between white disease (intrinsic) and predation (extrinsic) lesions.

Bacterial community study

Visualisation of bacterial communities associated with the disease lesion, adjacent tissues and exposed skeleton was conducted using fluorescence in situ hybridisation (FISH) (Ainsworth et al. 2006). Serial (4 μm) sections were used in the hybridisation with the universal eubacterial probes EUB338 mix (5′-GCTGCCTCCCGTAGGAGT-3′, 5′ GCAGCCACCCGTAGGTGT3′, 5′ GCTGCCACCCGTAGGTGT 3′). The Cy3 labeled oligonucleotide probes (Thermo Electon Corporation, Germany), were used in a standardised FISH protocol (Manz et al. 2000). The hybridization was conducted using 35% formamide concentration in hybridisation buffer (0.9 M NaCl, 0.01% SDS, 0.01 M Tris/HCl pH7.2) for 1.5 h at 46°C, followed by a 10 min wash in prewarmed wash buffer (0.08 M NaCl, 0.01% SDS, 0.01 M Tris/HCl, 0.05 M EDTA). A Zeiss Meta 510 confocal scanning laser microscope (Zeiss, Germany) combined with spectral emissions profiling of tissue autofluorescence was used for visualisation of FISH products in association with coral tissues and the disease lesion. The hybridization was repeated using a selection of bacterial group probes (Table 1) on adjacent tissue sections.

In situ analysis of cell death activity

To investigate mechanisms of cell death associated with WS, samples of tissue adjacent to the lesion and up to 3 cm away from the lesion were used for light microscopy. Harris's haematoxylin and eosin (with phyloxine B) (Sigma-Aldrich, cat. # HHS 32 and HT110-1-32) was used for evaluating the extent of mass tissue necrosis (swelling and lysis of cells, disruption of cell structure) and general tissue condition associated with the lesion and the adjacent tissues and in situ end labelling of fragmented DNA to investigate the presence and extent of PCD (In situ apoptosis detection kit S7101 Chemicon International, Inc. USA) (Dunn et al. 2002).

Cell counts of apoptotic (evident by red positive staining with in situ end labelling) and non-apoptotic cells (blue stain of nucleus with only hematoxylin) were conducted of all epithelial and mesentery cells within the tissue sections between the lesion and 2 cm away from the lesion, and in healthy tissue sections. Cell counts were performed on replicate tissue samples within each sampled colony ($n = 3$), and diseased ($n = 4$) and healthy ($n = 4$) colonies were analysed. In total 62,000 cells were counted microscopically, with 31,000 counted from healthy and 31,000 counted from diseased samples.

Table 1 Bacterial group probes used in FISH for identification of bacterial populations

Probe	Target group	Sequence	Formamide (%)	Reference
ALF969	α-proteobacteria	5′ TGGTAAGGTTCTGCGCGT3′	20	Manz et al. (1992)
BET42A	β-proteobacteria	5′GCCTTCCCACTTAGTTT3′	35	Manz et al. (1992)
GAM42A	γ-proteobacteria	5′GCCTTCCCACATCGTTT3′	35	Manz et al. (1992)
CF319	Cytophaga-flavobacterium	5′TGGTCCGTGTCTCAGTAC3′	35	Manz et al. (2000)
HCG69a	Actinobacteria	5′TATAGTTACCACCGCCGT3′	25	Roller et al. (1994)

Transmission electron microscopy

Samples of tabular *Acropora* healthy ($n = 6$) and symptomatic of WS ($n = 6$) were also preserved for transmission electron microscopy for investigation of bacterial communities and cell structure. Small fragments (0.2–0.5 cm^3) from each colony were immediately rinsed in 0.2 μm filtered and autoclaved artificial seawater and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer. Sample preparation used methods outlined in Le Tissier (1990), but was modified to include agarose embedding (Bythell et al. 2002) and decalcification in 2% formic acid over 3 days at 4°C. Sample grids with ultra-thin sections were viewed in a transmission electron microscope (JEOL 1010) at acceleration voltage 80 kV, and images taken using the Megaview III Soft Imaging system. Approximately 11,000 coral cells were viewed for general condition and determination of cell structures.

Results

Identification of WS in tabular Acroporid corals

Tabular acroporids displaying symptoms of WS were identified by the characteristic loss of tissue and the clear and distinct lesion boundary between white exposed skeleton and apparently healthy tissue (Fig. 1). Exposed skeletons were observed to become rapidly covered with benthic microflora and fauna (epifaunal community) at sites further from the lesion boundary.
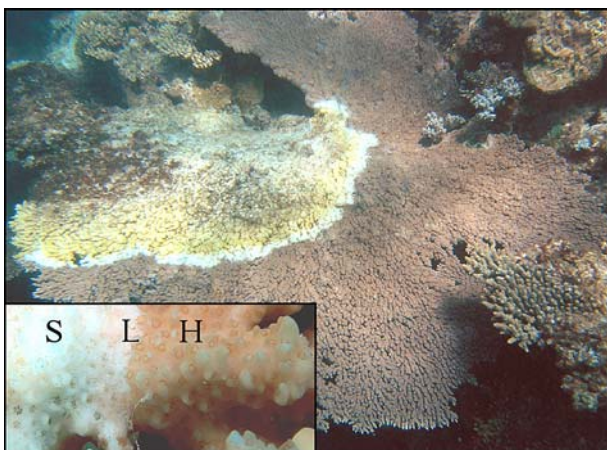

Fig. 1 White syndrome affecting tabular *Acropora* coral, with visible clear lesion (*L*) between recently exposed white skeleton (*S*) adjacent to health tissue (*H*) (*insert*)

Fluorescence in situ hybridisation

Bacterial populations were absent in all WS lesions and adjacent coral tissue (up to 3 cm away from the lesion border) in all tabular *Acropora* examined during this study (Fig. 2a–d). There was no evidence of binding of EUB338mix probes, or any of the six bacterial group probes in any of the WS lesions and adjacent tissues. Within the exposed skeleton behind the syndrome lesion (Fig. 2e) FISH-labelled bacterial communities were associated with the observed epifaunal communities. Identification of these bacterial communities, demonstrated the ability of the protocol utilised and the probes for visualisation of marine bacterial communities, and the effectiveness of agarose embedding for preserving the associated bacterial communities. Furthermore, using haematoxylin and eosin staining of adjacent WS tissue sections there was no evidence of other microbial communities within syndrome lesions and their adjacent tissues, apart from endolithic algal blooms of various intensities within skeletal regions and the epifuanal communities behind the lesion.

Interestingly, on the lesion borders resulting from predation by the coraliovore gastropod *Drupella* sp. bacterial communities were abundant. Dense bacterial populations were observed to penetrate from the *Drupella* sp. lesion into the surrounding coral tissues (Fig. 3a, b). These bacterial communities were also found on the surrounding coral tissue surface and within the gastrovascular canals (Fig. 3c, d).

In situ analysis of cell death activity

Haematoxylin and eosin staining of coral tissue sections showed no evidence of mass tissue necrosis associated with the syndrome lesion and surrounding tissues. The cells adjacent to, and up to 2 cm away, from the syndrome lesion displayed no signs of cellular swelling, rupturing or lysis, which are characteristic of necrotic cell death, and the cell membranes and cellular organisation of the tissues remained intact (Fig. 4c, d). In situ end labelling showed evidence of PCD in corals symptomatic of WS collected in both summer and winter 2004. PCD was evident by the dark red staining of apoptotic nuclei stained by the in situ end labelling, adjacent to normal nuclei (stained blue with haematoxylin) (Fig. 4a, b). This was consistent with the staining pattern evident within positive control vertebrate tissue sections. Also within diseased colonies tissues displayed cell morphologies consistent with macroscopic descriptions of PCD, including shrinkage and condensation of the nuclei while the cell structure, membrane and cytoplasm remained intact (Fig. 4c, d). Tissue samples of
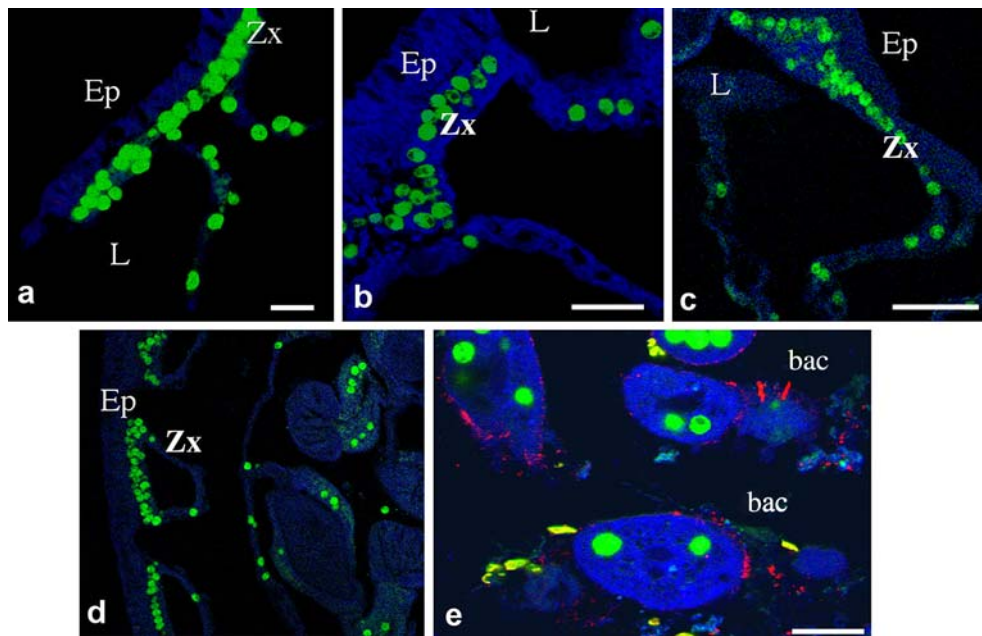
Fig. 2 Fluorescence in situ hybridisation (FISH) labelling of coral tissue section using Cy3 labelled universal bacterial probe EUB338 demonstrating the lack of bacteria associated with the coral tissue adjacent to the disease lesion in summer (**a**) and winter samples (**b**), or associated with the coral tissues (**c**) or healthy coral tissues (**d**), and bacteria (*red*) associated with epifaunal (ciliate containing predated zooxanthallae) community behind tissue lesion (**e**). Lesion (*L*), Zooxanthallae, *zx* (*green*); epithelium, *Ep*; bacteria, bact (*red*). Scale bar = 50 μm

healthy tabular colonies showed no morphological characteristics of PCD and no in situ end labelling of fragmented DNA within the cells (Fig. 4e–h).

Variability on the extent of in situ labelling of fragmented DNA within cells between diseased colonies was evident, with the percentage of apoptotic cells to normal cells within the epithelium ranged from 24 to 54%. There was less variability of in situ end labelling in the mesenterial cells, with 39–58% of cells stained (Fig. 5). There was no evidence for PCD or marking of cells by in situ end labelling within healthy coral colonies (Fig. 5), suggesting a lack of substantial PCD occurring with healthy colonies as well as a lack of non-specific binding by the in situ end labelling of DNA with the technique.

Transmission electron microscopy

Healthy tabular *Acropora* tissues appeared to be in good condition and showed intact characteristic cell structures including healthy and uncondensed nuclei (Fig. 6a, b). In contrast all WS affected tabular *Acropora* tissues investigated within this study showed areas with loss of cell to cell contact. Evidence of PCD was found in four of the six colonies investigated, with up to 25% of observed cells in each sample designated to display signs of PCD. These cells were characterized by condensation of the cytoplasm and the nuclear chro-matin (Fig. 6c–f), condensed cytoplasm and shrinkage (Fig. 6c, f), but intact cell structure and cell membrane (Fig. 6 c–f). Vacuolisation and blebbing of cytoplasmic contents were primarily observed in highly condensed cells where other features were unrecognisable. Condensed chromatin appeared either in a strong margin along the nuclear membrane periphery (Fig. 6c), or as one to several homogeneously condensed and regularly shaped nuclei (Fig. 6e). These cells were often rounded off and had lost contact with neighbouring cells (Fig. 6d). There was no evidence for PCD in either healthy colonies or in samples with areas of artificial lesions. Traces of cell debris from ruptured cells were occasionally observed in both healthy and WS samples, possibly an artefact of sample preparation.

Furthermore, there were no signs of bacterial or viral aggregates, protozoan or fungal microbes presence within the tissue or disease lesions. Bacteria-like structures were often observed on the outer side of epidermis or mucus layer within both WS affected and healthy tabular *Acropora* colonies but no observable difference was found between the two communities.

Discussion

This study is the first to provide evidence for the involvement of PCD in a disease-like syndrome affect-

Fig. 3 Bacterial communities (Bac, in *red*) identified by FISH using EUB universal bacterial probes, associated with the lesion of coral tissues predated by *Drupella* sp. (**a**) and penetrating into adjacent tissues (**b**), and on the tissue surface (**c**) and gastrovascular canals (**d**) of tissues adjacent to the lesion. Lesion *L*; epithelium *Ep*; zooxanthallae *Zx*; Bacteria *bac*. Scale bar = 50 μm
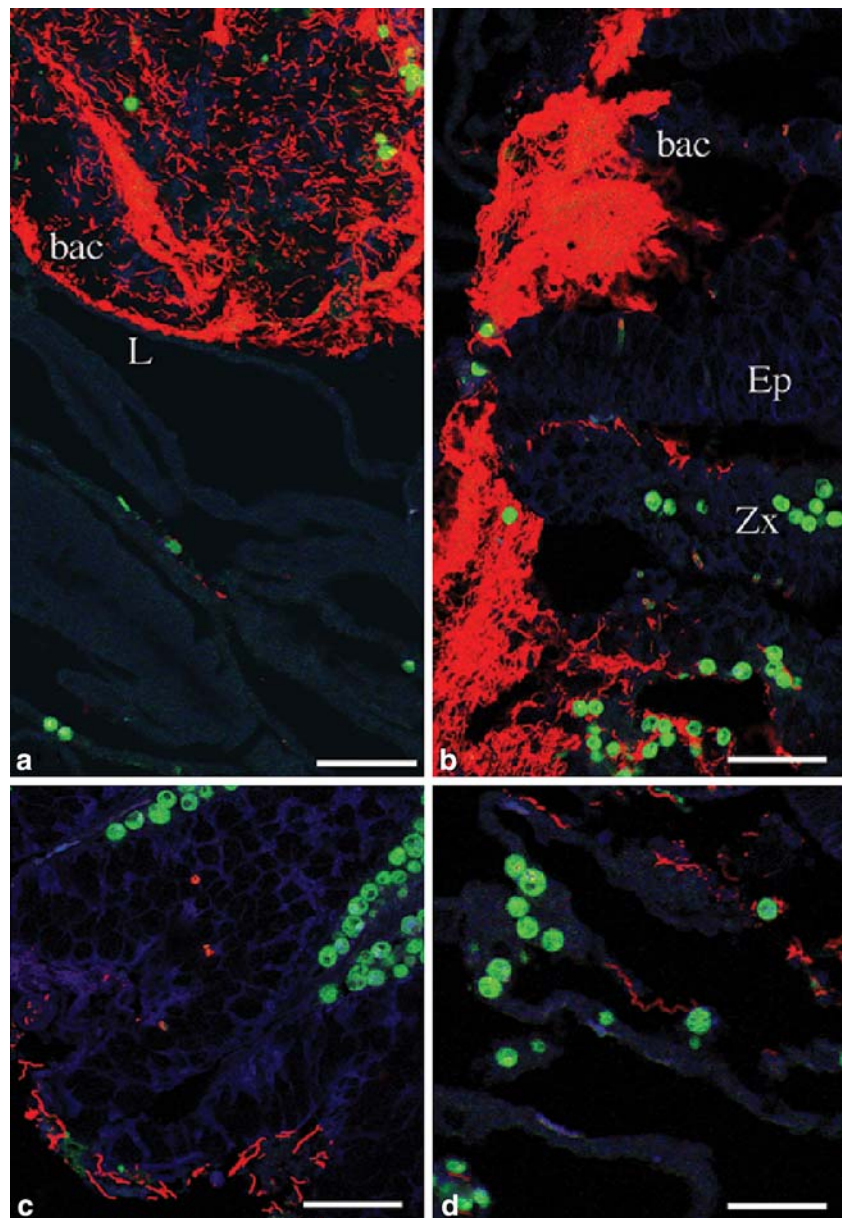

ing corals. The present study describes histopathological characteristics of WS affecting tabular *Acropora* on the southern GBR, which include: (1) a distinct absence of bacterial communities associated with the disease lesion and adjacent tissues; (2) a lack of evidence of necrotic cell death associated with the disease, with lesion boundaries bordered by apparently healthy tissue; and (3) evidence of PCD being the primary mechanism of cell death and tissue loss.

The use of oligonucleotide probes targeting the 16S rRNA and subsequent fluorescence anlaysis (FISH) is a rapid, reliable, and specific technique for detecting, identifying and quantifying bacteria (Amman et al. 1996; Thompson et al. 2004). The continued development of FISH in recent years has demonstrated the

accuracy and applicability of the technique, and it is now widely applied to identifying bacterial communities associated with various environments including the marine environment and environments with unculturable communities (Wagner et al. 2003; Thompson et al. 2004). Within the current study bacterial populations were identified within the denuded skeletal regions behind the lesions. Using a wide selection of bacterial FISH probes and histology techniques, consistently microbial communities were absent from the lesions of WS despite the presence of the mixed bacterial populations within adjacent denuded skeletal regions behind the lesions, there was no evidence of penetration of these communities into the syndrome lesion or adjacent coral tissues. This was also supported by a lack of

Fig. 4 In situ end labelling (**a**, **b**) and haematoxylin and eosin staining (**c**, **d**) of diseased tabular *Acropora* tissue sections, and in situ end labelling (**e**, **f**) and haematoxylin and eosin (**g**, **h**) staining of healthy tabular *Acropora* tissue sections, showing extensive programmed cell death within diseased tissues and no evidence of non-specific staining in healthy tissue. In situ end labelled nucleus, is (*red*); haematoxylin stained nucleus *Hx*; epithelium *Ep*; gastroderm *Ga*. Scale = 50 μm
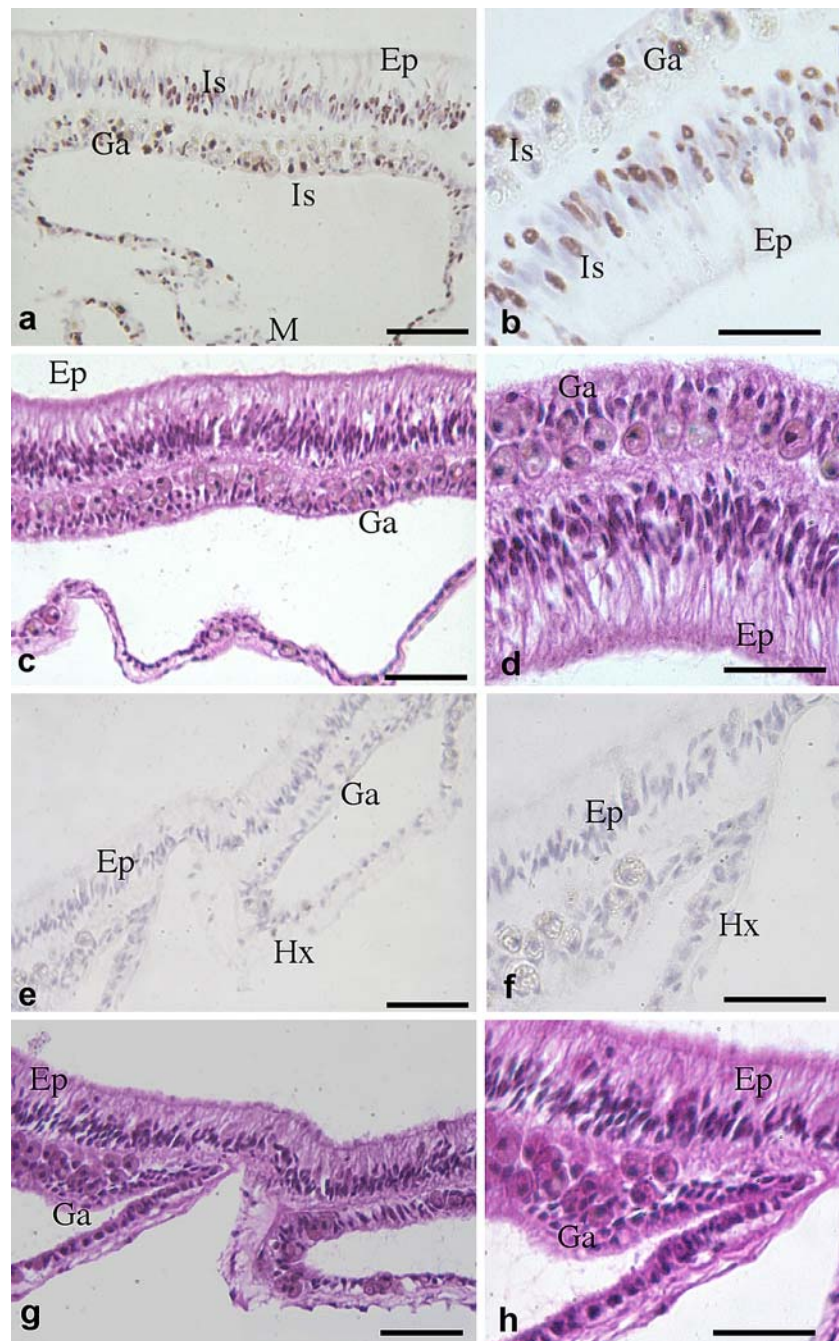
observable microbial communities associated with tissues or disease lesions using transmission electron microscopy. Comparatively, the present study did identify a dense mixed bacterial matt associated with the predation lesion on Acroporid coral created by *Drupella* sp., where dense, mixed bacterial communities were found penetrating the adjacent tissues and coating the surface of the adjacent coral tissues. Thus, the apparent lack of bacteria within the WS coral tissue or on the lesion is intriguing given the high degree of tissue loss on the coral colonies affected by this disease

throughout the year (Roff et al. 2006). Microbial communities associated with the lesion of corals affected by WS have been consistently hard to identify since the syndromes were first described; Antonius (1973) referred to a syndrome with a distinct similarity to WS as "shut-down reaction", noting an apparent lack of microbial association with rapid tissue loss in disease and stress; the first descriptions of white plague (type I) described the lesion boundary as being free from a build up of microbial communities (Antonius 1973; Bythell et al. 2002); and Peters et al. (1983) and Peters

Fig. 5 The proportion of apoptotic (by in situ end labelling) to normal epithelial and mesentarial cells associated with healthy tabular *Acropora* and tabular *Acropora* symptomatic of white syndrome
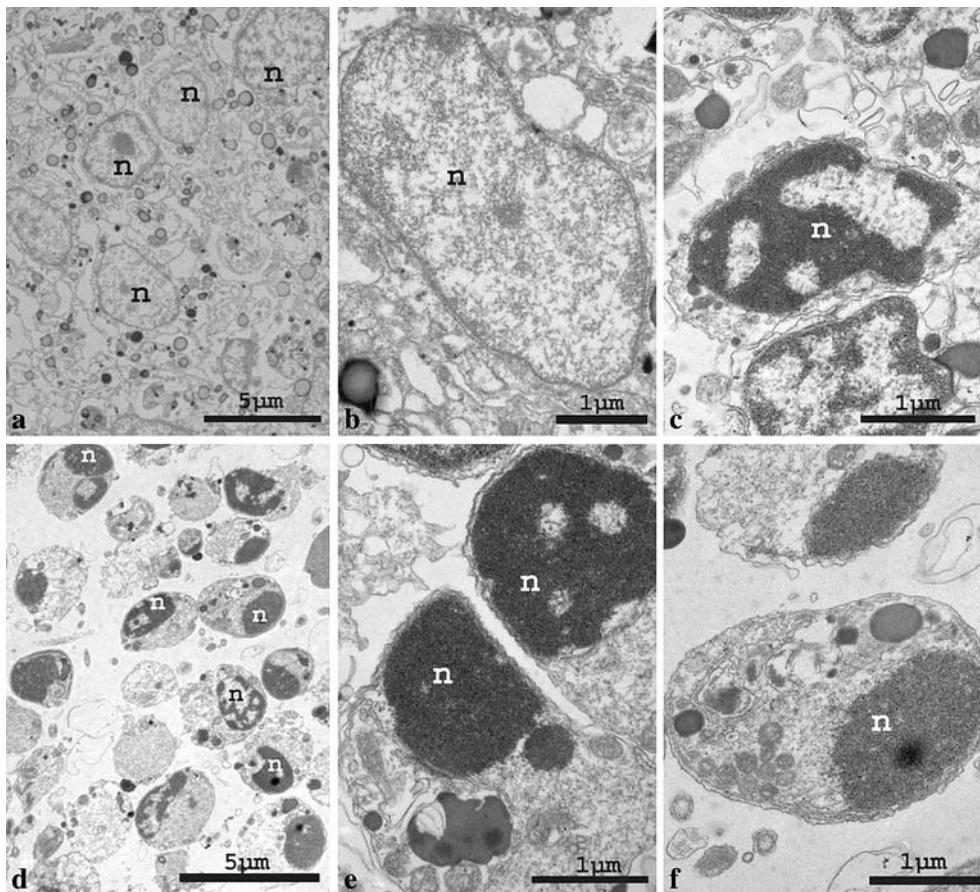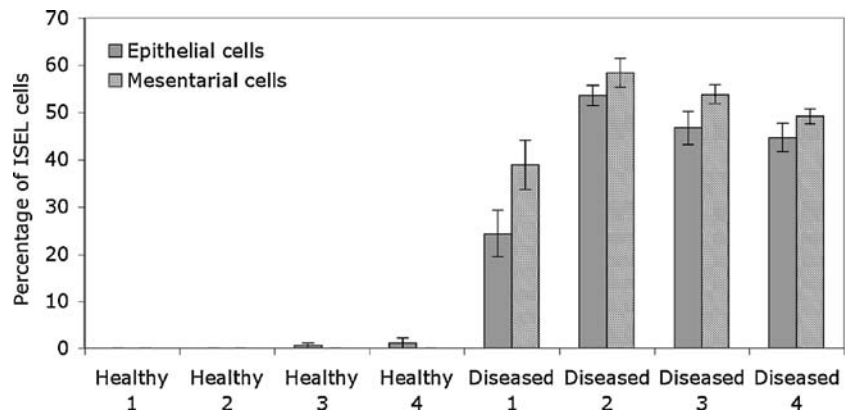

Fig. 6 Transmission electron micrographs of tissues from healthy tabular *Acropora* colonies in which cells contain uncondensed nuclei (**a**, **b**), and tissues of white syndrome affected tabular *Acropora* colonies with apoptotic-like cells displaying strong marginalisation of condensed chromatin within the nucleus (**c**), several apoptotic-like cells displaying rounded cell shape and loss of cell to cell contact (**d**), compact homogenously condensed nuclei and intact cell structure (**e**), and a single cell with condensed cytoplasm and nuclei but intact structure and contents (**f**). *n* nucleus

(1984) noted a distinct lack of microbial communities associated with white band in *Acropora palmata*. Recently, Bythell et al. (2004), also noted a distinct lack of microbial populations associated with tissue lesions of white band disease (type 1) on the coral *A. palmata*, despite the fact that they readily identified bacterial communities on lesion borders of corals with

black band disease. This study also noted the characteristically bacteria-free WS lesion was also associated with an apparent lack of tissue necrosis. Necrosis or necrotic lesions are terms commonly used within coral disease literature. However, in the present study, no morphological evidence of mass necrosis was found associated with WS. Tissue appeared healthy, with no

morphological characteristics of mass necrosis, such as membrane and cytoplasm swelling, cell extension, or mass loss of cell and tissue structure. As such, the distinct lack of bacterial communities and the lack of tissue necrosis associated specifically with the rapid and distinct tissue loss of WS, raises questions of the mechanisms of cell death, tissue loss and disease resistance that may be occurring within these syndromes, and specially of the involvement of an intrinsic or PCD in these processes.

The in situ end labelling of DNA breakage is a diagnostic feature of PCD (Kerr et al. 1972; Adle-Biassette et al. 1998), the technique has been widely used to demonstrate the presence of this mode of cell death across phyla, and has recently been used to monitor stress related cell death in sea anemones. Dunn et al. (2002, 2004) demonstrated the presence of PCD during early stages of heat stress and the use of in situ end labelling in lower organisms as effective for investigating mechanisms of cell death. In the present study, cells adjacent to the WS disease lesion displayed evidence of PCD discernible by in situ end labelling (up to 53% of epithelial cells and up to 58% of mesenterial cells), which was not seen in healthy control tissue samples. The extent of in situ end labelling in WS tissues was consistent between season's samples and was not found in either season healthy corals. The amount of in situ end labelling associated with the WS tissue lesion was extensive and evident in all cell types and tissue layers. Evaluations of PCD and associated cell structures were identified using morphological criteria described by Kerr et al. (1972), Wyllie and Kerr (1980) and Dunn et al. (2004). Using these criteria evaluations of general tissue structure, using standard haematoxylin and eosin staining and transmission electron microscopy, provided evidence of PCD consistent with previous morphological descriptions; in that tissues displayed normal cellular organisation and intact membranes but with condensed darkly stained nuclei evident of nuclei shrinkage. An indication of the presence and role of PCD in corals can also be drawn from Glynn et al. (1985), who noted an apparent proliferation of nuclei within the epidermis associated with tissue changes and loss during stress. This observed apparent nuclei proliferation may have been associated with extensive nuclei condensation (which occurs during PCD) leading to greater visualisation of nuclei within cell layers of uniform tissue preparations, and hence the apparent impression of proliferation in stressed samples. The demonstration of extensive PCD by in situ end labelling and transmission electron microscopy exclusively of cells associated with WS and previous descriptions in the literature suggests that an intrinsic mechanism

such as PCD may be important in coral disease and disease resistance and may play similar roles to that described in other lower organisms.

PCD has been shown to be a primary mechanism for the removal of pathogens and defence against microbial invasions across the phyla (Mittler et al. 1997; Gao and Kwaik 2000; Amersien 2002; van Doom and Woltering 2004). In plants, it has been proposed that PCD is induced to remove pathogens and develop a barrier of dead cells to prevent further pathogenesis. In the plant disease hypersensitivity response (HR), the disease is not necessarily caused by a specific invading organism but results from the activation of the apoptotic pathway (Mittler et al. 1997; Lam et al. 1999). This type of activation of apoptotic pathways and PCD has also been demonstrated in higher organisms; during development of the Hawaiian sepiolid squid (*Euprymna scopes*) the symbiotic bacterium *Vibrio fisheri* triggers an irreversible apoptotic signal within the first few hours of the association; in teleost fish activation of apoptosis occurs in response to viral infection; and the causative agent in Legionnaires disease induces apoptosis in macrophages (McFall-Ngai 1999; Foster et al. 2000; Essbauer and Ahne 2002; Gao and Kwaik 2000). Given the key role of PCD in pathogenesis across the phyla, there is the potential for PCD to also be involved in coral pathogenesis. This is particularly evident when considering the consistent reportage of an apparent lack of microbes or a specific pathogen identifiable with tissue loss and disease. Winkler et al. (2004) observed certain coral diseases appear to progress independent of a specific pathogen, and as also seen within this study. This may support the hypothesis of PCD in coral pathogenesis. The mechanisms of disease resistance have not been investigated in corals nor have mechanisms of pathogen removal. Given the recent increases in environmental stress and links between disease and stress related coral bleaching, there is the potential for disease resistance in corals to be compromised (Bythell et al. 2004; Sutherland et al. 2004; Harvell et al. 2002). Further investigation of the potential involvement and activation of PCD in coral disease and resistance should be considered.

This study strongly establishes the role of PCD in a disease-like syndrome of coral, and suggests that PCD maybe a key feature tissue loss and disease within WS currently affecting reef-building corals. This said, it is important to note that the lack of identifiable bacterial communities within the syndrome studied does not eliminate the potential involvement of pathogenic microbes especially in the early stages or activation of the syndrome. Just as with their presence, the absence of bacterial flora should not lead to the conclusion that

they are or are not involved in disease. Since bacteria have previously been shown to induce characteristic PCD responses, further studies are needed to distinguish the role of PCD resulting from increased stress and that triggered by pathogenesis.

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