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NOTE

Cellular processes of bleaching in the Mediterranean coral *Oculina patagonica*

T. D. Ainsworth · O. Hoegh-Guldberg

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Abstract Annual bleaching of *Oculina patagonica* on the Israeli Mediterranean coastline has been reported since 1993, although the cellular mechanisms underlying the bleaching have not yet been investigated. This survey examined 48 coral colonies of *O. patagonica* (bleached and unbleached) from various sites along the Israeli coast. Histopathological investigations of bleached lesions revealed a loss of endosymbionts, and an apparent in situ degradation of the endosymbionts. In situ end labelling of bleaching lesions did not provide evidence of apoptotic cell death. Electron microscopy of bleaching lesions also demonstrated an apparent in situ degradation and no evidence of apoptotic cell death of the host.

Keywords In situ degradation · Coral bleaching · Apoptosis · Histology

Introduction

Coral bleaching has been described in reef regions worldwide, and is considered a major impact to future sustainability of coral reefs (Hoegh-Guldberg 1999). Coral bleaching is defined as the paling in external coloration of tissues associated with the loss of the endosymbiotic dinoflagellates *Symbiodinium* sp. from the coral host or a loss of the algae's associated photosynthetic pigments (Coles and Jokiel 1977; Hoegh-Guldberg and Smith 1989; Kleppel et al. 1989). Bleaching in cnidarians is a generalised response to a range of stressors including: high sea temperature in corals (Hoegh-Guldberg and Smith 1989; Glynn and D'Croz 1990; Glynn 1991); low sea temperature in an anemone (Steen and Muscatine 1987); high light or UV in a zoanthid (Lesser et al. 1990); and possible microbial attack in coral (Kushmaro et al. 1996, 1997, 1998, 2001; Ben-Haim et al. 2002, 2003, b). Coral reefs are now considered to be undergoing a time of rapid change and increasing environmental and anthropogenic impacts (Hughes 1994; Hoegh-Guldberg 1999; Sutherland et al. 2004).

Oculina patagonica is a temperate coral species that has successfully invaded the Israeli Mediterranean coastline. The coral was first reported along the Eastern Mediterranean coastline in 1993 (Fine and Loya 1995), and has since been reported to bleach annually.

However, the cellular mechanisms by which endosymbiotic dinoflagellates are lost from these corals during bleaching have not yet been characterised. Gates et al. (1992) has proposed five different mechanisms that could underlie the bleaching of coral tissues, including: exocytosis of the endosymbionts from the gastrodermis, apoptosis of the host gastrodermis cell, necrosis of the gastrodermis, pinching off or budding of the endosymbionts from the distal portion of the host cell; and detachment of the gastrodermis cells containing the endosymbionts. Brown et al. (1995) later proposed that an array of cellular mechanisms occur during endosymbiont loss in naturally bleached tissues. This study investigated the cellular mechanisms of endosymbiont loss associated with the annual bleaching of the temperate coral *O. patagonica* in the Mediterranean. Light and electron microscopy were used to describe the histopathological characteristics and investigate the cellular mechanisms of *O. patagonica* bleaching.

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T. D. Ainsworth (✉) · O. Hoegh-Guldberg
Centre for Marine Studies, and The ARC Centre of Excellence for Coral Reefs Studies, The University of Queensland, St. Lucia, QLD 4072, Australia
e-mail: t.ainsworth@uq.edu.au

Materials and methods

Sampling design

Between 14th June and 22nd August 2005, bleached and control (healthy/unbleached) colonies of *O. patagonica* were sampled every 2 weeks at Sdot Yam (32° 28′N, 24° 53′E) on the Mediterranean coast, and at other sites including Ashkelon, Bat Yam and Acziv. A total of 16 colonies from each of bleached, unbleached and healthy colonies were sampled. Sampling involved six replicate cores (internal diameter 2.5 cm) from areas that were designated either healthy tissue (normally pigmented) ($n = 3$), or bleached lesion, which included the interface between normally pigmented and bleached/white tissues) ($n = 3$) (Fig. 1).

Tissue preservation and processing for histology

Coral cores were fixed in 4% paraformaldehyde in phosphate buffered saline, for 12 h then stored at 4°C in phosphate buffered saline. They were then decalcified in 20% (w/v) EDTA in PBS, and tissues were processed sequentially through 70, 80, 95 and 100% ethanol, three changes of each xylenes and paraffin (Paraplast Plus), each for 40 min, prior to paraffin embedding. Serial tissue sections (4 μm) were collected onto Superfrost Plus slides (Menzel, Germany). Histological sections were examined using light microscopy. Harris's haematoxylin and eosin (with phyloxine B) (Sigma-Aldrich, catalogue # HS32 and HT110-1-32) and Cason's Trichrome stain (or Mallory's triple stain) were used for evaluating the general tissue structure (swelling and lysis of cells, disruption of cell structure and distribution of endosymbionts) associated with the bleached lesion and the adjacent tissues. Confocal imagery and spectral profiling (Ainsworth et al. 2006) were also used to investigate endosymbiont distribution. In situ end labelling of fragmented DNA (In situ apoptosis detection kit S7101 Chemicon International Inc, USA) was conducted in adjacent sections to investigate the presence and extent of programmed cell death within sections of the bleaching lesions (Dunn et al. 2002; Ainsworth et al. 2007). Coral tissue in which apoptosis has previously been demonstrated were used as positive controls.

Tissue preservation and processing for transmission electron microscopy

For the electron microscopy, cores were taken from bleached and healthy *O. patagonica* colonies ($n = 6$) from 2 to 5 m depth on reefs adjacent in Sdot Yam in July 2005. Small fragments (2 cm^2) from were taken from colony cores and immediately fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer. Coral cores were decalcified in 20% EDTA at 4°C (Ainsworth et al. 2006), and samples were prepared using methods described in Le Tissier (1990). Ultra-thin 1 μm tissue sections were also stained using 1% toluidine blue (Sigma-Aldrich catalogue # 89640) to investigate general tissue structure and endosymbiont location, and were photographed using standard light microscopy. Sample grids with ultra-thin sections were viewed in a transmission electron microscope (JEOL 1010) at acceleration voltage 90 kV and images taken using the Megaview III Soft Imaging system.

Results and discussion

Endosymbiotic dinoflagellates were present only in the distal portion of the gastrodermis of bleached lesions adjacent to the gastrovascular cavity (Fig. 2a–c). In

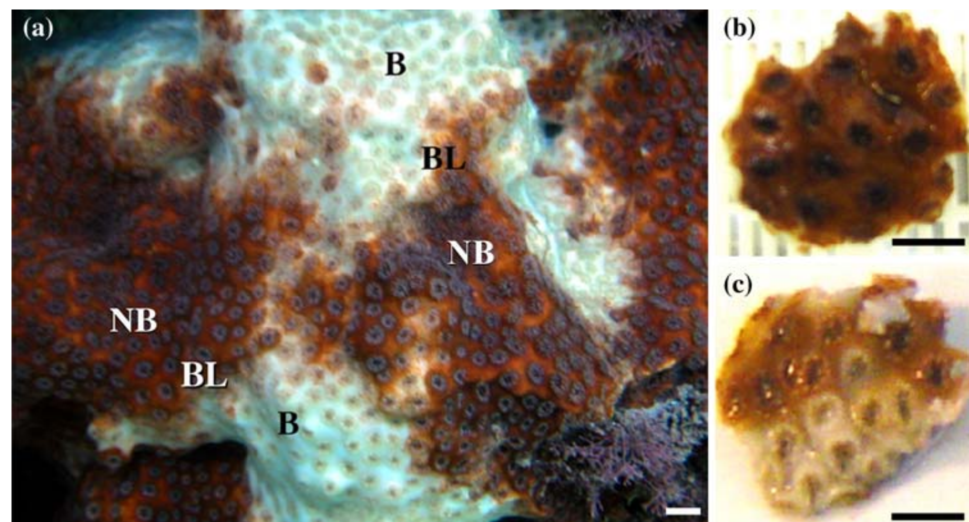

Fig. 1 Bleaching of (**a**) *Oculina patagonica*, (**b**) coral cores from unbleached tissue regions, and (**c**) bleaching lesion, showing the interface between normally pigmented and bleached tissue. B, bleached tissues with no apparent pigmentation. BL, bleaching lesions, designating the interface between unbleached and bleached tissue. NB unbleached tissue. Scale bars = 1 cm

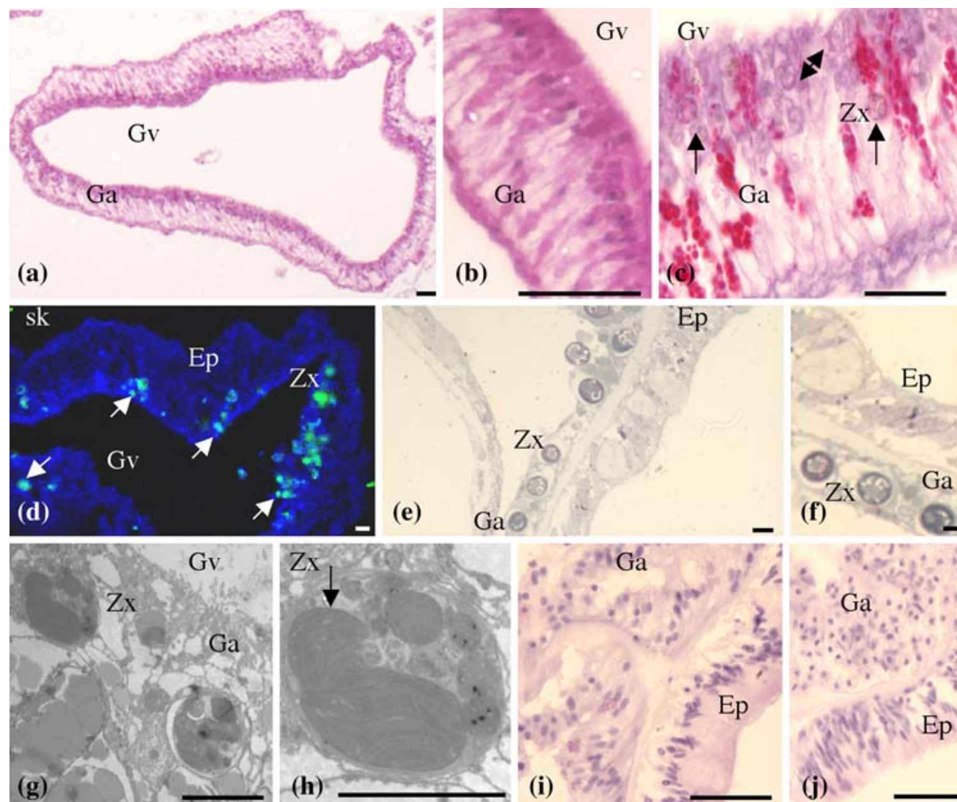

Fig. 2 Tissue sections from bleached lesions of *Oculina patagonica* showing endosymbiotic dinoflagellates clustered at the distal end of the gastrodermis adjacent to the gastrovascular cavity. (**a**) and (**b**) Light micrographs with tissue stained with haematoxylin and eosin, (**c**) tissue stained with Cason's trichrome, and (**d**) spectral profiling. (**e**) and (**f**) Toluidine blue staining of ultra-thin tissue sections demonstrating the low endosymbiont density. (**g**) and (**h**) Ultra-micrographs of the endosymbionts showing a disintegration of the thylakoid membrane (arrowed) of the endosymbionts adjacent to the gastrovascular cavity. (**i**) and (**j**) Bleached lesion section and healthy section, respectively, stained with in situ end labelling of fragmented DNA. Scale bars are all 5 µm. Endosymbiotic dinoflagellates, Zx; Gastrodermis, Ga; Epidermis, Ep.; Gastrovascular cavity, Gv; skeleton, sk

contrast in the gastrodermis of healthy tissues endosymbionts were randomly distributed (Fig. 3a–f). There was no evidence of host cell death or gastrodermis detachment, evidenced by the fact that the gastrodermis remained intact in bleached areas of low endosymbiotic dinoflagellates density (Fig. 2c, d). There was also no evidence for DNA fragmentation or programmed cell death of the host or endosymbiotic dinoflagellates, as demonstrated by a lack of binding to host or endosymbiotic dinoflagellate nuclei using in situ end labelling (Fig. 2g, h). In the bleached lesions, electron micrographs showed the endosymbionts clustered at the distal end of the gastrodermis (Fig. 2g), with disruption to the thylakoid membranes of the endosymbionts and degeneration of the endosymbionts (Fig. 2h) and an absence of staining indicative of host apoptosis (Fig. 2i, j).

In an earlier study on the sea anemone, *Aiptasia* sp, Steen and Muscatine (1987) described the cellular morphology of endosymbiotic dinoflagellates under environmental stress, defining the mechanisms of endosymbiont loss as exocytosis based on the shift of *Symbiodinium* sp. to the distal end of the host gastrodermal cells. They described the host cells remaining intact while many of the *Symbiodinium* sp. were in various stages of disintegration. Similar morphological observations were seen in the present study of the temperate coral *O. patagonica*, which may therefore be circumstantial evidence of exocytosis. These morphological changes may also be driven by in situ degradation of the endosymbiont cell. Endosymbiont stacking in distal regions to avoid higher light from the bleached tissues is unlikely given the evidence of symbiont thylakoid disintegration, which suggests that the algal symbionts were degrading in situ. In contrast, the endosymbionts in healthy tissues were evenly distributed within the gastrodermis, as seen by Steen and Muscatine (1987), and remained intact, with an evident algal membrane. The lack of evidence for either programmed cell death or necrosis of the host, and an apparent degradation and a possible exocytosis of a damaged endosymbiont supports the suggestion that bleaching in *O. patagonica* results from an impact to the symbiont rather than the host.

Characterising the cellular mechanisms associated with the gross morphological bleaching response provides

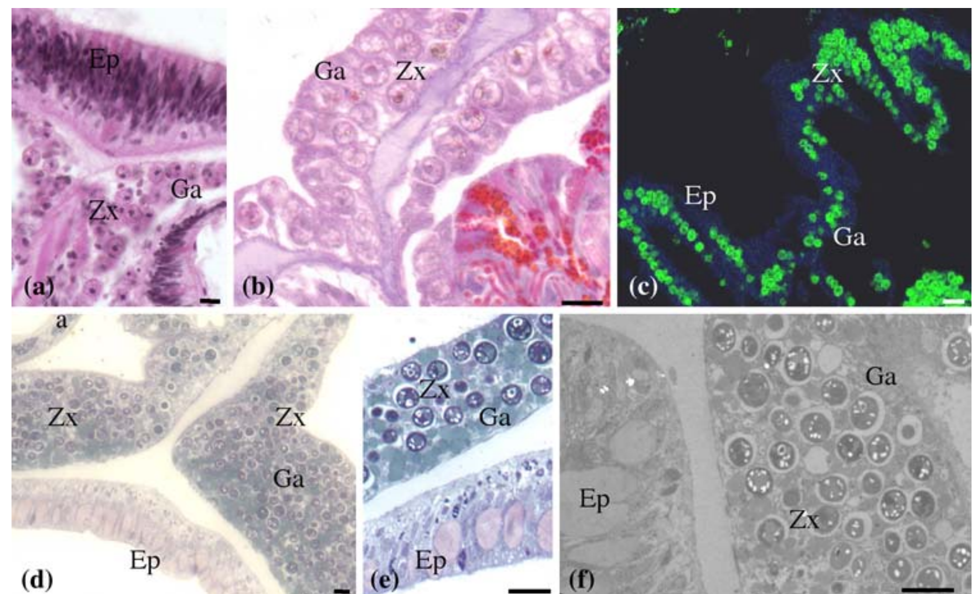

Fig. 3 Histological and ultra-structural tissue sections of healthy *Oculina patagonica* showing random distribution of the endosymbionts within the gastrodermis. Using (**a**) haematoxylin and eosin stain, (**b**) Cason's trichrome, (**c**) spectral imaging, (**d**, **e**) toluidine blue staining of ultra-thin sections, and (**f**) transmission electron micrographs of healthy *Symbiodium* sp. Scale bars all 20 µm. Endosymbiotic dinoflagellates, Zx; Gastrodermis layer, Ga; Epidermis, Ep

insights into the processes by which very similar macroscopic observations of changes occur in coral bleaching. Glynn et al. (1985) described necrosis of coral tissues associated with coral mortality in Panama. Patchy coral mortality was evident and histological examination and morphological characterisation by the authors showed that the tissue loss was associated with necrosis of the host, while the endosymbionts remained normal and intact. These morphological descriptions highlight clear differences between processes of coral tissue death, such as lysis and necrosis of host tissues, and coral bleaching, a disassociation of the symbiosis and loss of the endosymbionts, as observed in the present study. Differentiating cellular mechanisms of coral bleaching and coral death are very important in determining and differentiating the cause of coral mortality. The initial impact of the dissociation may be highlighted through investigation and characterisation of cellular morphology and understanding cellular processes. For example, host cell detachment has been linked to exposure to extreme conditions and described as a mechanism of coral death (Brown et al. 1995). Patterns of host necrosis resulting in bleaching, such as that described by Glynn et al. (1985) have also been linked to mechanical disruption such as exposure to low salinity (Gates et al. 1992). Gates et al. (1992) and Brown et al. (1995) call for studies investigating the processes, and the progression, of cellular changes associated with coral bleaching to be further investigated and utilised in defining bleaching. The example of *O. patagonica* bleaching also highlights the importance of understanding the cellular mechanisms underlying the different process of coral bleaching (symbiont loss) and coral death (host processes). The bleaching of *O. patagonica*, which begins annually in precisely mid-June (Kushmaro et al. 1996) before coral mortality later in the summer, is associated first with a breakdown of the symbiosis with the coral host tissues remaining intact, and followed later by the breakdown of bleached host tissues. This study shows that the bleaching of *O. patagonica*, may in fact relate to two disparate events. Firstly of coral bleaching followed later by coral tissue loss and necrosis. By differentiating the causes of distinct cellular changes and their association with coral bleaching, disease, death and colony mortality we gain insights into the true underlying process governing the fate of coral colonies during times of environmental stress.

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