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REPORT

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Characterizing lesions in corals from American Samoa

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Abstract The study of coral disease has suffered from an absence of systematic approaches that are commonly used to determine causes of diseases in animals. There is a critical need to develop a standardized and portable nomenclature for coral lesions in the field and to incorporate more commonly available biomedical tools in coral disease surveys to determine the potential causes of lesions in corals. We characterized lesions in corals from American Samoa based on gross and microscopic morphology and classified them as discoloration, growth anomalies, or tissue loss. The most common microscopic finding in corals manifesting discoloration was the depletion of zooxanthellae, followed by necrosis, sometimes associated with invasive algae or fungi. The most common microscopic lesion in corals manifesting tissue loss was cell necrosis often associated with algae, fungi, or protozoa. Corals with growth anomaly had microscopic evidence of hyperplasia of gastrovascular canals, followed by necrosis associated with algae or metazoa (polychaete worms). Several species of apparently normal corals also had microscopic changes, including the presence of bacterial aggregates or crustacea in tissues. A single type of gross lesion (e.g., discoloration) could have different microscopic manifestations. This phenomenon underlines the importance of using microscopy to provide a more systematic description of coral lesions and to detect potential pathogens associated with these lesions.

Keywords Coral · Pathology · Lesion · Fungi · Algae · Protozoa

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Introduction

Disease in corals has taken on an increasing importance in the light of massive degradation of coral reef ecosystems, particularly in the Atlantic (Green and Bruckner 2000). However, the study of coral disease has suffered due to the lack of systematic approaches to elucidate the causes of mortality. One reason is that there has been a disconnect between those who study and elucidate the causes of animal disease (veterinary medicine) and those who study coral biology and ecology. As a result, many coral diseases are described based only on gross descriptions. A review of coral disease literature confirms this. Much effort is spent counting diseased corals and assigning names to gross lesions, with comparatively less effort spent on developing standardized nomenclature or delving further into determining what may cause particular lesions. To date, only two diseases of corals, black band in the Caribbean (Richardson et al. 2001) and *Vibrio*-associated bleaching in the Mediterranean and Red Sea (Rosenberg et al. 1999), have been extensively characterized.

Resources to investigate diseases as thoroughly as black band or *Vibrio*-associated bleaching are limited. On the other hand, there is a need to progress beyond gross descriptions (Bruckner and Bruckner 1997; Metalpa et al. 2000) when describing lesions in corals, particularly when such descriptions are open to subjective interpretation and make comparisons between sites problematic. Histopathology (Peters 1984) can provide an additional layer of detail in morphologic descriptions of disease and has the added benefit of detecting potential causal agents of the lesion. We set out to evaluate the use of underwater photography combined with histopathology to characterize lesions of corals in American Samoa. Our specific objectives were to: (1) develop a simplified and consistent nomenclature for lesions encountered in the field and (2) characterize these lesions at the microscopic level.

Methods

Survey sites were in American Samoa on the islands of Tutuila, Ofu, and Olosega and were monitored for distribution of fish and corals by Samoa Department of Marine and Wildlife Resources (DMWR), National Oceanic and Atmospheric Administration (NOAA), and National Park Service (NPS). Haphazard dives were done using scuba or snorkeling. Corals were photographed using a Nikonos V underwater camera with a 20 mm lens and twin Ikelite 50 strobes, and an Olympus C3030 digital camera in an Olympus underwater housing attached to a single Ikelite 50 strobe. Close-up photos were taken with a Nikonos V using a two to one framer and 35 mm lens. The film used for analog cameras was Kodak 100 ASA Ektrachrome.

Gross lesions in corals were placed in three categories. Discoloration included corals exhibiting color changes or lack of pigmentation in tissues typically exemplified by a white color. Tissue loss included corals manifesting absence of tissues with or without intact skeleton. Growth anomaly included corals exhibiting excessive or apparently uncontrolled growth of skeleton or soft tissues in relation to adjacent polyps on the same colony. Categories of gross lesions were not mutually exclusive.

Samples of corals were taken using bone shears, or hammer and chisel, and placed in seawater into labeled plastic bags. In cases where lesions were sampled, care was taken to collect both normal and abnormal tissue bordering the lesion. Corals were preserved in Hellys fixative (Barszcz and Yevich 1975) with sufficient added salt to make a 5 M NaCl solution and allowed to fix for 24 h. The fixative was decanted and the coral allowed to soak in fresh tap water for 24 h with a change of water once after 12 h. Subsequently, samples were stored in 70% ethanol, decalcified with Cal-ex II (Fisher Scientific), placed in cassettes, processed for paraffin embedding, trimmed at 5 μm, and stained with hematoxylin and eosin. Grocott's methenamine silver or Taylor's gram stains were used on tissue sections to identify fungi or bacteria, respectively (Prophet, et al. 1992). Those structures staining negative with silver stain were classified as algae whereas those staining positive with silver were classified as fungi. Slides were examined using light microscopy at magnifications ranging from 20 to 400×.

On microscopy, lesions were classified as depletion of zooxanthellae, uncomplicated necrosis, necrosis associated with algae, fungi, or ciliates, hyperplasia of gastrovascular canals, or skeletal thickening associated with metazoa.

Results

We examined 71 samples from 50 coral colonies comprising 29 species and 15 genera. Samples were collected from 42 colonies manifesting discoloration, tissue loss or growth anomaly (Table 1); the remaining samples were collected from apparently normal corals.

Discolored corals were characterized grossly as normal tissue bereft of pigmentation overlying normal skeleton. Discoloration patterns could be divided into focal (Fig. 1a), or diffuse (Fig. 1b). Tissue loss was characterized by areas bereft of coral tissue leaving recently exposed white skeleton or skeleton colonized by algae giving it a brown or green color (Fig. 1c, d). Growth anomalies were characterized by focal areas of smooth to rugose aberrant growth of coral skeleton. The tissue overlying these growths was discolored white or purple, and polyps were either absent, or if present, appeared small or mis-shapen with lack of tentacles (Fig. 1e, f).

Depletion of zooxanthella

The hallmark of this change was the absence of zooxanthellae from the gastrodermis (Fig. 2a, b). In two cases, the remaining zooxanthellae were atrophied with a hypereosinophilic fragmented cytoplasm. Varying de-

Table 1 Species of corals with gross lesions and instances where a particular microscopic finding was seen associated with a particular gross lesion

Species	Gross morphologic diagnosis		
	Discoloration	Tissue loss	Growth anomaly
Acropora abrotenoides	D (1)	C (1)	H(3)
Acropora cytherea		C (1)	H(3)
Acropora digitifera			H
Acropora hyacinthus	D (1)		H(3)
Acropora samoensis			F (1)
Diploastrea heliopora	N(2)	N(1) F(1)	
Echinopora lamellosa		F (1)	
Favia stelliger		F (1)	
Goniastrea sp.	N (1) F (1)		
Hydnophora microconos		D (1)	
Leptoria phrygia	D (1)		
Massive *Porites*	D(3)	F (1)	
Millepora sp.		N (1)	
Montipora nodosa		F (1)	
Montipora sp.		F (2)	
Montipora turtlensis			M (1)
Palythoa sp.		N (1)	
Pavona minuta	D (1)		
Platygyra daedala	N (1)		
Platygyra sp.		D (1) N (1)	
Pocillopora eydouxi	D (1)		
Pocillopora meandrina	D (1)		
Pocillopora verrucosa			H (1)
Total	14	15	13

Key to microscopic findings: D, depletion of zooxanthella; N, uncomplicated necrosis; F, necrosis associated with algae or fungi; C, necrosis associated with ciliates; M, necrosis associated with metazoan; H, hyperplasia of gastrovascular canals. The following species (*n*) of corals were examined but did not have gross lesions: One each in *Acropora gemmifera*, *Cladiella* sp. *Lobophyllia hemprichii*, *Rumphella* sp., *Sarcophyton* sp., *Pocillopora eydouxi*, *Pectinia lactuca*. Numbers in parenthesis refer to the number of instances when a lesion was seen in a particular species.

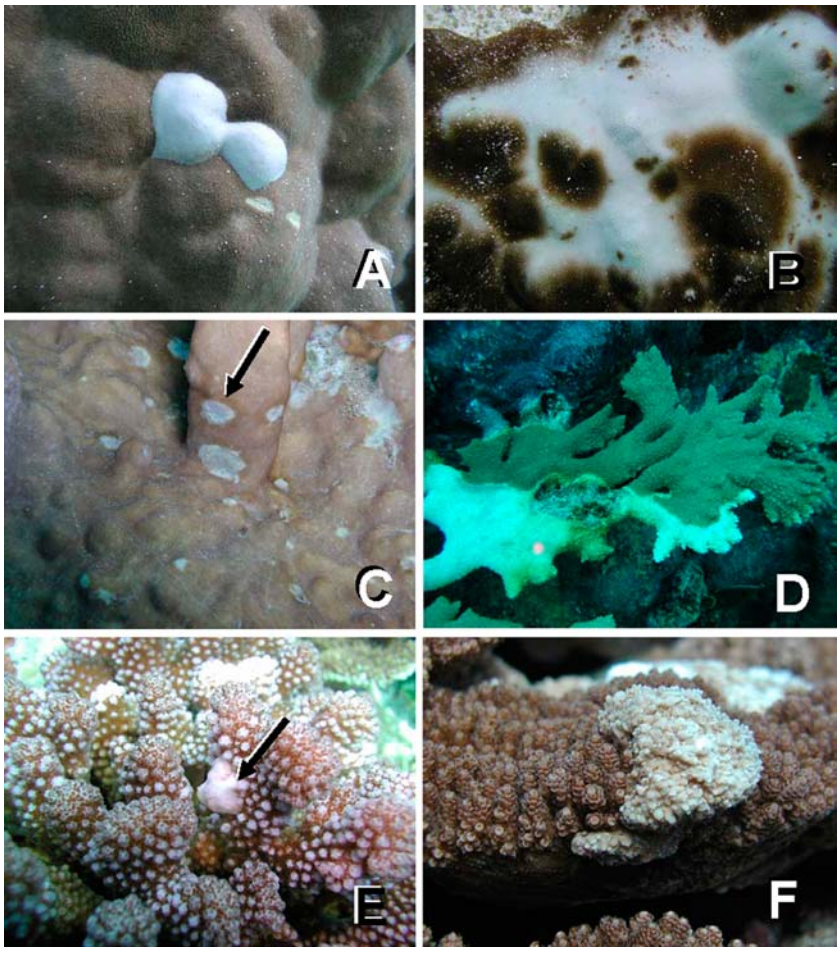

Fig. 1 Focal discoloration in *Porites evermani* (**a**); Diffuse discoloration in massive *Porites* sp. (**b**); Tissue loss in *Millepora* sp. (**c**); Tissue loss in *Acropora abrotenoides* ; note recently exposed white skeleton bereft of algal colonization (**d**); Growth anomaly in *Pocillopora verrucosa* (*arrow*) (**e**); *Acropora hyacinthus* (**f**)

grees of atrophy of gastrodermis, mesoglea, and epidermis were seen including general collapse of tissue architecture. Grossly, all but two corals with this microscopic lesion manifested as discoloration (Table 1).

Necrosis

Microscopic evidence of tissue necrosis was seen in corals manifesting gross evidence of discoloration, tissue loss or growth anomaly. Uncomplicated necrosis comprised those cases where coral cells manifested cytoplasmic hypereosinophilia or fragmentation with karyorrhexis (fragmentation of cell nuclei), karyolysis (dissolution or swelling of cell nuclei), or pyknosis (shrinkage of cell nuclei) with no associated organisms (Fig. 2c). Microscopic evidence of uncomplicated necrosis was seen equally in the category of corals manifesting gross evidence of tissue loss and corals manifesting discoloration (Table 1). Cell necrosis associated with microorganisms was seen in corals exhibiting all three types of gross lesions (discoloration, tissue loss, or growth anomaly). Organisms most commonly associated with cell necrosis were filamentous algae or fungi, the latter identified by positive staining with silver stain (Prophet et al. 1992), infiltrating into the gastrovascular canals. Calicoblastic epithelium, gastrodermis, or cells of the mesenterial filaments adjoining these organisms exhibited cytoplasmic fragmentation hypereosinophilia and pyknosis. In *Montipora* sp., there was evidence of a significant cellular host response against invasive algae manifested by an increase in number and size of eosinophilic granular cells (Fig. 2d, e). In *Acropora cytherea*, gastrodermis adjoining fungal elements was atrophied, depleted of zooxanthellae, or necrotic (Fig. 2f). In *Acropora hyacinthus* and *Acropora abrotenoides*, tissue necrosis was associated with ciliates replete with zooxanthella invading intact gastrovascular canals, gastrodermis, and epidermis (Fig. 2g, h). In one case of grossly visible growth anomaly in *Montipora turtlensis*, microscopic examination revealed a marked increase in space between gastrovascular canals that surrounded a polychaete among infiltrates of filamentous branching algae.

Hyperplasia of gastrovascular canal

The histologic hallmark of this lesion was a marked proliferation of the gastrovascular canal network (Fig. 3a, b) with occasional proliferation of gastrodermal cells. Within these areas of gastrovascular canal proliferation, mesenterial filaments were missing or markedly atrophied, and polyps were usually missing, or

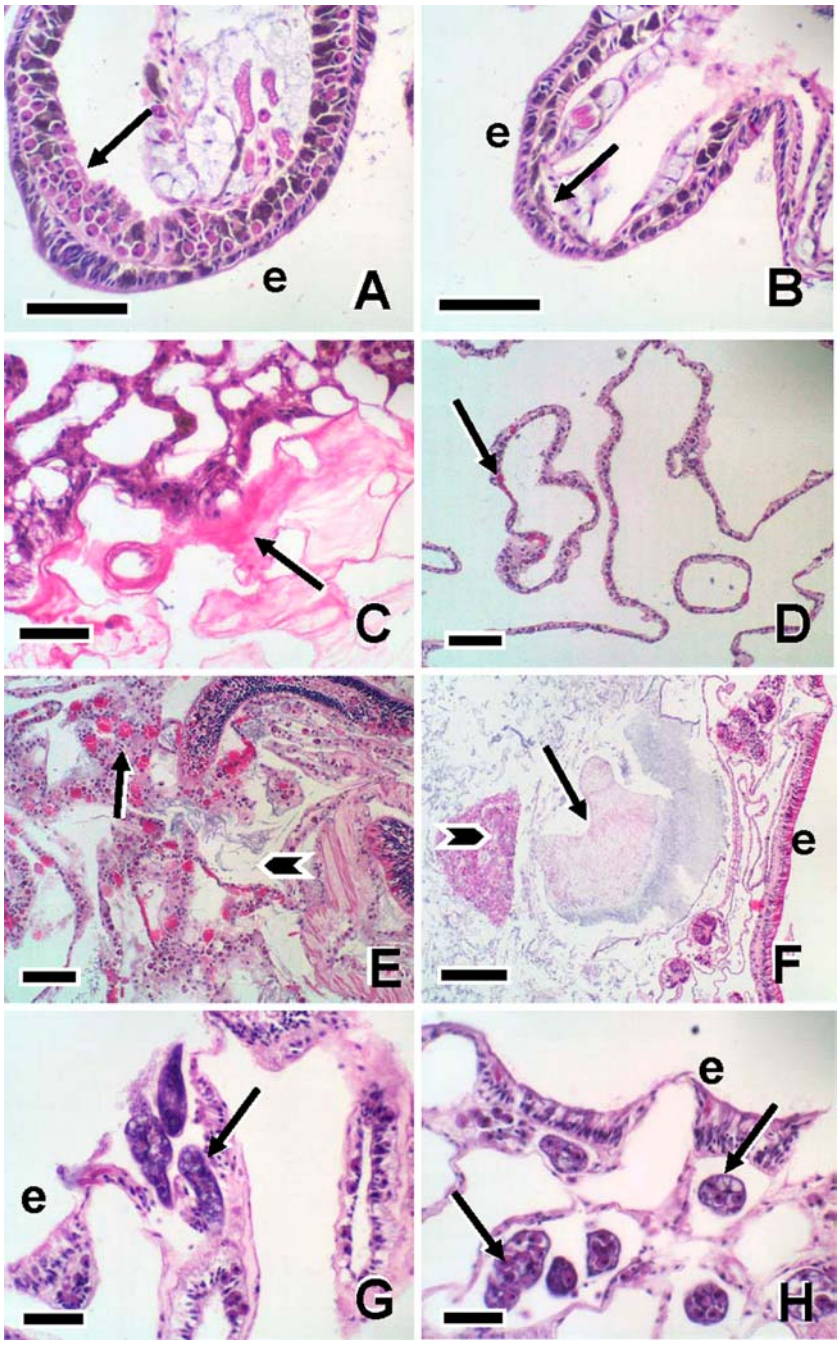

Fig. 2 Coenosarc *Goniastrea* sp. (**a**) Note normal tissue on left with gastrodermis replete with zooxanthella (*arrow*), bar = 50 μm; (**b**) Note gastrodermis bereft of zooxanthella and diffuse atrophy of coenosarc (*arrow*), bar = 50 μm.(**c**) *Millepora* sp. Uncomplicated tissue necrosis. Note diffuse coagulation necrosis of tissue (*arrow*) (Skeleton is to the upper left), bar = 100 μm; (**d**, **e**) *Montipora nodosa*, bar = 50 μm; (**d**) Normal tissue, note sparse eosinophilic granular cells in mesoglea of gastrovascular canals (*arrow*); (**e**) Areas of algal infiltration (*arrowhead*). Note infiltrates of hypertrophied eosinophilic granular cells (*arrow*), bar = 50 μm. (**f**) *Acropora cytherea*. Note organized mass of fungal organism (*arrow*) and adjacent clump of necrotic tissue (*arrowhead*), bar = 50 μm. Note: *e* = epidermis. (**g**, **h**) *Acropora abrotenoides*; (**g**) Note ciliates with ingested zooxanthella (*arrows*) invading intact epidermis, bar = 50 μm; (**h**) note ciliates (*arrows*) distended with zooxanthellae within gastrovascular canals, bar = 50 μm

when present, appeared deformed with the absence of tentacles. Gastrodermal cells within the lesion were uniformly bereft of zooxanthellae, and in many cases, epidermis and underlying gastrodermis appeared atrophied with absence or atrophy of mesenterial filaments. *Acropora* sp. were over-represented in this category although a similar lesion was seen in one *Pocillopora verrucosa* (Fig. 3c, d).

Corals with no gross lesions

Microorganisms not associated with visible cellular pathology were also seen in normal coral tissue. The most notable was the presence of gram-negative aggregates of putative bacteria within the gastrodermis or epidermis of a variety of corals (Fig. 3e, f). These aggregates were well defined and surrounded by normal tissue. In rare cases, aggregates displaced normal tissue and were occasionally partially surrounded by a clear space. These aggregates were most often seen in *Acropora* $n = 8$) including *Acropora abrotenoides*, *Acropora digitifera*, and *Acropora hyacinthus*, and one each *Goniastrea* sp., *Porites* sp., *Platygyra* sp., *Pocillopora eydouxi*, and *Pocollopora meandrina*. Other organisms seen associated with normal tissue were crustacea within the actinopharynx of polyps in *Rumphella* sp. or massive

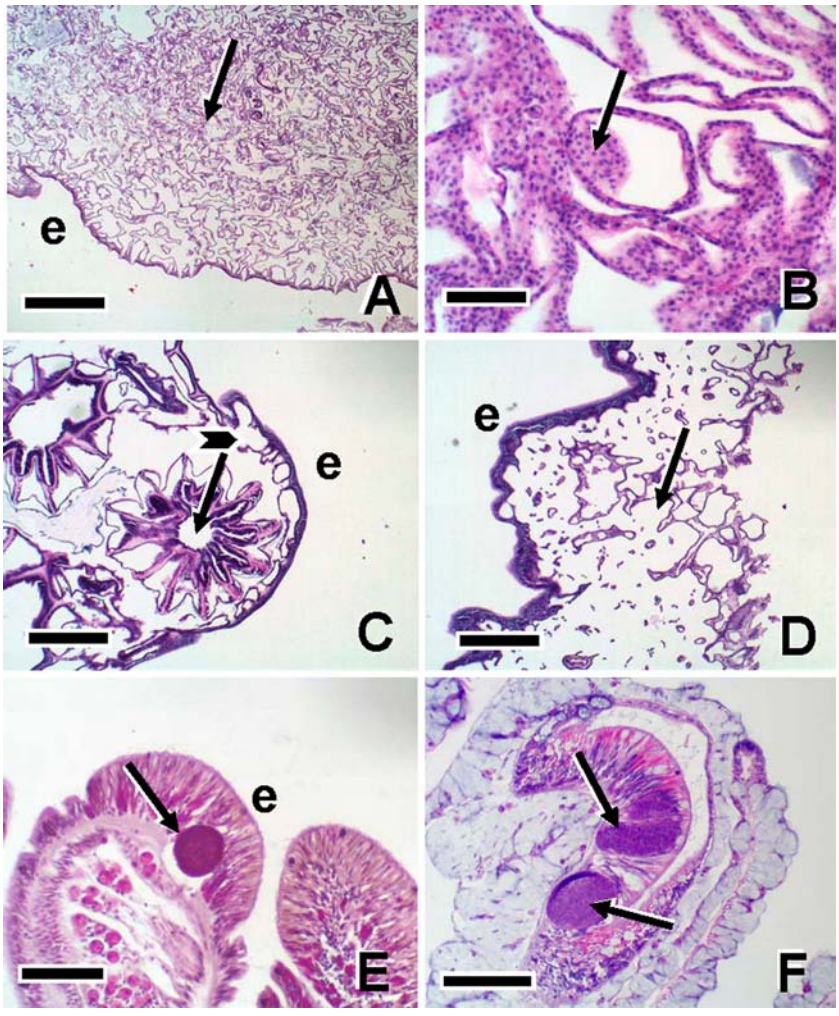

Fig. 3 **(a)** *Acropora cytherea* growth anomaly, note massive proliferation of gastrovascular canals (*arrow*), absence of polyps, and absence of zooxanthellae in gastrodermis, bar = 500 μm; **(b)** *Acropora abrotenoides* growth anomaly, note hyperplasia of gastrodermis within gastrovascular canals (*arrow*), bar = 50 μm; **(c, d)** *Pocillopora verrucosa;* **(c)** normal tissue, note polyps (*arrow*) and organized architecture of parallel gastrovascular canals (*arrowhead*), bar = 500 μm; **(d)** growth anomaly, note gastrodermis bereft of zooxanthellae, and proliferation and disorganization of gastrovascular canals (*arrow*). The tissue is also bereft of any normal polyp structure; bar = 500 μm. Note: *e* = epidermis. **(e)** *Pocillopora meandrina* Gram-stain, note aggregates of gram-negative bacteria (*arrow*) at junction of mesoglea and epidermis, bar = 50 μm; **(f)** *Platygyra* sp. H&E stain. Note bacterial aggregates in epidermis of tentacle (*arrows*) bar = 100 μm. Note: *e* = epidermis

Porites sp., within the mesoglea of *Pectinia lactuca*, and polychaete worms within gastrovascular canals of *Montipora* sp. and *Echinopora* sp.

Discussion

This paper provides a simple, but standardized and relatively unambiguous system of nomenclature for gross lesions in corals (discoloration, tissue loss, growth anomaly) that should theoretically be equally interpretable regardless of species and geographic location. Using this system, our results revealed that a particular gross lesion could have multiple microscopic manifestations.

For corals manifesting gross evidence of discoloration, the most common microscopic finding was depletion of zooxanthellae from atrophied gastrodermis. Similar changes were seen in bleached corals from the Pacific coast of Panama (Glynn et al. 1985) and from Thailand (Brown et al. 1995). Several factors are known to cause loss of zooxanthellae in corals including elevated temperature (Coles and Jokiel 1978) and infection with Vibrio sp. (Kushmaro et al. 2001). We saw no bacteria in bleached corals, however, some bleached corals had necrosis in deep tissues, sometimes associated with fungi or algae with loss of zooxanthella in gastrodermis underlying an intact epidermis (e.g., Fig. 2f). This suggests that some cases of bleaching are associated with the pathology of underlying tissues and may not be only associated with the simple absence of zooxanthellae from the gastrodermis.

For corals manifesting gross evidence of tissue loss, tissue necrosis associated with filamentous algae or fungi was the most common microscopic lesion. Coral-algal interactions are common elsewhere such as the Caribbean (Peters 1984). In one instance, fungal/algal invasion of coral tissue resulted in a marked cellular response in *Montipora* sp. as evidenced by increased eosinophilic granular cells in gastrovascular canals. Very little is known about immune defenses of coral (Hildeman et al. 1975) and determining the ultrastructure of these cells may help understand mechanisms of coral immunology. Knowledge about fungal pathogens in scleractinian corals is also limited. Fungi are commonly found in both normal and unhealthy corals, particularly in the skeleton (Ravindran et al. 2001). Raghukumar and Raghukumar

(1991) implicated fungi as the cause of necrotic lesions in several species of scleractinians in the Andaman islands of India, and Ramos-Flores (1983) found fungi responsible for black lesions on *Montastraea annularis* Montastraea annularis in Venezuela. In both cases, fungi were implicated as pathogens based on their association with dead tissue and invasion of coral tissue with fungal hyphae.

In two instances, corals manifesting gross evidence of tissue loss had active infection with ciliates. In contrast to opportunistic saprophytic ciliates that are commonly seen in tissue debris, ciliates in these cases were considered likely primary pathogens based on their invasiveness into intact tissue. Ciliates in corals have been documented in Caribbean *Porites porites*, *Porites astreoides*, and *Acropora palmata* with the latter case exhibiting epithelial necrosis (Peters 1984). Cerrano et al. (2000) implicated ciliates as a cause of mortality in gorgonians in the Mediterranean. Confirming this would necessitate experimental infections to assess the pathogenesis and pathophysiology of ciliate infections in corals.

There were several instances of corals manifesting tissue loss with microscopic evidence of tissue necrosis that could not be associated with any visible organism (uncomplicated tissue necrosis). While we suspect that predation may account for some instances of uncomplicated tissue necrosis, there were cases where necrosis was limited to particular structures (e.g., gastrodermis and mesenterial filaments). Such tissue specific episodes of tissue necrosis need further investigation as to potential etiologies, such as viruses, not visible on microscopy. One tool that may shed light on this is the examination of ultrastructural morphology with electron microscopy.

Growth anomalies were common in *Acropora* sp., and on microscopy, usually manifested as hyperplasia of gastrovascular canal. There was no evidence of neoplasia, which is usually characterized by an uncontrolled growth of anaplastic pleomorphic cells with prominent nucleoli, mitotic figures, and occasional tissue loss. Peters et al. (1986) characterized growth anomalies in Caribbean *Acropora* sp. as calicoblastic neoplasms based on proliferation of calicoblastic epidermis resulting in complete loss of normal polyps. That hyperplasia of gastrovascular canals predominate in *Acropora* sp. may be related to the phenomenon that this is a fast growing species of coral. Physiologic mechanisms responsible for these lesions, whether they are neoplastic, and whether they pose a detriment to coral colonies remains to be seen. Other instances of skeletal growth anomalies were due to infestation with polychaetes or fungi/algae. Polychaetes as a possible cause of growth anomalies was documented in *Montipora* sp. from Israel (Wielgus and Glassom 2002) indicating that some species of corals may respond to invasion by organisms by excessive skeletal growth.

Presence of basophilic Gram-negative aggregates of putative bacteria was noted in several species of corals. The role of these organisms in coral reef physiology or disease is unknown; however, attempts should be made to culture and identify them. Current evidence does not implicate them as causing disease in corals from the Pacific because none were associated with gross lesions. However, Peters et al. (1983) implicated similar aggregate-forming bacteria as the possible causal agent of white band disease in Caribbean acroporids. Crustaceans were seen in polyp actinopharynx suggesting they were being ingested as food. Polychaete worms in skeleton of apparently normal corals were seen in several species and their role in coral biology is unknown. The presence of crustacea in normal tissue mesoglea of *Pectinia* was unexpected, however, crustacea have been documented in other normal scleractinians (Stock 1975).

In order to ensure replication and standardization, nomenclature of gross lesions as used in this study was necessarily crude. The advantage of this system is that it focuses on describing the lesion and is less subjective to interpretation. As more data are gathered, it is likely that a more refined nomenclature of gross lesions will emerge which will continue to focus on systematically describing morphology of lesions in corals not interpreting causes. Histopathology provides a first step in identifying microbial agents associated with gross and microscopic lesions but has limitations in that it currently provides few clues on the temporal process of disease (e.g., Does this organism cause the lesion?). Answering this will require experimental studies that incorporate careful gross and microscopic descriptions of disease progression thereby elucidating the nature of host-pathogen interactions, etiology and pathophysiology of coral disease.

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