INTRODUCTION

Diseases are an emerging issue in coral reef ecosystems, but little fundamental knowledge exists on their morphology, their causes or options for their management. This has been partly due to lack of standardized case definitions, descriptions and nomenclature (Work & Aeby 2006). One easily recognizable disease of scleractinian corals is growth anomalies (GAs) that present as protuberant masses on the coral skeleton. GAs were first seen in Madrepora kauaiensis collected in 1902 from Hawaii, USA (Squires 1965). Since then GAs have been reported in a variety of coral genera from both the Caribbean and the Indo-Pacific (Peters 1997, Sutherland et al. 2004). In Acropora GAs have been seen in the Florida Keys (Peters et al. 1986), Caribbean (Bak 1983), Gulf of Oman (Coles & Seapy 1998), American Samoa (Work & Rameyer 2005), Guam, Eniwetok (Cheney 1975) and Australia (Willis et al. 2004). Acropora GAs are characterized by reduced colony growth (Cheney 1975, Bak 1983), decreased density of coral skeleton (Cheney 1975, Bak 1983) and reduced numbers of zooxanthellae (Cheney 1975, Peters et al. 1986). Peters et al. (1986), in the first systematic descriptions of GAs cellular morphology, found them to consist mainly of basal body wall (the tissue apposed to skeleton and comprising calicodermis, mesoglea and gastrodermis) with reduced or absent polyp formation, increased cellular density and decreased numbers of
MATERIALS AND METHODS

Field surveys. FFS is part of the Northwestern Hawaiian Islands and consists of a barrier reef partially enclosing a lagoon with 2 permanent emergent low islands (Tern and East Island) and several ephemeral sand bars. Tern and East Island experienced heavy activity during World War II (WW II), and Tern Island was a US Coast Guard LORAN (Long Range Aids to Navigation) station until the early 1980s. FFS is now a national wildlife refuge (NWR) and was considered heavily in use during WW II and the Vietnam War (Amerson & Shelton 1980). FFS is part of the Northwestern Hawaiian Islands and consists of a barrier reef partially enclosing a lagoon with 2 permanent emergent low islands (Tern and East Island) and several ephemeral sand bars. Tern and East Island experienced heavy activity during World War II (WW II), and Tern Island was a US Coast Guard LORAN (Long Range Aids to Navigation) station until the early 1980s. FFS is now a national wildlife refuge (NWR) with a small (<5 person) staff on Tern Island (Amerson 1980). Dominant corals at FFS include Pocillopora, Acropora and Montipora (Maragos & Jokiel 1986). Tutuila is a volcanic island within American Samoa surrounded by shallow-water fringing coral reefs. Tutuila supports a high human population (ca. 1300 people km<sup>–2</sup>), and its coral reefs harbor a large diversity of corals reflective of a tropical Indo-Pacific reef (Mundy 1996, Green et al. 1999).

Surveys were conducted at multiple sites at FFS, Johnston Atoll and Tutuila in 2004 (Table 1). At each site 2 consecutive 25 m lines, separated by ca. 5 m, were laid out along depth contours (3 to 15 m). Coral community structure was documented along the transect lines by recording coral colonies by size class (0 to 5, >5 to 10, >10 to 20, >20 to 40, >40 to 80, >80 to 160 and >160 cm). Width of the belt transect for colony counts was either 1 or 2 m depending on colony density and time available for surveys. All Acropora with GAs were described, enumerated and photographed along a 25 × 6 m belt that overlapped the transect used for colony counts. We estimated the total number of Acropora colonies surveyed for disease based upon the average number of colonies m<sup>–2</sup> found within the 25 × 1 or 2 m belt transect where colony counts were done as: (avg. no. of corals m<sup>–2</sup>) × (total area [m<sup>2</sup>] surveyed for disease). Prevalence of Acropora GAs for each site was calculated as: [(no. of Acropora with GAs)/(total no. of Acropora)] × 100. Frequency of Acropora GAs disease occurrence (FDO) was calculated as: [(no. of sites with Acropora GAs)/(total no. of sites surveyed containing Acropora)] × 100. Percent coral cover was estimated using the line-intercept method (documenting substrate every 10 cm along the transect line) at FFS and Tutuila and visually at Johnston Atoll.

Morphology. Description and histology of GAs were based on photographs and collection of samples from studies in each of the 3 regions between 2002 and 2006. Corals with GAs were photographed and collected as described in Work & Rameyer (2005) and classified based on gross morphology (Work & Aeby 2006). Tissue samples were preserved in Zinc-Formaldehyde solution (Z-Fix, Anatex) diluted 1:5 in seawater, decalcified in CalEx-II (Fisher Diagnos-

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>Dates surveyed</th>
<th>No. of sites</th>
<th>Mean depth (m) (SE)</th>
<th>Coral cover (%)</th>
<th>Colonies m&lt;sup&gt;–2&lt;/sup&gt; (SE)</th>
<th>Acropora GAs (%) (SE)</th>
<th>FOD (%)</th>
<th>Disease prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>French Frigate Shoals</td>
<td>23°N, 166°W</td>
<td>Sep 2004</td>
<td>11</td>
<td>7 (1)</td>
<td>1 to 53</td>
<td>0.41 (0.19)</td>
<td>19.4 (8.9)</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Johnston Atoll</td>
<td>16°N, 169°W</td>
<td>Jan 2004</td>
<td>12</td>
<td>8 (0.9)</td>
<td>10 to 80</td>
<td>0.42 (0.08)</td>
<td>9.6 (2.6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tutuila</td>
<td>14°S, 170°W</td>
<td>Jun 2004</td>
<td>7</td>
<td>7 (0.5)</td>
<td>23 to 48</td>
<td>0.58 (0.11)</td>
<td>10.4 (2.2)</td>
<td>58</td>
<td>0 to 3</td>
</tr>
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</table>
Acropora growth anomalies in the Pacific

RESULTS

Acropora cover ranged from a low of 9.6% at Johnston Atoll to ca. 20% at FFS, but average Acropora colony density and survey depths did not differ significantly across regions (Table 1). Tutuila had the highest FDO and highest prevalence of Acropora GAs. Although Acropora GAs were seen at Johnston Atoll and FFS, they were not found within the belt transects we surveyed, thus explaining the zero prevalence for both regions (Table 1).

GAs were found on more than 10 species of Acropora representing the major colony morphs of this genus (plating, encrusting, branching and corymbose) (Table 2). The size of colonies affected by GAs ranged from <50 cm to >2 m. Numbers of GAs per colony ranged from 1 to >100, and size of individual GAs ranged from <1 to >35 cm in diameter. For branching colonies GAs were found on all parts of the colonies, whereas for plating colonies GAs were distributed chiefly on the upper surface. Two colonies with GAs photographed in February 2005 and January 2006 experienced varying levels of tissue loss, and the numbers of GAs increased from 13 to 40 in one instance and 11 to 20 GAs in another during 11 mo. Tissue loss was most often seen in larger GAs (Fig. 2B,F). Within colonies distribution of GAs was significantly clustered (p < 0.05) for 2 plating Acropora from Johnston Atoll and Tutuila. Regression of GAs over time was not seen.

Seven morphologic types of Acropora GAs were observed (exophytic, bosselated, crateriform, nodular, vermiciform, fimbriate and annular) (Fig. 1). Based upon a representative sub-sample of 54 GAs photographed in the field, the most common type of GA was exophytic (44.4%) with this morphology found on 8 Acropora species including encrusting (100%), branching (62%) and plating (52%) colonies; bosselated GAs were most commonly found on corymbose colonies. All 7 morphologies of GAs were found in Tutuila, compared to 2 types of GAs found at FFS (exophytic and vermiciform) and only 1 type of GAs (exophytic) found at Johnston Atoll (Table 2).

Polyps were quantified for 41 paired GAs and normal tissues, and GAs had significantly fewer polyps per unit area than normal tissues (Mann-Whitney U, T = 2486, p < 0.0001). Microscopic cellular changes were quantified for paired tissues from 16 normal and 16 GAs including 2 nodular, 3 cratiforms, 5 bosselated and 6 exophytic GAs. Compared to normal tissues, tissues from GAs had significantly fewer zooxanthellae in the upper body wall of the coenenchyme (Mann-Whitney U, T = 359, p < 0.001) and a lower percent of mesenterial filaments based on total tissue area (Mann-Whitney U, T = 169, p < 0.001). We saw no significant difference in the
numbers of spirocysts or numbers of calicodermal or gastrodermal nuclei between GAs and normal tissues (Table 3).

Five of the 7 morphologic types of GAs (exophytic, bosselated, crateriform, nodular and vermiform) showed a consistent pattern of microscopic morphology, which included hyperplasia of cells of the polyp’s basal body walls (consisting of the calicodermis, mesoglea and gastrodermis covering the exoskeleton of scleractinian coral) with reduced to absent polyp structures and lack of or reduced numbers of zooxanthellae (Fig. 3A,B). Significantly more necrosis ($\chi^2 = 10.96, p < 0.001$) was present in GAs (56%) versus normal tissues (0%), whereas significantly fewer gonads ($\chi^2 = 9.64, p = 0.002$) were present in GAs (0%) versus normal tissues (50%). Within GAs 3 patterns of necrosis were evident. Mild to severe selective necrosis of partial or entire mesenterial filaments was present in 3 exophytic and 1 nodular GAs and was characterized by cellular rounding, pyknosis, cytoplasmic hyper eosophilia, dissociation and karyorrhexis (Fig. 3C). Mild to severe diffuse necrosis of basal body walls including mesenterial filaments was seen in 1 annular, 2 bosselated and 6 exophytic GAs (Fig. 3D), of which 2 cases of necrosis were accompanied by mixed fungi and algae infiltrating skeleton and tissues. Mild to severe diffuse necrosis of basal body wall with deposition of layers of hyaline material was seen in 1 nodular (Fig. 3E), 1 fimbriate, 4 bosselated and 8 exophytic GAs, of which 7 cases of necrosis were accompanied by fungi or filamentous algae infiltrating skeleton and tissues. In 1 crateriform GA

Table 2. *Acropora* spp. Number of colonies partitioned by morphology of growth anomalies (GAs), colony morphology and species for French Frigate Shoals (FFS), Johnston Atoll and Tutuila (American Samoa)
large masses of endolithic sponges were present within the skeleton (Fig. 3F). We saw no recognizable difference in microscopic morphology between fimbriate GAs and normal tissue, and diffuse necrosis associated with hyaline material was the only significant finding in the single annular GAs examined. Anaplasia, prominent nucleoli, cellular invasiveness or mitotic figures were not seen in any of the GAs.
Table 3. *Acropora* spp. Mean (SD); median (italics) cellular morphometrics for paired tissues from areas with (n = 16) and without (n = 16) growth anomalies (GAs). cg: coenosarc gastrodermis; bbw: basal body wall; percent mesenterial filaments based on total tissue area. *Significant differences between tissues without and with GAs (Mann-Whitney U, p < 0.01)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Polyps per unit area</th>
<th>Zooxanthellae per unit length cg</th>
<th>Spirocyts per unit length cg</th>
<th>Coral cell nuclei per unit length bbw</th>
<th>Percent mesenterial filaments</th>
</tr>
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<tr>
<td>Normal</td>
<td>0.195 (0.11)*; 0.182</td>
<td>1.191 (1.198)*; 1.189</td>
<td>0.493 (0.737); 0.184</td>
<td>1.769 (1.685); 1.9</td>
<td>0.234 (0.141)*; 0.2</td>
</tr>
<tr>
<td>GAs</td>
<td>0.023 (0.0313)*; 0.0122</td>
<td>0.329 (0.358)*; 0.223</td>
<td>0.329 (0.537); 0.0563</td>
<td>1.944 (1.023); 1.649</td>
<td>0.0773 (0.0893)*; 0.0415</td>
</tr>
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</table>

Fig. 3. *Acropora* spp. Tissue sections of corals stained with haematoxylin and eosin. (A) Corymbose normal tissue; note polyp (black arrow) and mesenterial filaments (arrowhead); scale bar = 200 µm. (B) Bosselated growth anomalies (GAs) from same coral in (A); note lack of mesenterial filaments and polyps and generally uniform presence of basal body wall forming gastrovascular canal network; scale bar = 200 µm. (C) Exophytic GAs from *A. abrotanoides*; note selective necrosis of mesenterial filaments exemplified by cytoplasmic rounding, hypereosinophilia and pyknosis (arrows) compared to normal filaments (arrowhead); scale bar = 400 µm; inset is close-up of necrotic (arrowhead) and intact (left) mesenterial filaments (arrowhead); scale bar = 20 µm. (D) Same as (C); note coagulation necrosis (black arrow) in an exophytic GA and deposition of hyaline layers of organic matrix (arrowhead); scale bar = 60 µm. (E) Same as (A); note hyaline material deposition (black arrow) and necrosis in nodular GAs; scale bar = 30 µm. (F) Crateriform GAs in *A. monticulosa* with endobionts; note sponges (black arrow) associated with necrosis and deposition of hyaline laminae (arrowhead) in coral tissue; scale bar = 400 µm.
DISCUSSION

Acropora GAs were found in all 3 regions, but the reefs of Tutuila had the widest distribution and largest variety and number of morphological types. Host density does not appear to explain this phenomenon because the average density of Acropora was similar in the 3 regions. Similarly, Willis et al. (2004) found GAs to be uncommon on the Great Barrier Reef even though Acropora is the dominant coral.

The etiology of Acropora GAs is unknown, but a number of hypotheses have been proposed. Peters et al. (1986) and Coles & Seapy (1998) suggested that damage to cells from ultraviolet (UV) radiation was a potential mechanism contributing to formation of GAs in corals. Peters et al. (1986) found GAs on A. palmata at a reef in the Florida Keys that was subject to environmental stressors such as high levels of sedimentation, turbidity and seasonal temperature extremes and suggested that environmental factors may have a role in formation of coral GAs. Environment may partly explain the relatively higher prevalence of GAs in Tutuila; Tutuila differs from the other 2 sites in that it is a high island with high human populations and extensive watersheds (with attendant runoff and siltation) compared to Johnston Atoll and FFS, which are both low atolls. Similarly, Aebly et al. (2006) found Porites GAs to be much more common on the reefs of the inhabited main Hawaiian Islands as compared to the relatively more pristine reefs of the Northwestern Hawaiian Islands.

The reefs of Tutuila had all 7 morphologic types of GAs as compared to only 2 morphologic types found at FFS and 1 found at Johnston Atoll. Tutuila also has the highest number of Acropora species (n = 25 species) (Mundy 1996) as compared to Johnston Atoll (n = 10 species) and FFS (n = 7 species) (Maragos et al. 2004). Presumably, a greater number of different host types could lead to greater diversity of GAs. Of the 7 types of GAs, exophytic GAs appeared to be the most common and were found on branching, encrusting and plating colonies. However, we had not devised our morphologic classification of GAs during the surveys, and so our examination of the frequency of occurrence of particular types and their relationship with specific coral colony morphologies was based on a sub-sample of the GAs encountered on the reef. Further studies are needed to verify these findings. Unlike Cheney (1975), we did not see GAs in A. formosa or other species of the staghorn morphology.

Hyperplasia of basal body wall with significantly reduced numbers of polyps and zooxanthellae in Acropora from the Indo-Pacific confirmed the findings of Peters et al. (1986) in A. palmata. Reduced numbers of zooxanthellae would explain the translucence of tissues (white color) seen in most GAs, although some corals with decreased zooxanthellae were gray. Unlike Peters et al. (1986), we did not see significant increases in cellularity or decreased nematocysts in GAs. Methodology may account for the discrepancy. Peters et al. (1986) quantified cellularity and nematocysts over a surface area, whereas we quantified cellularity along a linear contour. Hyperplastic basal body wall in GAs quantified over a surface area may result in multiple tangential sections having the appearance of hypercellularity potentially producing artificially increased cell counts. Alternatively, Peters et al. (1986) quantified GAs from a single species of Acropora, while we pooled different GAs from different species to increase statistical power and to answer the more general question of what differences existed between GAs and normal tissues in Acropora. Clearly, future studies will need to assess whether patterns seen in the present study can be applied to particular morphologies across a wider range of corals and geographic areas. A final possibility is that, compared to Acropora in the Pacific, GAs in A. palmata from the Caribbean have a different microscopic morphology.

Peters et al. (1986) showed that GAs in Acropora palmata are progressive leading to death of surrounding normal tissue, and the present study confirms this phenomenon, at least in the colonies where we had sequential data. Larger GAs seemed to preferentially undergo tissue loss; however, confirming the generality and mechanisms of these phenomena awaits more targeted studies. Microscopic evidence of apparently spontaneous necrosis in 41% of intact GAs in the absence of associated organisms such as fungi or algae provides a clue into possible early stages of degeneration of these GAs. Selective necrosis of mesenterial filaments may explain the reduced number of polyps in GAs (e.g. polyp structures die before the full polyp can develop or mature). Rounding of cells in some GAs was morphologically suggestive of apoptosis (Vermeulen et al. 2005), and use of molecular markers to localize apoptosis signaling molecules in coral cells may shed light on this phenomenon (Hewitson et al. 2006). Why necrosis in GAs assumes 3 forms (cellular rounding, diffuse necrosis and diffuse necrosis with deposition of hyaline matrix layers) remains open to question; however, necrosis in GAs is not necessarily dependent on invasive organisms such as algae or fungi.

In higher vertebrates, necrosis of tumors typically occurs either because rapidly growing neoplastic cells outgrow their blood supply (anoxia) or because toxic factors associated with tumor invasiveness lead to cell death (toxemia) (Cheville 1976). Coral polyps absorb oxygen directly from the water, so anoxia would not be a likely explanation for necrosis. Like Peters et al. (1986), we found continuity in gastrovascular canals between normal and GA tissues, but circulation within
gastrovascular canals of GAs could be compromised and this topic merits further analyses. Toxemia due to altered metabolism (Cheney 1975) of GA tissue or inadequate food supply for such rapid growing tissue are other possible explanations of necrosis. In sum, tissues from GAs are compromised functionally (reduced numbers of polyps leading to reduced food capture), structurally (necrosis) and reproductively (reduced development of gonads), exerting a negative cost on the colony. Costs in terms of reduced growth due to GAs have also been found (Cheney 1975, Bak 1983). As such, the effect of Acropora GAs on the overall health of reef systems is a concern.

Spatial analysis of GAs on 2 acroporid colonies showed a clustered distribution of GAs within colonies. Whether these 2 colonies represent the usual distribution of GAs on acroporids or are the exception needs to be verified; however, we commonly see this pattern in the field. Clustering of GAs within colonies may be explained either by metastasis (dissemination of GA cells from Point A to B) or by de novo generation of GAs. In the present study the absence of mitotic figures, hypercellularity and invasiveness in tissues from GAs argue against metastasis (Cheville 1976). On the other hand, little is known regarding morphologic indicators of neoplasia and metastasis in invertebrates, so this phenomenon cannot be ruled out completely. A second hypothesis is that factors locally secreted from GAs promote growth of GAs in the immediate area (paracrine effect). A final possibility is that a locally communicable agent (such as a virus that is transmitted cell to cell) is responsible for clustered growth patterns of GAs within colonies. However, attempts to transmit GAs among corals have been unsuccessful (Cheney 1975, Peters et al. 1986).

We chose to describe the cellular changes seen in Acropora GAs as hyperplasia because we judged that they did not fit the classic definition and morphologic criteria of neoplasia or uncontrolled cell growth (Cheville 1976). The concept of neoplasia continues to elicit uncertainty even for vertebrate medicine (Cheville 1976). Peters et al. (1986) concluded that GAs in A. palmata were neoplasias based on observations of irreversible growth leading to death. Dawe (1969) and Sparks (1972) urged caution in applying the criteria used to define neoplasia in vertebrates for invertebrates. In spite of these reservations, vertebrate medicine currently offers the best and most complete criteria for defining neoplasia in animals, and thus may provide an adequate basis for determining the nature of GAs in corals. Cheville (1976) noted that all neoplasias share certain commonalities including (1) cell surfaces designed for movement, (2) simplified energy production, (3) failure of differentiation (nuclear pleomorphism and prominent nucleoli); and (4) inconsistent presence of mitotic figures. The latter 2 criteria highlight the important role that morphology plays in characterizing neoplasia, and these features were not seen in samples from the present study. Mix (1986) noted that the hallmark characteristic of vertebrate neoplasms (e.g. metastasis) were not always present in so-called neoplasms of mollusks and other invertebrates. Nevertheless, it has become generally accepted that neoplasia is a condition probably found throughout the animal kingdom.

A significant factor in the uncertainties surrounding GAs in corals is lack of information (Peters 1997). Given current data, certain commonalities of GAs in corals of the family Acroporidae are emerging. At the gross level these include more rapid growth compared to normal tissues (Cheney 1975, Peters et al. 1986), irreversible growth leading to reduced colony growth, partial or complete death of the colony (Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000, the present study), reduced polyp density (Peters et al. 1986, Yamashiro et al. 2000, the present study) and lower skeletal density (Cheney 1975, Peters et al. 1986, Yamashiro et al. 2000, the present study). At the cellular level GAs are exemplified by reduced mesenterial filaments (Peters et al. 1986, Yamashiro et al. 2000) and lack of anaplasia (Peters et al. 1986, the present study). Future efforts should focus on confirming or refuting these morphologic commonalities and critically evaluating whether they fit the criteria used to define neoplasia for vertebrates. Meanwhile, given the current uncertainties regarding this topic, ‘growth anomaly’, rather than ‘tumor’, may be a preferable term for this condition in corals since the latter implies neoplasia.

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