IV. STATE OF KNOWLEDGE IN THE PACIFIC—WHAT DO WE KNOW AND WHAT HAVE WE LEARNED?

OVERVIEW OF ISSUES UNIQUE TO THE PACIFIC: BIOLOGICAL & SOCIAL PERSPECTIVES

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Pacific Islands

The term "Pacific Islands" in the context of this paper arbitrarily refers to those tropical islands of the central and western Pacific Ocean which support shallow hermatypic coral reefs, but excluding the Hawaiian Archipelago, which is covered in other papers. The tropical Pacific Island nations and territories all support coral reefs and, no doubt, harbor coral diseases, although these have not been scientifically documented in many of the islands. In fact, as part of the U.S. National Action Plan to Conserve Coral Reefs, surveys in 2002 and 2004 of coral reef academic scientists, resource managers, government agencies and NGOs recorded that in the U.S. Pacific islands they perceived "no threat" from coral disease, although American Samoa registered an increase to perception of "moderate threat" in the 2004 survey (Waddell, 2005). This lack of concern partially reflects a lack of information on the status of diseases in many islands. Wilkinson (2004, p. 405) notes that in American Samoa and Micronesia "Coral bleaching and disease were either rare or undocumented in 1994, but are now clearly evident and considered a serious threat to many reefs in the region."

The Pacific island coral reefs range from veneers on newly emergent volcanic islands, to platform-like fringing reefs, to barrier reefs with lagoons, to atolls, and include non-emergent isolated banks.

The islands associated with the reefs vary from large high islands, such as in Fiji and New Caledonia, to small low atoll islands rising less than two meters above sea level, as in the entire nations of Tuvalu and the Republic of the Marshall Islands. Although the availability of land resources has limited human settlement and development in these islands, even some of the small atoll areas have been inhabited continuously for thousands of years. The long term and current native residents of the islands are generally defined as Polynesian, Melanesian and Micronesian, based upon their ancestry, culture and languages and their geographic association within these island archipelagoes.

Similarities to Atlantic Areas

Before European contact, the Atlantic and Pacific coastal residents and islanders (except for those far in the interior of high islands), depended on coral reef resources for survival and managed these resources in a sustainable way. But Western development brought major changes to land use, which impacted on surrounding reefs. As in the Western Atlantic area of coral reefs, the Pacific Islands have had similar political histories of colonialism and plantation agriculture exploitation over the last few centuries. Both areas were colonized by European nations from the Sixteenth Century to the present, although most island states have become independent countries in the decades since World War II.

During the early periods of colonization, diseases, warfare and forced relocation decimated local populations. During the last century, populations have re-established or been replaced by immigrants and are in many cases exceeding the traditional self-sustainable carrying capacities. Economic development bases have been shifting from plantation monoculture agriculture to tourism and, to a limited degree, mining, fisheries, and logging (which is typically not sustainable). Impacts of related coastal pollution are similar world-wide.

Atlantic and Pacific reefs are influenced by similar weathers patterns with the same water temperature ranges, variable annual precipitation from very dry to some of the wettest places on earth and frequent violent tropical storms (hurricanes, typhoons or cyclones). Trans-oceanic wind-driven continental dust may be reaching parts of the North Pacific from Asia, but this is not as apparent as African wind driven pollutants entering the Caribbean.

Differences between the Pacific and Atlantic

Island and Reef Numbers:
Although both oceans have atolls and barrier reefs, the numbers of islands and atolls and the lengths and areas of their reefs are much greater in the Pacific (Wilkinson, 2004). Although they are relatively small, the islands of Micronesia alone number over one thousand. Compared to the few atolls in the Caribbean, the Pacific has over fifty. The barrier reefs of Fiji, New Caledonia, Papua New Guinea and other Pacific islands together exceed the extent of the Mesoamerican Barrier Reef System, without considering the world's largest barrier reef in Australia.

Ocean Size:
Distances across the vast Pacific and between its far-spread reefs dwarf those of the Caribbean. The entire Caribbean, if it could be moved over the Pacific, would be lost between Hawaii and Guam without even overlapping the larger and broader tropical Pacific waters south of the equator.

Fisheries:
These great expanses of the Pacific support industrial fisheries fleets supplying over one third of the annual worldwide catch of tropical tuna. And the artisanal and subsistence catches of reef fishes continue to supply the basic protein needs of hundreds of thousands of Pacific islanders.

Biodiversity:
The greatest marine bio-diversity lies in the western Pacific. This diversity diminishes from island to island, crossing the Pacific to the east. For example, the corals of the Hawaii Islands have been intensively studied and found to have similar species numbers to the Caribbean (approximately 60 and 2 massive soft corals) (Waddell, 2005). But the Hawaiian Island Archipelago is isolated from the central triangle of highest marine diversity among western Melanesia, Indonesia and the Philippines (Carpenter and Springer, 2005). The Marianas including Guam, also fairly well studied, lie closer to this center, and typical of many Pacific islands, have recorded over 400 species in 108 genera and 21 families of hard corals and over 30 species of massive soft corals (Paulay, 2003). Higher diversity progresses towards this center through Fiji, Palau and the Great Barrier Reef of Australia.

Traditional Knowledge:
The ancient pre-colonial knowledge of coral reef resources, which must have resided with the aboriginal Caribbean residents, apparently has been lost through most of those islands. But the descendents of Pacific island fishermen have retained much of their ancestors' knowledge of their reefs, which has been accumulated and passed down over thousands of years. The sustainable management practices for reef resources that evolved over these thousands of years remain active in some of the islands, although western ideas and their misplaced or unsuccessful management approaches have replaced traditional controls in many islands. Traditional knowledge retained by Palauan fishermen has been partially recorded in the book "Words of the Lagoon" compiled by Robert Johannes (1981), after residing in Palauan villages for over a year to seek out this knowledge. This gives an example of the actual scientific knowledge base that exists among island fisherfolk. Although their knowledge is usually focused on edible or harvestable species, it includes ecological information that can relate directly to coral health.

Many traditions regarding coral reefs still strongly influence the daily lives of islanders and must be recognized and respected by scientists wanting to study the reefs. For example, in many islands, submerged reef resources are privately owned or are strictly managed by chiefs or families (Gawel, 1984). This ownership and control is not simple in any case and involves complicated overlapping and changing rights to use various resources. These systems have succeeded in managing many of the key reef resources for centuries, but are being eroded often because of modern aspirations for commercial development of marine and coastal resources. Entry to some reef areas or taking of scientific samples may sometimes not be allowed. Even taking of a dead coral or piece of rock may be taboo at times in certain places. Gender distinctions separating how men and women relate to coral reef activities strongly persist in many islands, especially

related to subsistence uses. And diving on Sunday may be offensive or prohibited in some islands due to European derived religious beliefs, which are treated as strong tradition in many islands. Consequences of violating traditional laws can be very severe in certain island communities. Value of conservation or research efforts to the traditional users and to the health of the reefs may not always be recognized in decisions by traditional leaders. But their authority can remain unchallenged because of broader aspects of custom and traditional life.

War:
The impacts of World War II and pre-war activities in the Pacific islands contrast with that war's impacts on Atlantic reefs. American, European colonial and Japanese harbors and fleets were developed and destroyed across the coral reef islands. Serious damage was caused and, although most coral reefs have recovered over the last sixty tears, millions of pounds of explosives, fuel and toxic chemicals remain in the ships sunk in coral reef areas. An unfortunate result of war practices is the use of old recycled explosives to illegally harvest fishes by blasting coral reef areas.

Current Political Ties:
Although Guam and parts of Micronesia had been Spanish colonies for hundreds of years, and subsequently these islands (excluding Guam) were German and then Japanese owned, their ties with these mother countries have been greatly over-shadowed by the American influence since WWII. This has led to English being the most widespread language of Micronesia. South of the equator, France has retained three island groups within its nation and Australia and New Zealand have replaced former European nations as having the greatest alliances with the former colonies. (Note: the three Tongan archipelagoes had never been colonized and retain their traditional king.) The recently independent Pacific island countries are members of the United Nations and have attracted assistance and attention from East Asian neighbors including Japan, China, Taiwan and South Korea (Crocombe, 2001).

Authorities for Management:
Under modern laws in force in the Pacific islands, regulations and permit systems to manage marine resources uses and activities that impact coral reefs exist at national levels. For example, taking of endangered species like turtles may be prohibited and dredging for a new reef passage or harbor may require environmental impact assessments and permits with conditions. But these legal controls apply mostly to centers of population where more damaging impacts to the reefs tend to occur (Birkeland, 1997). Fortunately, the more remote reef areas remain more pristine although modern legal controls are less recognized by outer island populations. Traditional and local community controls continue to function more in areas of low development and population pressure. Even in national centers and developed areas, the approach of involving communities in coral reef conservation is proving to be the best approach. The U.S. Coral Reef Task Force is pursuing support of "Local Action Strategies" to locally develop strategies and projects at more of a community level for coral reef conservation, to be implemented with federal funding (Wilkinson, 2004). One of the nationally applied LAS issue areas is Coral Bleaching and Disease (Waddell, 2005). Those islands

not under the U.S. flag have other sources of support for coral reef conservation such as the Global Environment Facility and non-governmental organizations such as TNC.

Scientific Resources in the Pacific

A number of centers of coral reef scientific expertise have developed in the Pacific and new ones are arising. The following tables list some of the major academic, research and data centers in the tropical Pacific, excluding many from Hawaii and Australia.

Universities and Colleges:

- University of the South Pacific
- University of French Polynesia
- University of New Caledonia
- University of Papua New Guinea
- Community College of Micronesia
- College of the Northern Marianas
- College of the Marshall Islands
- Palau Community College
- University of Guam

Regional Institutions and Centers:

- Secretariat of the Pacific Regional Environmental Programme (SPREP)
- South Pacific Applied Geoscience Commission (SOPAC)
- Secretariat of the Pacific Community (SPC)
- Coral Reef Initiative for the South Pacific (CRISP)
- University of the South Pacific (USP)
- University of Guam (UOG)
- US Coral Reef Task Force: All Islands Group
- Marine Resources Pacific Consortium (MAREPAC)
  - American Samoa
  - Republic of the Marshall Islands
  - Federated States of Micronesia
  - Republic of Palau (Palau International Coral Reef Center)
  - Commonwealth of the Northern Marianas
  - University of Guam

Global and Regional Research and Data Organizations:

- Australian Institute for Marine Sciences
- The Global Coral Reef Monitoring Network
- ReefBase
- Reef Check
- Hawaii Institute of Marine Biology, University of Hawaii
- Institute of Marine Resources, University of the South Pacific
- University of Guam Marine Lab
- Palau International Coral Reef Center
- CRIOBE Research Center Moorea

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BASELINE LEVELS OF CORAL DISEASE IN THE NORTHWESTERN HAWAIIAN ISLANDS

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ABSTRACT

There has been a worldwide increase in the reports of diseases affecting marine organisms. In the Caribbean, mass mortalities among organisms in reef ecosystems have resulted in major shifts in community structure. However, our ability to fully understand recent disease outbreaks is hampered by the paucity of baseline and epidemiological information on the normal disease levels in the ocean. The Northwestern Hawaiian Islands (NWHI) is considered one of the last relatively pristine coral reef ecosystems remaining in the world. As such, it provides the unique opportunity to document the normal levels of disease in a coral reef system exposed to limited human influence.

In July 2003, baseline surveys were conducted at 73sites throughout the NWHI to quantify and characterize coral disease. Ten disease states were documented with the most common disease found to be *Porites* trematodiasis. This disease was widespread and is known to exclusively affect *Porites* sp. coral. Numerous other conditions were observed but at much lower levels of occurrence. Numbers of colonies affected by *Porites* trematodiasis were not enumerated but other types of conditions were counted with the average prevalence of disease estimated at 0.5%. Several of the observed disease states were distinct from what has been described from other coral reef systems. Coral genera exhibited differences in types of syndromes and prevalence of disease. Pocilloporids, common corals on the reefs of the NWHI, were comparatively resistant to disease. In contrast, acroporids showed the greatest damage from disease and the highest estimated prevalence of disease.

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Report 2: French Frigate Shoals Reef Health Survey, included in this document below.

Report 2. *French Frigate Shoals Reef Health Survey*

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INTRODUCTION

French Frigate Shoals (FFS) is one of the refugia comprising the Northwest Hawaiian Islands National Wildlife Refuge (NWHINWR). French Frigate Shoals was discovered by La Perouse in the late 18th century; however, the atoll was most notable as a naval air station during World War II when the US Navy dredged Tern Island into an airstrip, and the US Coast guard established a LORAN station on East Island. After the war, the LORAN station was moved to Tern Island where it remained until the Coast Guard vacated in 1979. Since then, the US Fish and Wildlife Service (USFWS) has managed Tern Island-FFS as a wildlife refuge with a full time staff presence (USFWS, 2001).

French Frigate Shoals consists of a large (31 nm) fringing reef partially enclosing a lagoon. A basalt pinnacle (La Perouse Pinnacle) arises approximately halfway between the two ends of the arcs of the fringing reefs. Tern Island is situated at the northern end of the lagoon and is surrounded by a dredged ship channel. The lagoon becomes progressively shallower from west to east and harbors a variety of marine life including corals, fish, marine mammals, and sea turtles (Amerson 1971). In 2000, an interagency survey of the northwestern Hawaiian Islands was done to document the fauna and flora in FFS (Maragos and Gulko, 2002). During that survey, 38 stations were examined, and 41 species of stony corals were documented, the most of any of the NW Hawaiian islands (Maragos and Gulko 2002). In some of these stations, corals with abnormalities were observed. The present study aimed to expand on the 2000 survey to evaluate the lesions in areas where they were documented.

METHODS

Survey areas:

Because of the ocean conditions, patchy distibution of corals, and lack of water clarity, all surveys were done using SCUBA. Survey locations were chosen based on observations of Jim Maragos during the 2001 NOWRAMP surveys (Fig. 1). Specifically, he had noted bulbous white tumors in *Montipora patula* on site R31 and *Porites lobata* with dead lesions in sites R33 and 39. Emphasis was placed on these locations and those areas where coral cover was substantial (>40%) and that were accessible by boat.

Corals were photographed using a Nikonos V underwater camera with a 20 mm lens and twin Ikelite 50 strobes or a digital camera in an underwater housing. Close-up photos were taken with a Nikonos V camera with a single Ikelite 50 strobe and a 2:1 extension tube. Coral samples were taken using bone shears, or hammer and chisel, and placed into labeled plastic bags in seawater. Gross lesions were broadly characterized as growth anomaly, tissue necrosis, algal infiltration, trauma, or a combination thereof.

Corals were preserved in Helleys fixative (Barszcz and Yevich, 1975) with added salt and allowed to fix for 24 hr. The fixative was decanted and the coral rinsed with fresh water once every 12 hr for 24 hr. Subsequently, coral was stored in 70% ethanol, decalcified

with Cal-ex II (Fisher Scientific), placed in cassettes, processed for paraffin embedding, trimmed at 5 um, and stained with hematoxylin and eosin. Slides were examined using light microscopy at magnifications ranging from 20-1000X. Normal histology of species of corals not yet in USGS-Hawaii Field Station archives was described. Lesions were classified as neoplasia, algal infiltration, necrosis, inflammation, atrophy or parasites.

RESULTS

For some of our survey, we were limited to doing shore dives from Tern Island because of wind conditions that rendered small boat operations in the lagoon unsafe. A review of available wind speed data for 1989-93 and 2000-1 revealed that the best times to conduct open water operations on Tern Island was late March-early April, late May-early June, late July mid-August, and early September-mid October (Fig. 2). We did a total of 16 dives half of which were shore dives from 18-28 March, 2002. Coral cover was generally patchy throughout all dive sites but seemed most sparse around the ship channel and most rich near La Perouse Pinnacle.

Lesions

Of 44 coral samples examined, 34 had lesions. Of the samples with lesions, algal infiltration of coral tissue was the predominant diagnosis (26) followed by bleaching (4), tumors (2), and parasites (1). The remainder of corals was healthy individuals used for reference purposes.

Algae-coral interactions:

Invasion of coral tissue by marine algae was seen in *Acropora cytherea, Porites lobata, P. evarmanni, P. compressa, Leptastrea purpurea, Montipora capitata, and Pavona duerdeni*. In *P. duerdeni* and *L. purpurea*, algal infiltrates had a distinct border with presence of macro-algae within the algal mats (Figs. 3A-B). Algal infiltrates in *A. cytherea* were characterized by ill-defined discolored areas with occasional greenish or yellow tinge or complete bleaching (Figs. 3C-D). In some instances, algal infiltrates were accompanied by exuberant skeletal growth (Figs. 3E-F). In *P. lobata* and *P. evarmanni*, algal infiltrates had less distinct borders, and coral tissues adjacent to infiltrates were bleached or tinged with pink coloration (Figs. 3G-H). Gross lesions of algal infiltration in *M. capitata* consisted of firm, smooth to rugose raised areas (Figs. 4A-D).

On microscopy, a mixed assemblage of algal organisms infiltrated coral tissue. In *A. cytherea*, reaction to algal infiltration was much less marked and generally characterized by focal tissue death and focal thickening of the gastrodermis of the gastrovascular canal near the algal infiltrates (Figs. 4E-F). In *Porites* sp. and *P. duerdeni*, within gastrovascular canals, gastrodermal cells formed rosettes and clumps among algal filaments. In many instances, there was localized thickening of calicoblast layer adjacent to algae. Clumps of sloughing and necrotic gastrodermal cells appeared constricted by

linear bands of unidentified granular grey material. In many cases, there were clumps of necrotic debris associated with algae (Figs 4G-H; 5A-D). In *Montipora* sp., algal infiltrates were often accompanied by necrosis and a prominent cellular response comprising calicoblast and pigment cells (Figs. 5E-H).

Parasites:

The only instance of parasitism was in *P. lobata*. Grossly parasites were manifested by multiple small bumps scattered throughout the surface (Figs. 6A-B). On histology, these foci were characterized by necrosis of epithelium (Fig. 6C). Within the gastrovascular canal network, there was erosion of epithelium and proliferation of calicoblast and pigment cells around putative coccidia (Figs. 6C-F). As the lesion progressed, it appeared that these coccidia became encapsulated leading to eventual loss of sporozoites (Figs. 6G-H). In many cases, parasite-induced lesions were accompanied by algal infiltrates (Fig. 6H).

Tumors:

Tumors were seen only in *A. cytherea*. One type of tumor was cauliflower-like and had focal distribution on the coral (Fig. 7A). Another type of tumor was also localized but appeared vermiform (Fig. 7B). On histology, both tumors revealed a marked disorganized proliferation of gastrodermal cells with no mitotic figures and no necrosis (Figs. 7C-D).

Miscellaneous:

Other lesions included the burrows of the skeleton-dwelling symbiotic crab *Pseudocryptochirus kahe* McCain and Coles (1979) (Fig. 7E) and bleaching (Fig. 7F) in *P. eydouxi* and trauma of *P. evermani* due to fish bites. Bleaching of *P. eydouxi* was characterized by lack of pigmentation of tissues, and on histology, bleached tissue was markedly atrophied with loss of mesoglea and zooxanthellae (Fig. 7H). Lesions caused by *P. kahe* consisted of distinct holes within coral skeleton surrounded by hyperpigmented tissue. On microscopy, each hole contained a single crab surrounded by mats of algae. Fish bites in *P. evarmanni* consisted of localized areas of tissue ablation revealing white skeleton beneath.

Normal histology:

Palythoa tuberculosa (Figs. 8A-D)

Colonies consisted of closely apposed large polyps encrusting on the substratum. Coenosarc epithelium was composed of columnar cells mixed with holotrichous isorhizas, clusters of eosinophilic granular cells, scarce zooxanthella, and large vacuoles. Tentacle epithelium consists of closely apposed columnar cells mixed with spirocysts, granular pigment cells, and zooxanthella. Deeper down, near the mesoglea, there was a mesh-like filigree of cells and delicate filaments. Pharyngeal epithelium was composed

of closely apposed ciliated columnar cells with aggregates of granular eosinophilic and brown pigment cells at the base.

Tentacle gastrodemis was composed of cuboidal cells replete with zooxanthellae. Gastrovascular canals course through the large mesoglea in a haphazard manner and are lined by similar cells as those found in tentacles. Mesenteric filaments was composed of a cnidoglandular cap consisting of closely apposed columnar cells, eosinophilic granular cells, few holotrichous isorhizas, and a base consisting of columnar cells mixed with large numbers of zooxanthella and granular eosinophilic cells.

Mesoglea consisted of a large network of connective tissue enveloping a mesh of skeleton. Gastrovascular canals course through the mesoglea that forms the structural matrix. Several different types of cells were noted within the mesoglea including eosinophilic granular cells, stellate cells with basophilic cytoplasm, denegerating zooxanthella, and small amphophilic cells with small dark granules in a clear cytoplasm. At the base where the zooxanthid contacted the substrate, the mesoglea was composed of eosinophilic debris mixed with branching septated organisms.

Trabeculae of skeleton coursed through mesoglea. Calicoblast layer was almost not discernable and consisted of a single layer of eosinophilic granular cells. Occasional hyphae and filamentous organisms were seen in the skeletal space.

Cyphastrea ocellina (Figs. 8E-F)

Large brown encrusting colony with large calices. Coenosarc epithelium consisted of columnar cells mixed with vacuoles and pigment cells. Epithelium of polyps consisted of closely apposed columnar cells mixed with vacuoles and batteries of spirocysts. Pharynx epithelium consisted of closely apposed ciliated columnar cells. Mesoglea of coenosarc was thin and somewhat thickened with mesogleal pleats within mesenteric filaments.

Gastrodermis of coenosarc was vacuolated and contained moderate numbers of zooxanthella and granular brown pigment cells. Gastrodermis of polyps was massively distended with zooxanthella. Gastrodermis of gastrovascular canals contained granular brown pigment cells. Cnidoglandular cap of mesenteric filaments consisted of closely apposed columnar cells with spirocysts. Occasional planula were noted within gastrovascular canals. Calicoblast consisted of single squamous layer of cells.

Leptastrea purpurea

Coral was encrusting, brown, and has large contiguous calices reminiscent of brain coral. Epithelium consisted of columnar ciliated cells underlaid focally by larger supporting cells. Spirocysts were scattered throughout although these were clumped and more numerous within tentacles. Occasional gray vacuolar cells are noted. Pharyngeal epithelium consists of closely apposed columnar ciliated cells mixed with larger dark globular cells.

The mesoglea formed an arching structure that was overlaid externally by epithelium and internally by gastrodermis forming a single layer canal network. Mesogleal pleats were prominent. The gatrodermis underlying epithelium was composed of pseudostratified columnar epithelium containing numerous zooxanthellae. Mesenteric filaments contained typical closely clumped columnar cells with prominent eosinophilic granular cells at cnidoglandular cap. Some mesenteric filaments contained batteries of holotrichous isorhizas. Gastrodermis of gastrovascular canal contained numerous mucous and pigment cells. The calicoblast consisted of a single layer of squamous cells that were focally hyperplastic.

DISCUSSION

Algal-coral interactions made up the preponderance of lesions encountered in corals at FFS. It is likely that some of the necrotic lesions in Porites sp. and the tumor-like lesions in Montipora observed by Maragos during the 2000 NOWRAMP survey were due to invasion of coral tissue by algae. Insufficient time and weather conditions precluded our doing manta-tows to assess spatial distribution of lesions. As such, we were unable to determine if certain sites had higher incidence of coral-algal interactions than others. However, cursory observations did not indicate a particular site having unusual numbers of lesions.

Different species of corals appeared to respond to algal invasion differently. All species responded by increasing thickness of gastrodermis and calicoblast layers adjacent to algae. Peters (1984) described blistering necrosis of cells in Caribbean corals infiltrated by algae. The appearance of sloughing rosettes of gastrodermis appeared to be a response limited to *Porites* sp. and *P. duerdeni*. Exuberant growth of skeleton in response to algae was characteristic of *Montipora* sp. giving them a gross appearance of a tumor-like growth. Although similar responses were seen in some *A. cytherea,* this was far less common. A similar manifestation to coral invasion was seen in Montipora from Johnston Atoll (Work et al., 2001). *Montipora* sp. were one of the few species that also showed microscopic evidence of a distinct cellular inflammatory response to algal invasion. Most studies of coral immunity have involved grafting experiments (Jokiel and Bigger 1994, Hildeman et al. 1975). Microscopic evidence of inflammation in corals is a rarely documented phenomenon that merits further study. Likewise, the algal assemblage infiltrating coral tissues appeared to be a mix of different species. There is a need to elucidate what species of algae are associated with these lesions.

The manifestation of parasitism in *P.lobata* was markedly different than that observed in the main Hawaiian Islands. In the latter case, the parasite was a trematode that is common in the main Hawaiian Islands (Aeby 1991). In this study, the parasites were compatible in morphology to coccidia and were similar in appearance to *Gemmocystis* sp. recorded by Upton and Peters (1986) in *Porites* sp., *Montastrea* sp., *Diploria* sp. and *Meandrina* sp. from the Caribbean. The coccidia seen here contained a single sporozoite, and the coral appeared to mount a response to these parasites via proliferation of calicoblast cells. In later infections, there appeared to be encapsulation of coccidia

suggesting a possible mechanism of parasite clearance by the coral. Other parasites that have been documented from corals elsewhere in the Caribbean include ciliates, nematodes, and amoeba (Peters 1984).

The tumors in *A. cytherea* were both gastrodermomas based on tissue morphology and location of cell proliferation. Criteria used to define these lesions as tumors were similar to those used by Work et al. (2001). One type of tumor was identical to that seen in *A. cytherea* from Johnston Atoll and classifed as type 1 tumor (Work et al., 2001). The second type of tumor was also a gastrodermoma but manifested as a vermiform rather than a cauliflower-type growth. Two coral heads were seen with a type 1 tumor whereas only one coral was noted with the vermiform tumor. The paucity of tumors on *A. cytherea* suggests that they did not appear to pose a major threat to this species.

Remaining lesions were incidental findings. The crab-induced lesions in *P. eydouxi* were similar to those observed on the main Hawaiian Islands (Work and Rameyer, 2001). The numbers of corals affected with this lesion appeared small, and interestingly, corals that were infested with crabs appeared to have high numbers with tissue that appeared paler than normal. The crab-induced lesions are common on thick-branched *Pocillopora* corals in the Hawaiian Islands (McCain and Coles, 1979) and Johnston Atoll (Work et al., 2001). Fish bites in *Porites* sp. were also similar to those observed in the island of Hawaii.

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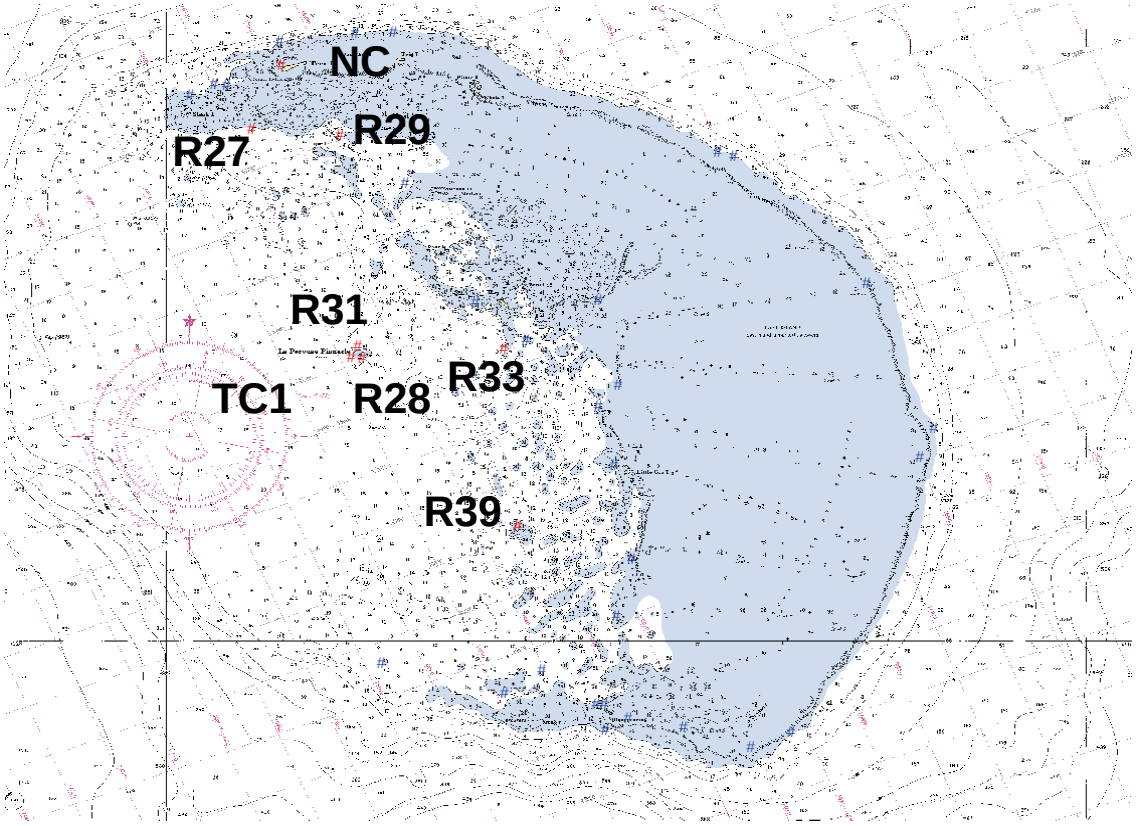

Figure 1. NOWRAMP 2001 survey locations for FFS. Survey sites for this study in red

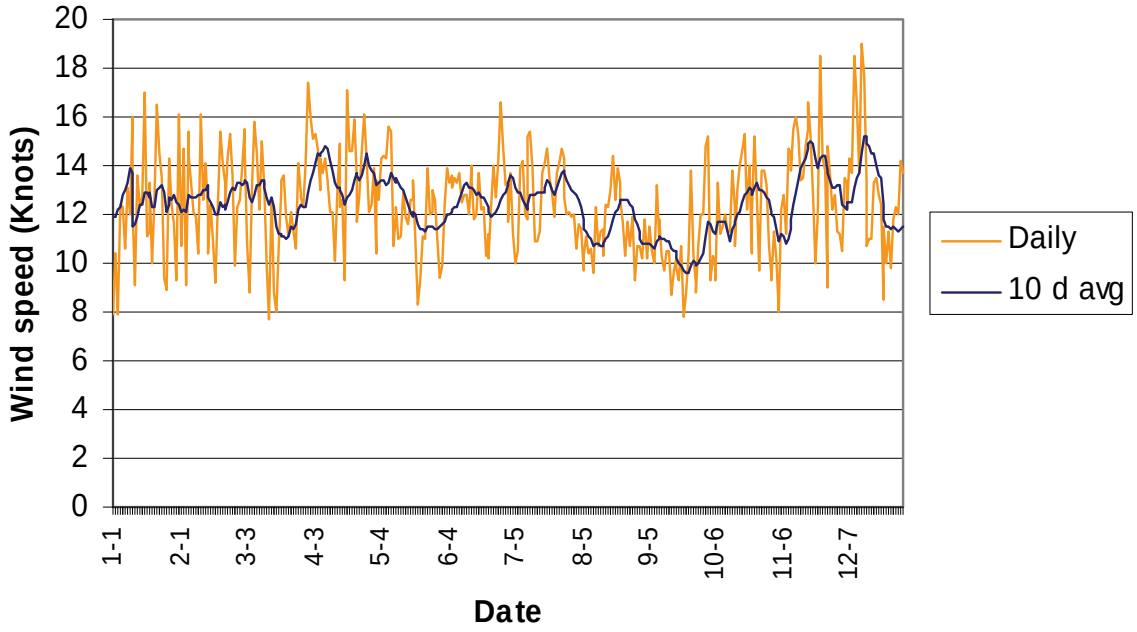

Figure 2. Daily average wind speed (orange) and 10 day running average wind speed for 1989-93; 2000-11, FFS.

Figure 3. *P. duerdeni*. Note clear demarcation between normal tissue (right) and alga (left) (A). *L. purpurea.* Note clearly defined patches of algal growth (arrow) (B). *A. cytherea* (C-F). Note algal infiltration with bleaching (C) and pigmentation (D). Note algal growth (arrow) accompanied by skeletal proliferation (E). Close up of C (F). *P. lobata* (G-H). Note ill defined areas of depigmentation (G) and raised pigmented areas on edge of lesion (H).

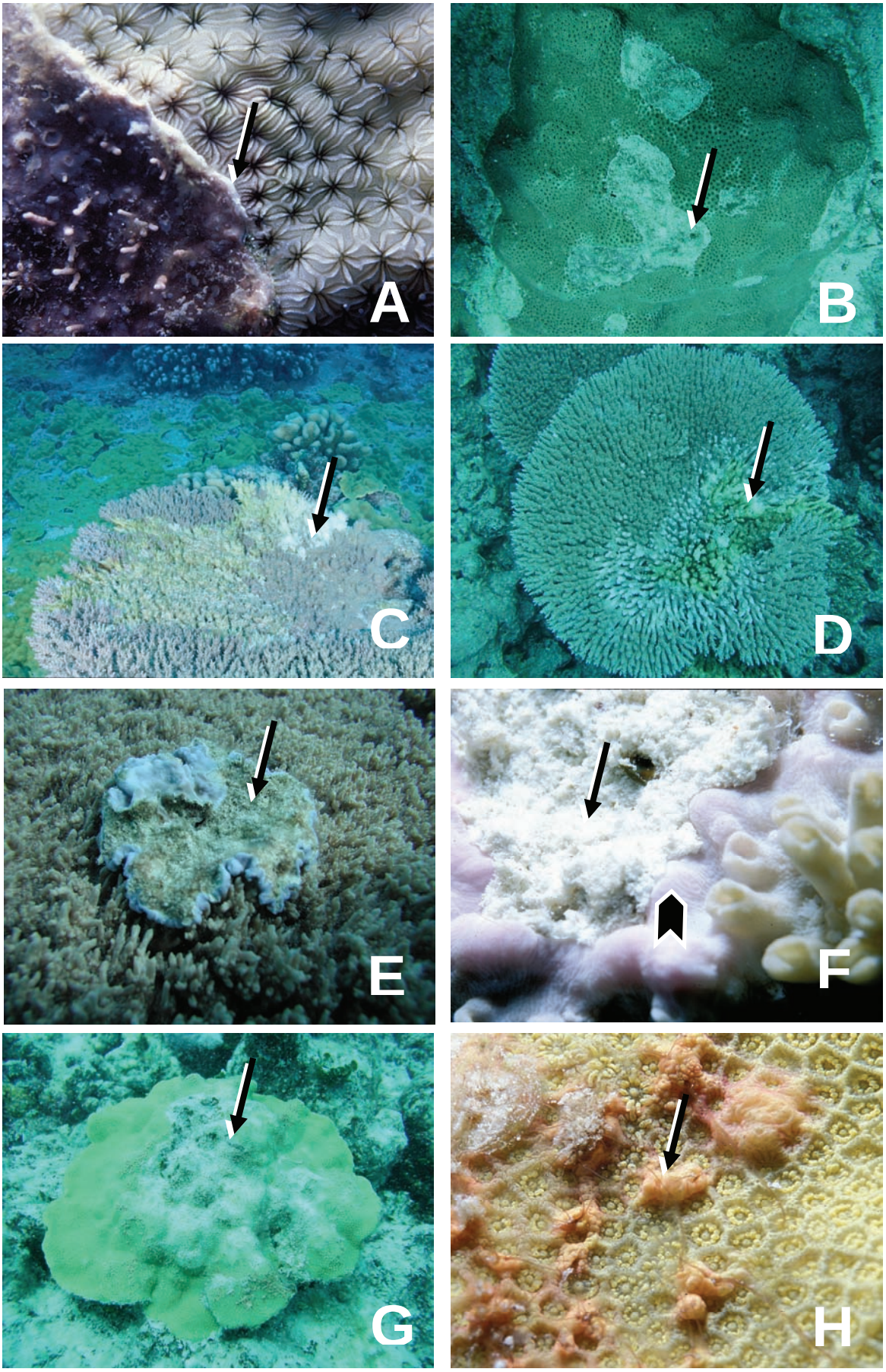
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Figure 4. ***M. capitata*****, note growth anomalies (arrows) (A-D*). *A. cytherea* (E-F). Note focus of necrotic tissue (arrow) and algal filaments (arrowhead), bar=50 µm (E). Hyperplastic gastrodermis (arrow) and algal filaments (arrowhead), bar=100 µm (F). *P. lobata* (G-H). Polyp being invaded by algae (arrowhead), bar=100 µm (G). Sloughing gastrodermal cells forming rosettes (arrowhead), bar=50 µm (H).**

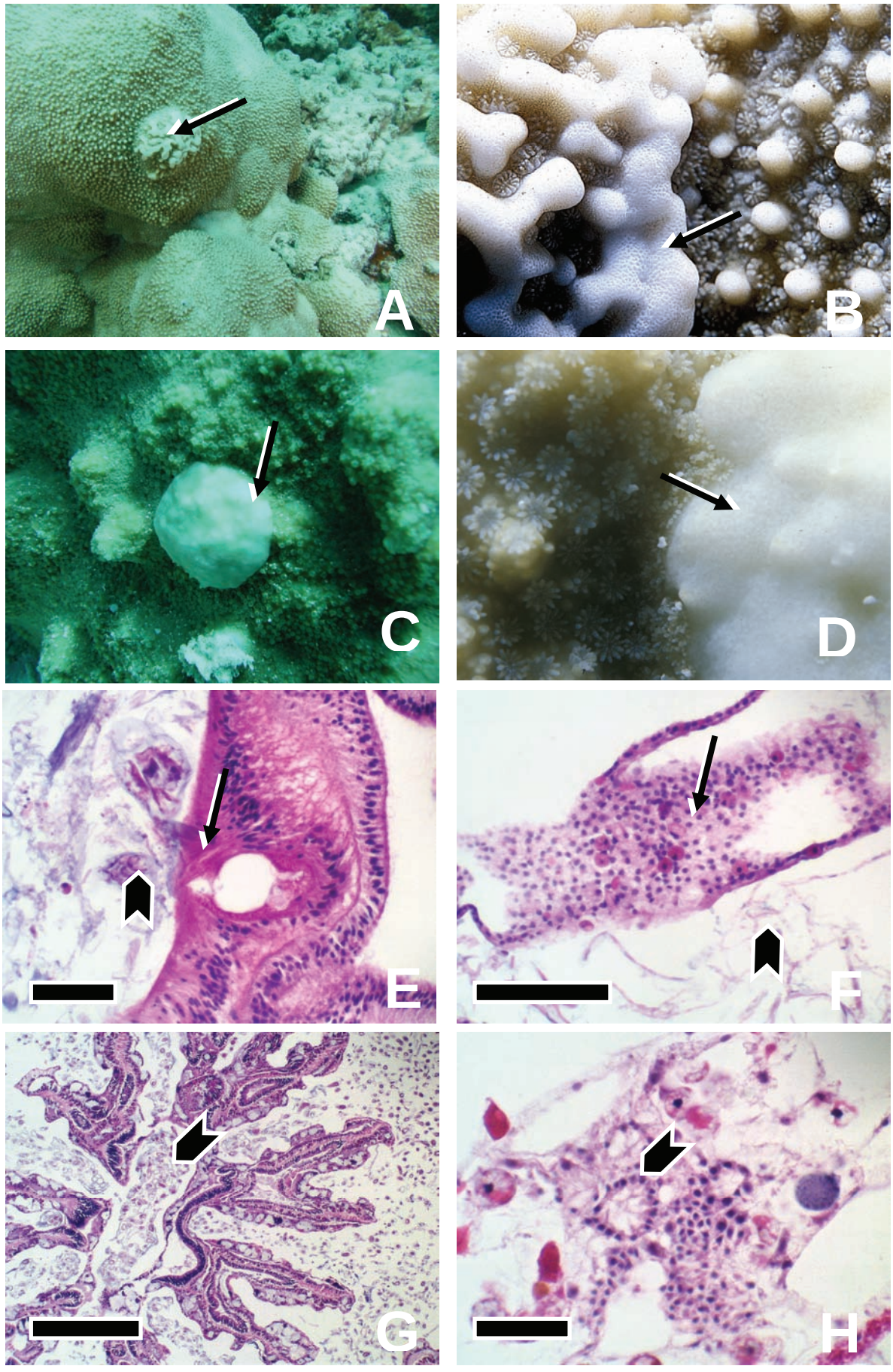
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Figure 5. ***P. lobata*** **(A-D). Note clump of alga surrounded by red capsule (arrow) and reactive calicoblast cells (arrowhead), bar=50 µm (A). Note alga (arrowhead) and reactive calicoblast (arrow), bar=100 µm (B). Clump of necrotic gastrodermal cells among algal filaments. Note bands of grey material (arrow), bar=100 µm (C). Close of of band of grey material (arrow) and algal filament (arrowhead), bar=100 µm (D). M. capitata (E-H). Note thickened gastrodermis in area of algal infiltration (arrowhead), bar=200 µm (E). Note sparse algal infiltrates in skeletal matrix (arrowhead) overlaid by thin squamous membrane (arrow), bar=200 µm (F). Note thickened gastrodermis in gastrovascular canal (arrowhead) and granular red inflammatory cells (arrow), bar=50 µm (G). Granular red cells (arrow) among thickened gastrodermal cells and algal filaments (arrowhead), bar= 50 µm (H).**

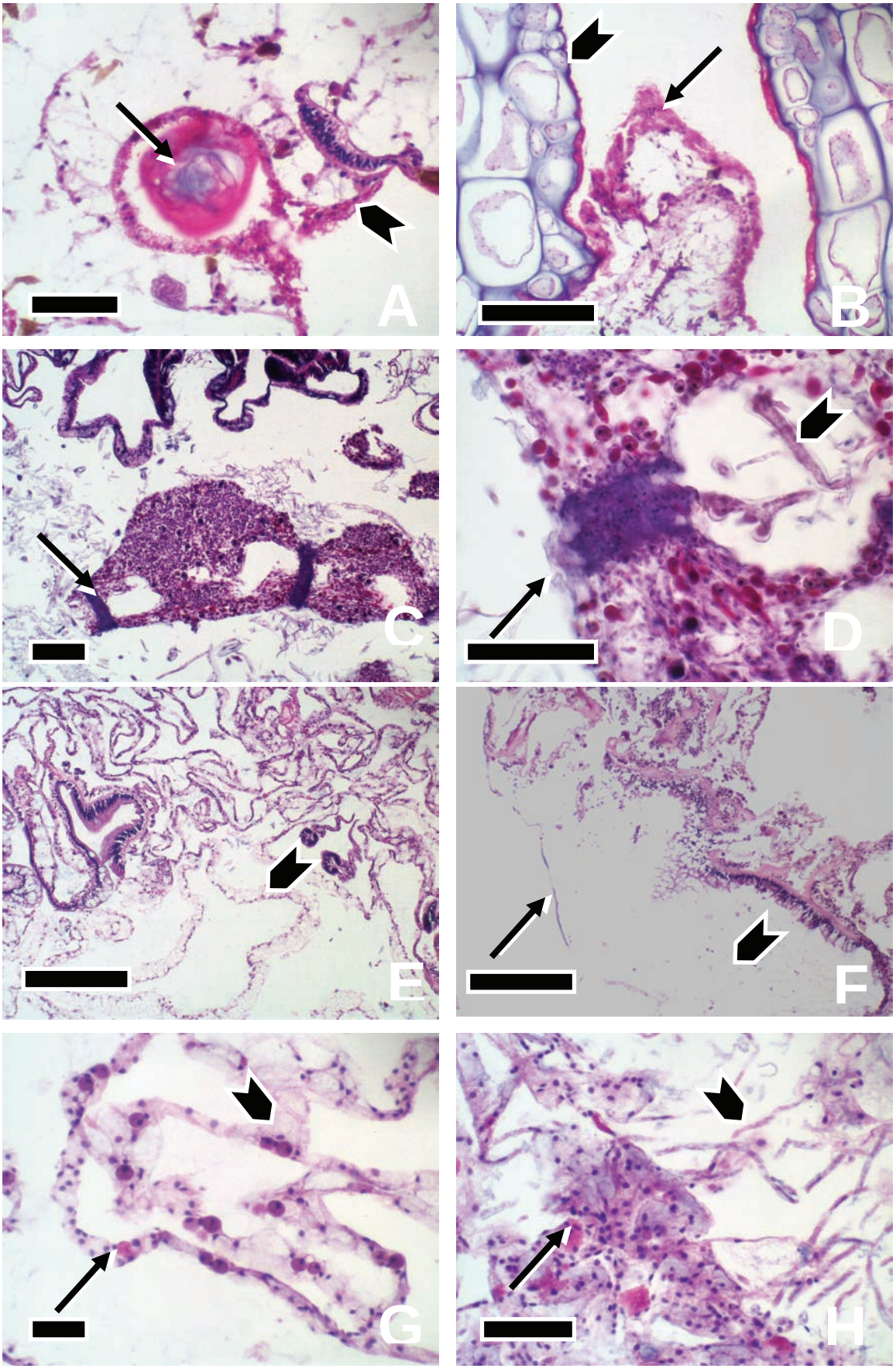
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Figure 6. ***P. lobata*** **(A-H). Coral with focal lesions pan view (A) and macro (B). Coenosarc epithelium. Note necrosis (arrow), bar=100 µm (C). Reactive calicoblast (arrowhead) and pigment cells (arrow), bar=10 µm (D). Clump of gastrodermal cells, loose zooxanthella and pigment cells (arrow), bar=20 µm (E). Coccidia containing single sporozoite (arrow) surrounded by reactive calicoblast cells, bar=20 µm (F). Similar coccidian as in F (arrow) surrounded by laminar capsule (arrowhead), bar=20 µm (G). Encapsulated coccidian minus sporozoites.**
Note algal filaments (double arrow), bar=20 µm (H).

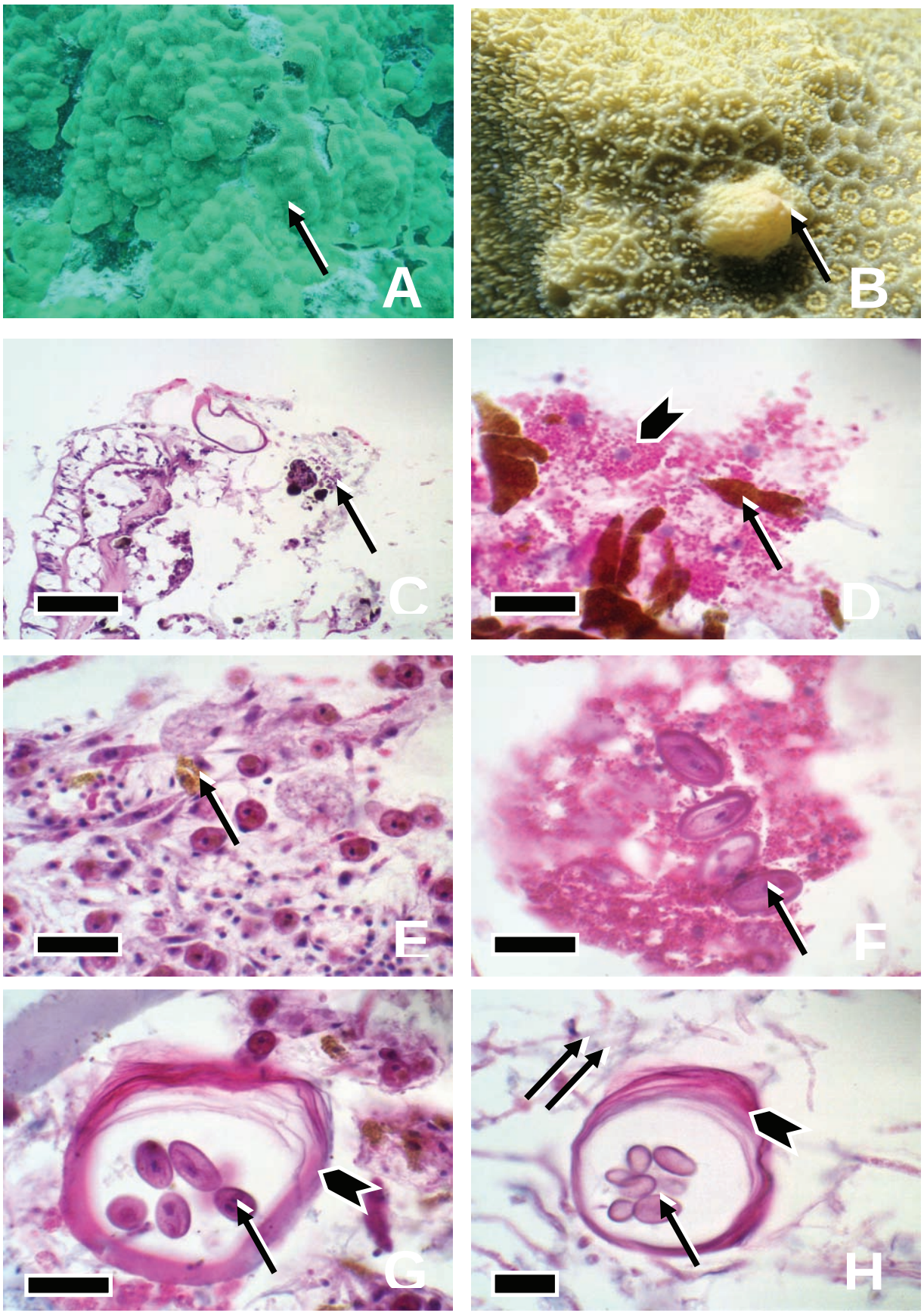
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Figure 7. ***A. cytherea*** **(A-D). Type 1 tumor (A) and vermiform tumor (B). Note proliferation of gastrodermis, bar=100 µm (C-D).** ***P.eydouxi*** **(E-H). Note holes with pigmented rim of tissue (arrow) (E). Note areas of bleaching (arrow) (F). Normal epithelium (arrow) and gastrodermis (arrowhead), bar=50 µm (G) and atrophied epithelium (arrow) and gastrodermis (arrowhead), bar=50 µm (H).**

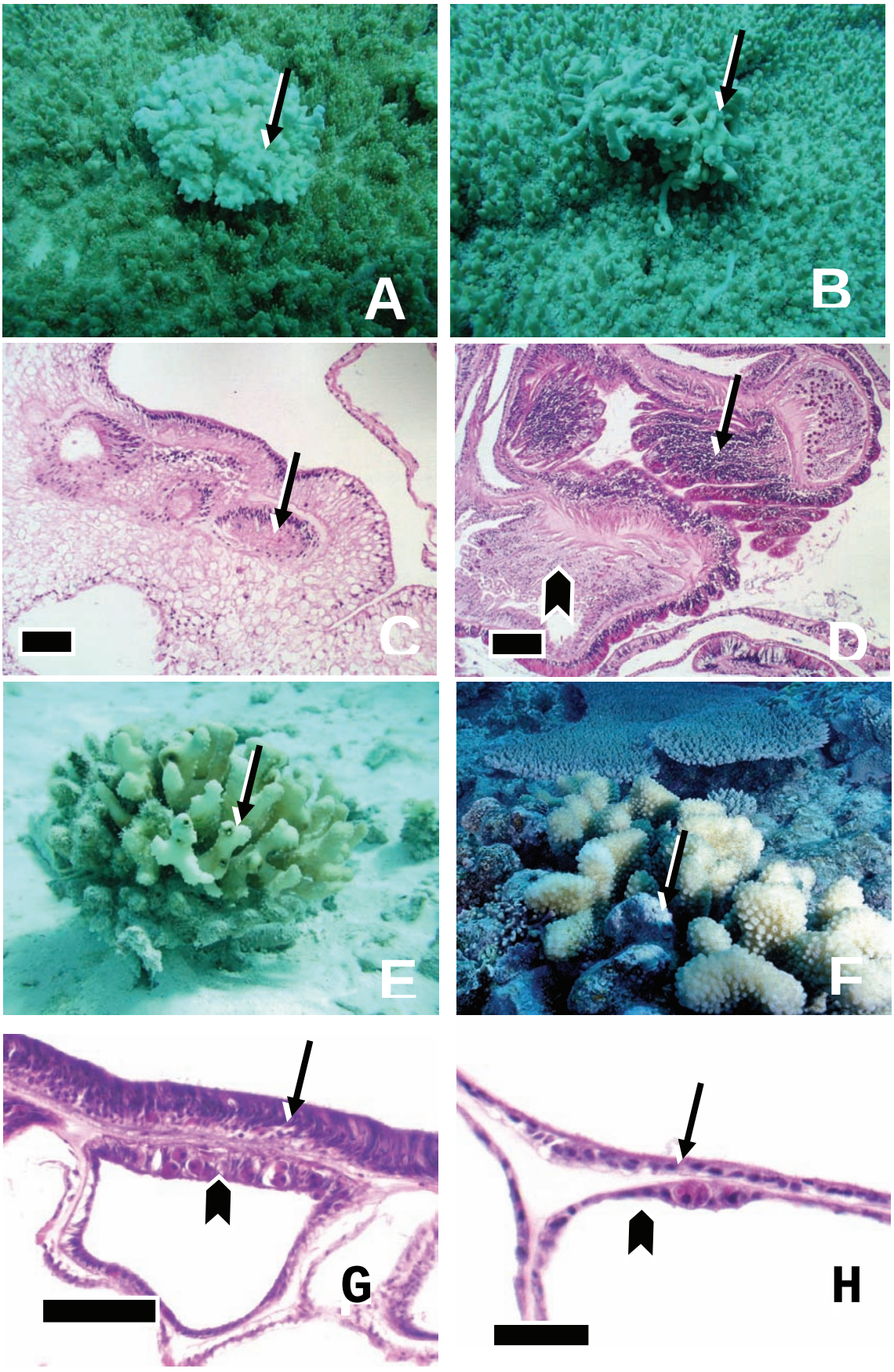
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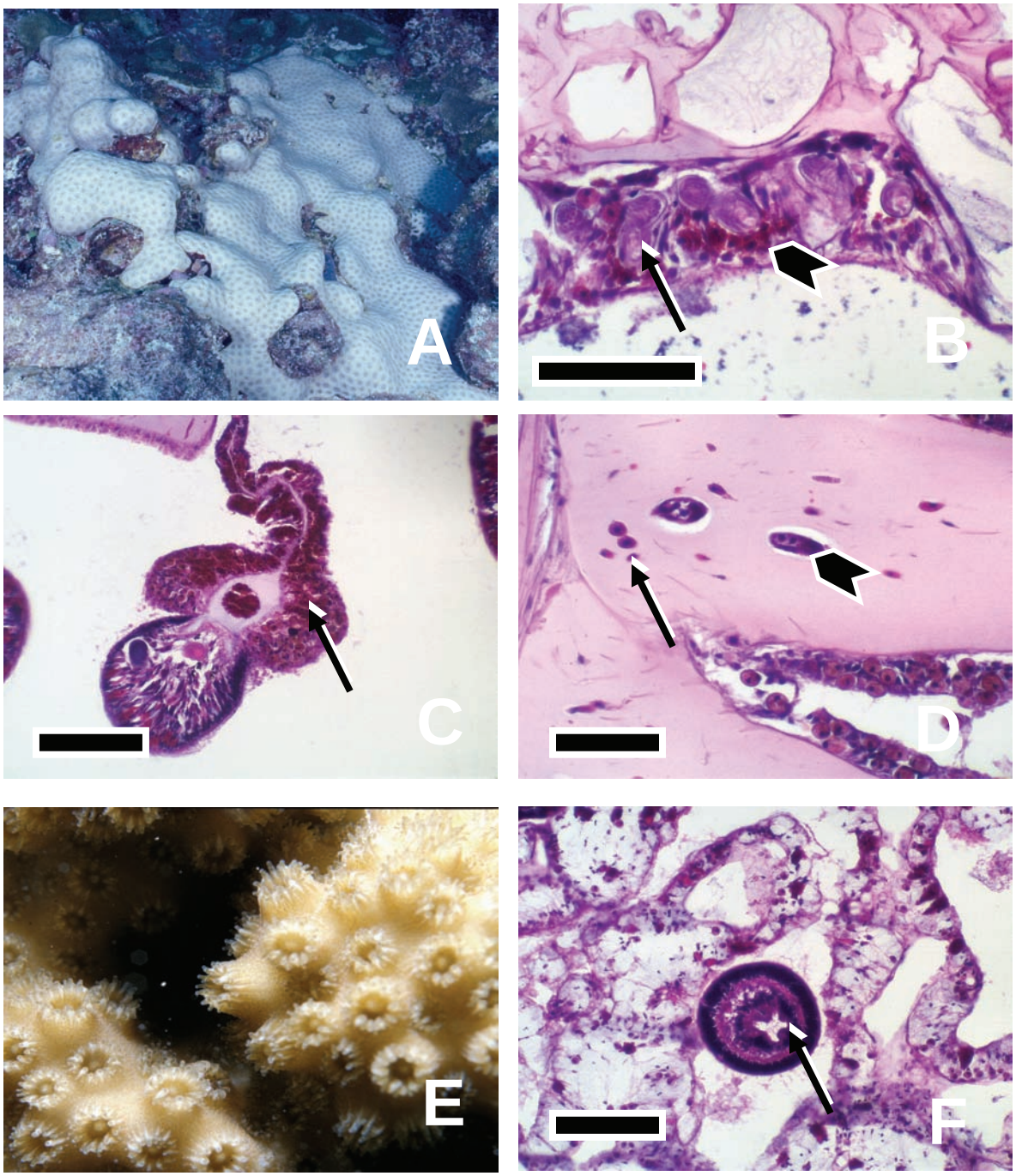
Figure 8. Blue-gray zooanthid (A-D). Epithelium (bottom) and mesoglea (top). Note holotrichous isorhizas (arrow) and red granular cells (arrowhead) in epithelium, bar=100 µm (B). Mesenteric filament. Note the base replete with zooxanthella and red granular cells (arrow), bar=100 µm (C). Mesoglea. Note gastrovascular canal (arrowhead) and mesogleal cells (arrow), bar=100 µm (D). Cyphastrea ocellina (E-F). Note planula (arrow) in gastrovascular canal, bar=100 µm (F).



CURRENT KNOWLEDGE OF DISEASES IN US TERRITORIES/FREELY ASSOC. STATES

Thierry Work

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PO Box 50167
Honolulu, HI 96850

Report 1: CORAL AND CRUSTOSE CORALLINE ALGAE DISEASE ON THE REEFS OF AMERICAN SAMOA
Greta Aeby, Thierry Work, Eva Didonato

Report 2: JOHNSTON ATOLL REEF HEALTH SURVEY
Thierry M. Work, Steve L. Coles, Robert A. Rameyer

Report 1. CORAL AND CRUSTOSE CORALLINE ALGAE DISEASE ON THE REEFS OF AMERICAN SAMOA

Greta Aeby, Hawaii Institute of Marine Biology
Thierry Work, USGS, National Wildlife Health Center, Hawaii Field Station
Eva Didonato, National Park Service, American Samoa

Submitted October, 2005

INTRODUCTION

The world's coral reefs are in serious decline (Wilkinson 2004). The effects of overfishing and pollution from agriculture and land development have been a major force accelerating decreases in abundance of coral reef species (Hughes et al. 2003, Pandolfi et al., 2003). With increased human populations the scale of human impacts on reefs has grown exponentially. Within American Samoa, alone, the population has risen 22% in the last ten years (Turgeon et al., 2002) and thus so has the potential for damage to the near shore resources. Compounding these anthropogenic stressors are the impacts of global climate change which is predicted to result in more frequent bleaching episodes and higher levels of disease (Hughes et al., 2003). As such, reef managers are faced with the challenge of developing strategies to maintain these reefs in the face of these changing conditions and it becomes clear that research in support of management is urgently needed.

The reefs of American Samoa support more than 200 species of corals and their conditions have been affected by both natural disturbances (crown-of-thorns starfish invasion, hurricanes and mass bleaching events) and human-induced impacts (pollution and over fishing) (Turgeon et al., 2002). The reefs of American Samoa suffered mass bleaching in 1994 (Birkeland et al., 2000) with reports of bleaching also occurring in both 2002 and 2003 (Peter Craig, pers. comm.). Coral disease has been reported from these reefs (Work & Rameyer 2002) but no surveys to quantify disease on a spatial scale had been conducted. An important component of monitoring the health of reefs is to have baseline 'before' data with which to compare 'after' conditions (Porter et al. 2001; Santavy et al. 2001). Unfortunately, investigations on the role of disease in animal populations are often done only after problems are noticed and out of control. However, American Samoa is in the unique position of not having suffered major catastrophic declines of reefs due to disease. Furthermore, significant effort is planned by American Samoa Department of Marine and Wildlife Resources to conduct monitoring programs on reefs throughout Manua and Tutuila. As such, American Samoa is well placed to develop a baseline assessment of the health of their reefs. The objectives of this study were to: 1) document the baseline levels of bleaching and disease in the major genera of corals and coralline algae; 2) compare incidence of disease in coral and coralline algae across a gradient of levels of reduced water quality based on watershed population; 3) systematically describe gross and microscopic morphology of lesions in reef corals and develop a standardized nomenclature for identifying and designating coral disease.

METHODS

The first task of the proposed research was to conduct a baseline assessment of the abundance and distribution of bleached and diseased corals and crustose coralline algae at seven sites in American Samoa (Vatia (National park), Tafeu Cove (National park), Fagaitua, Faga'alu, Fagatele Bay (National marine sanctuary), Leone and Maloata (see Table 1, GPS coordinates, and Fig. 1, map).

Figure 1. Map showing the seven sites surveyed for bleaching and disease in corals and crustose coralline algae in June, 2004.

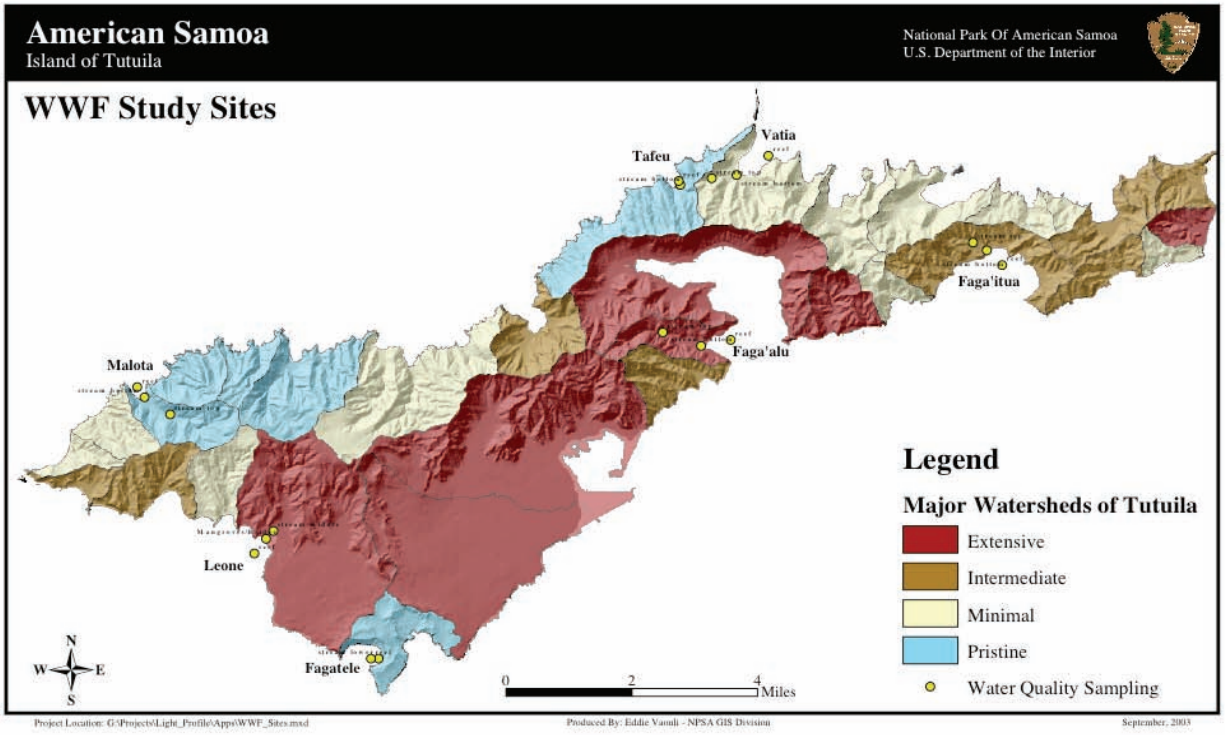


Table 1. Coordinates of sites surveyed for bleaching and disease of coral and coralline algae on Tutuila in June, 2004. Coordinates were taken by GPS unit onboard boat at start of transect 1

Site	Date	Depth (ft)	Latitude (S)	Longitude (W)	Notes
Fagaitua1	6/8/04	27	14 16.342'	170 36.728'	fringing reef off Fagaitua village- WWF site
Fagaitua 2	6/8/04	22	14 17.005'	170 36.393'	fringing reef off Alofau village
Fagatele Bay 1	6/9/04	18	14 21.944'	170 45.736'	Fagatele Bay National Marine Sanctuary-WWF site
Fagatele Bay 2	6/9/04	22	14 21.817'	170 45.708'	other side of the Bay from WWF
Tafeu 1	6/10/04	20	14 15.142'	170 41.338'	fringing reef- WWF site
Tafeu 2	6/10/04	21	14 15.158'	170 41.498'	other side of the embayment from WWF
Vatia 1	6/14/04	20	14 14.774'	170 40.076'	fringing reef- WWF site
Vatia 2	6/14/04	26	14 14.704'	170 40.262'	fringing reef other side of the embayment from WWF
Leone	6/15/04	32	14 20.592	170 47.328	fringing reef- WWF site
Maloata	6/15/04	25	n/a	n/a	fringing reef- WWF site
Faga'alu	6/16/04	19	n/a	n/a	fringing reef- WWF site

A project funded by the World Wildlife Federation (WWF) has been documenting water quality parameters at these sites, providing a gradient of sites with presumed anthropogenic impacts (Lara Hansen, personal communication). We documented baseline levels of coral bleaching and disease at each of the sites using two 25 m x 2 m belt transects with visual counts (total 100 m^2 area of reef). The two transect lines were laid end to end along depth contours (20-30 ft) separated by approximately 5 meters. A team of two divers swam along the transect, with one diver identifying and enumerating coral colonies, while the other diver recorded incidence of bleaching and disease. Corals were identified to the genus level and assigned to one of seven size classes (0-5cm, 6-10cm, 11-20cm, 21-40cm, 41-80cm, 81-160cm and > 160cm). These size classes and protocols were adapted from Mundy (1996) who used them for broad-scale surveys in American Samoa. They have also been used to examine coral community structure in the NWHI (Maragos et al., 2004). Diseased corals and coralline algae were photographed and a general description of the condition was recorded. Samples of diseased coral (and healthy portions for controls) were collected for laboratory investigations using standard histopathological techniques. Substrate characteristics were documented by line-intercept method whereby the substratum underlying the tape measure was recorded at 10 cm intervals. At some of the sites (Vatia, Tafeu, Fagatele, Faga'itua) a 2nd station was surveyed as described above. Surveys from both stations were combined at these sites for analysis. Surveys were conducted June, 8-16, 2004.

Another task of the proposed research was to systematically describe gross and microscopic morphology of lesions in corals and crustose coralline algae and develop a standardized nomenclature for identifying and designating disease. For characterization of gross lesions, corals with abnormalities were photographed with a digital camera (Olympus C5050) contained in an Ikelite® housing, and attached to an Ikelite® 50 substrobe. Lesions were photographed from two aspects. A pan photograph encompassed the larger colony to gauge the extent of the lesion. Close-up photos were done of the same lesion to gain detail on polyp morphology. The following data were recorded: date, location, and depth of collection.

Grossly, lesions were classified by distribution as focal, locally extensive, or diffuse. Lesions were further classified as tissue loss, discoloration, or growth anomaly.

Tissue loss included those cases where tissue was missing leaving exposed skeleton. In such cases, we recorded if the skeleton was intact or damaged (eroded) and characterized as acute, subacute, and chronic. Acute tissue loss was those cases where skeleton was bare (eroded or intact). Subacute tissue loss included cases of algal growth on skeleton separated from intact tissue by bare skeleton. Chronic tissue losses were those cases where skeleton was completely covered by algae or sediment. Discoloration included those lesions where tissues were abnormally colored and was further subdivided into bleaching and non-bleaching discoloration. Growth anomaly included those lesions exhibiting anomalous growth of coral skeleton.

For histopathology, sections of corals were fixed in Z-Fix (Anatech Ltd.) according to manufacturer instructions. Briefly, Z-fix was diluted 1:5 with seawater and placed in 100 cc plastic jars. Coral were placed in the fixative and allowed to fix for at least 24 h. Corals were decalcified in dilute formic acid/formaldehyde solution (CalExII, Fisher Scientific) until the skeleton was completely dissolved. Tissues were dehydrated in alcohol series, embedded in paraffin, sectioned at 5 µm, placed on microscope slides and stained with hematoxylin and eosin. Special stains were used as appropriate to identify fungi, bacteria, algal filaments, or protozoa. Tissues were examined using light microscopy at magnifications ranging from 40X-1000X.

On histology, lesions were classified as depletion of zooxanthella, atrophy, uncomplicated necrosis, necrosis associated with fungi, algae, protozoa, or metazoa, and hyperplasia of gastrovascular canals. Depletion of zooxanthella included cases where gastrodermis was depleted of zooxanthella. Uncomplicated necrosis included evidence of cytoplasmic fragmentation and hypereosinophilia, pyknosis, or karyorrhexis. Organisms were classified as algae if they were filamentous and stained negative with silver or if cell walls were present or fungi if they stained positive with silver (Prophet et al. 1992).

RESULTS

Surveys

Coral community structure

Based on colony counts within transects, the four dominant coral genera on the reefs of Tutuila were *Montipora* (39.9% of the total colonies), *Galaxea* (12.9%), *Pocillopora* (11.4%) and *Acropora* (10.1%). Coral community structure varied among the seven sites as did the coral cover (Table 2).

Table 2. Coral community structure at seven sites around Tutuila surveyed for bleaching and disease in June, 2004. Percent coral cover was estimated using line-intercept. Coral community was calculated from colony counts along belt transects. Data show the percentage of colony counts along the transect represented by each coral genera. Dominant coral genera shown in bold for each site.

	Fagatele	**Maloata**	**Tafeu**	**Vatia**	**Faga'itua**	**Leone**	**Faga'alu**
coral community (%)							
Acropora	8.7	4.6	3.1	**19.7**	10.7	15.3	10.6
Astreopora	0	0.57	0.77	0.24	0.39	0	0
Coscinaraea	0	0.57	2.3	0.72	0.65	0.4	0
Cyphastrea	0	0	0	0	0.13	0	0
Diploastrea	0	0	0	0	0	0	0
Echinophyllia/Oxypora	0.25	0	0	0	0.65	0	0
Echinopora	0.13	0	0	0	0.91	0	0
Favia/Favites	4.9	4.6	3.8	3.6	1.7	1.2	0.33
Fungiidae	1.6	0.57	0.38	2.2	0.78	0	0.66
Galaxea	**38.6**	9.7	1.2	1.9	10.5	0	0
Goniastrea	0.5	0	0	0.24	2.2	0	0
Goniopora/Alveopora	0.13	0	0	0.24	0	0.4	0
Hydnophora	0	0	0.19	0	0.52	0	0
Leptastrea	0.38	2.3	11.2	0.48	0.65	1.2	0.33
Leptoseris/Pachyseris/Coe	0.63	0	0	0	0	0	0
Lobophyllia	0.25	0	0	0.24	3.8	0	0
Merulina/Scapophyllia	0	0	0	0	0	0.8	0
Millepora	0	0	0	0.24	0	0	0
Montastrea	0	4.6	0.77	0.96	0.26	0.4	1.3
Montipora	20.8	**39.4**	**51.9**	41	**39.7**	**70.7**	**46.4**
Pavona	2.4	10.3	14	13.4	11.2	2.4	5.6
Pocillopora	7.8	21.7	4	9.4	12.9	3.6	33.4
Porites	12.9	1.1	6.3	5.5	2.5	3.6	0.66
Psammocora	0	0	0	0	0	0	0.66
avg. coral cover (%)	46.3	22.8	45.7	48.2	28.4	40.9	32.6

Overall occurrence of bleaching and disease

Within belt transects an estimated 16,824 coral colonies from 24 different genera were examined for bleaching and disease. Bleaching was found at very low levels affecting less than one percent of the overall colonies. Six different coral disease states as well as a number of lesions not associated with disease were documented from four coral genera on the reefs of Tutuila (Appendix I). Three diseases affected *Acropora*: *Acropora* white syndrome, *Acropora* ciliate disease, and *Acropora* growth anomalies. *Montipora* was affected by one disease: *Montipora* growth anomalies. *Porites* was affected by one disease: *Porites* tissue loss syndrome. *Lobophyllia* was affected by one disease: *Lobophyllia* tissue loss syndrome. Coral disease was found at all seven sites but the overall proportion of colonies examined that had signs of disease (prevalence) was low (0.143%) (range = 0.029-0.40%). The crustose coralline algae (CCA) disease,

coralline lethal orange disease (CLOD) was found at 4 of the 7 sites (57% of the sites). The number of CLOD infections per m^2 of CCA ranged from 0 to 0.24 (Table 3).

Distribution and prevalence of each disease state

Distribution of the different coral diseases varied (Table 3). *Acropora* white syndrome was found to be the most widespread disease occurring at 5 of the 7 sites (71.4%). *Acropora* growth anomalies occurred at 4 of the 7 sites (57.1%) with the other diseases only occurring at one site each (14.3%). The proportion of coral colonies affected by each disease (prevalence) also varied with both *Acropora* white syndrome and growth anomalies being the highest (0.624% each). Prevalence of the other diseases was lower; Acropora ciliate disease (0.07%), *Porites* tissue loss syndrome (0.095%), and *Montipora* growth anomalies (0.031%). Prevalence of *Lobophyllia* tissue loss syndrome was not calculated as the diseased colony was not found within the transect area.

Prevalence of disease differed among the affected genera with *Acropora* having the highest overall prevalence (1.2%) compared to the other genera; *Montipora* (0.031%), *Porites* (0.095%).

Relationship between disease and watershed usage

The seven sites were originally selected based on a population gradient within each watershed used as an indicator of anthropogenic stress. The number of different diseases present within each site varied as did the overall prevalence of disease (Table 3). However, no patterns emerged suggesting that disease levels were directly related to anthropogenic watershed stress.

Table 3. Distribution and prevalence of different coral diseases and density of coralline algae diseases around Tutuila in June, 2004. Coral disease prevalence=(# diseased colonies/# colonies examined)*100
Coralline lethal orange disease (CLOD) density =# CLOD infections/est. m^2 of CCA at site
X* Disease present at site but prevalence not calculated as affected colony was not within belt transect
Human usage rating based on watershed populations.

human usage	pristine	pristine	pristine	minimal	intermediate	extensive	extensive
site	**Fagatele**	**Maloata**	**Tafeu**	**Vatia**	**Faga'itua**	**Leone**	**Faga'alu**
Acropora white syndrome	0.24		2.08	1.08	0.54		0.52
Acropora ciliate disease				0.27			
Acropora growth anomalies	X*	4.2		1.6		5.3	
Montipora growth anomalies		0.48					
Porites tissue loss syndrome	0.16						
Lobophyllia tissue loss syndrome					X*		
# CLOD/m^2 CCA	0.24	0.02	0	0.009	0	0.004	0
overall coral disease prevalence	0.031	0.286	0.032	0.266	0.029	0.402	0.055

Histology (gross and microscopic findings)

We examined tissue specimens from 8 sites comprising 67 samples from 59 colonies comprising at least 20 species of corals (Table 4). Of these, 10 were from apparently normal coral and the remainder from lesions. The most common gross lesions included tissue loss and growth anomalies, and for these, *Acropora* sp. and *Montipora* sp. were over-represented (Table 5).

Table 4. Number of coral samples collected for histopathology at various sites around Tutuila, June 2004.

Species	Faga'itua2	Faga'alu	Fagatele	Faga'itua1	Leone	Malato	Tafeu1	Vatia1	Total
Acropora abrotenoides				1				2	3
A. cytherea			1					7	8
Acropora sp.					1				1
plate *Acropora*		1					2	7	10
corymbose *Acropora*		1			3			1	5
encrusting *Acropora* sp.	2		1			1			4
Astreopora sp.							1		1
Favia sp.			1	1			2		4
Galaxea sp.			2						2
Goniastrea sp.	1								1
Leptoria phrygia			1						1
Lobophyllia corymbosa			2						2
Lobophyllia sp.	2		2						4
massive *Porites* sp.	1						2		3
Porites rus			2						2
Montastrea sp.							1		1
Montipora sp.	1	1	2	3	1	1	1	1	11
Pocillopora eydouxi				2					2
Pocillopora meandrina								1	1
Pavona sp.				1					1
Total	**7**	**3**	**14**	**8**	**5**	**2**	**9**	**19**	**67**

Table 5. Gross lesions found in various genera of corals in Tutuila, June 2004.

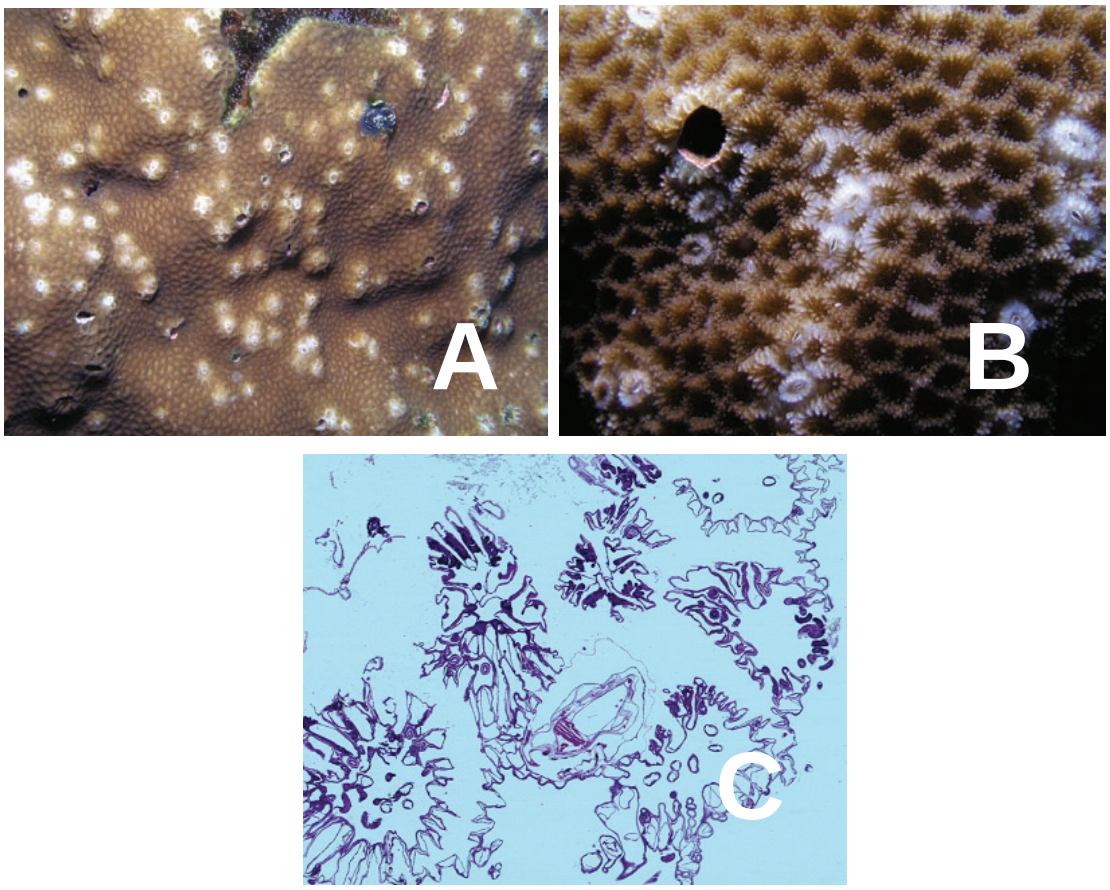
Genus	Discoloration	Growth anomaly	Tissue Loss	Normal	Total
Acropora	1	11	14	5	31
Astreopora	1				1
Favia	4				4
Galaxea	1			1	2
Goniastrea			1		1
Leptoria	1				1
Lobophyllia	1		2	3	6
Montastrea	1				1
Montipora		3	8		11
Pavona			1		1
Pocillopora	1		1	1	3
Porites	4		1		5
Total	15	14	28	10	67

Tissue loss:

The following patterns were seen for tissue loss:

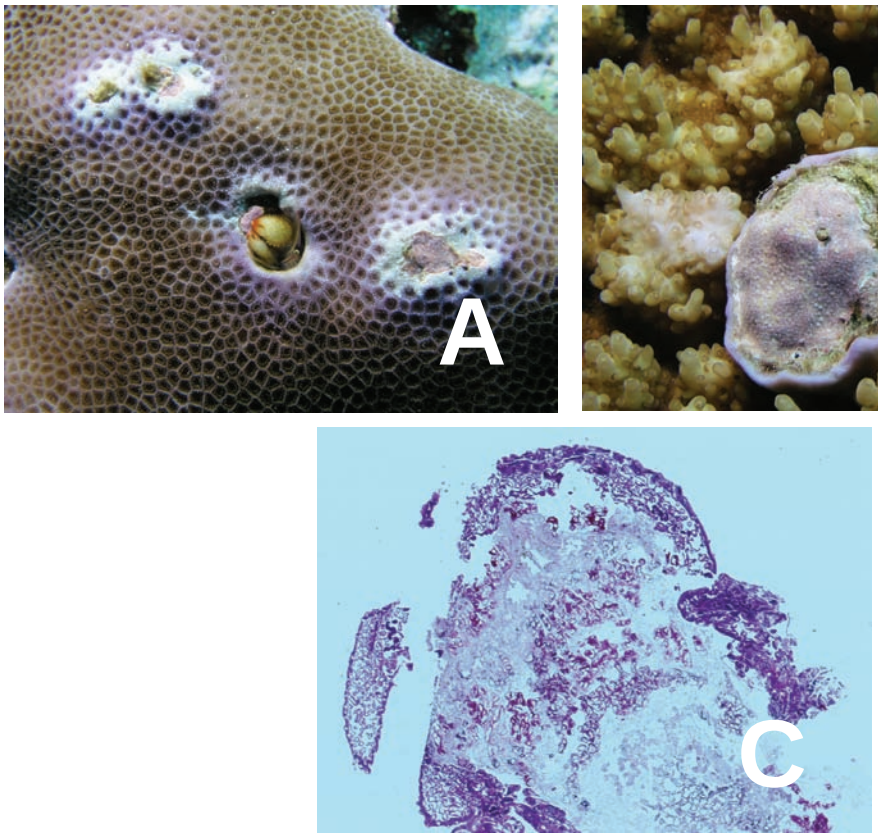
Barnacle infestation: This was seen in *Goniastrea* only and was characterized by small white foci encompassing approximately the diameter of one polyp (Fig. 2A). The center of the area contained a barnacle with bleaching of tissues immediately around the barnacle (Fig. 2B). Microscopy revealed a crustacean surrounded by normal coral tissue (Fig. 2C). In some cases, a mix of algae and sponges infiltrated into the coral tissue leading to cell fragmentation and necrosis.

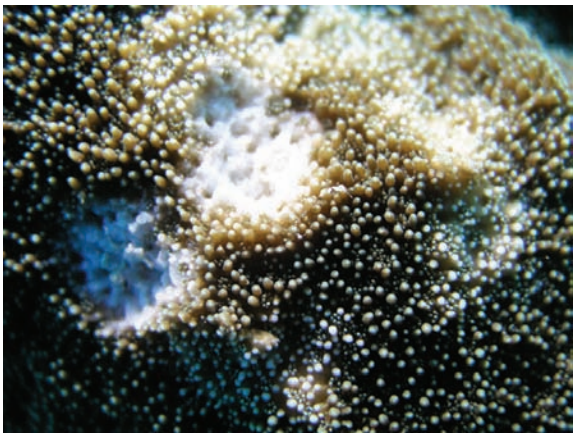
Figure 2. A) *Goniastrea* sp. with barnacles. B) Note barnacles (arrowhead) surrounded by bleached tissue. C) Photomicrograph of barnacle (arrowhead) among coral tissue.



Chronic tissue loss and excessive skeletal growth: This was seen in massive *Porites* sp. and *Acropora cytherea* and was characterized by a focus of coralline or turf algae surrounded by exuberant skeletal growth. On microscopy, these lesions manifested as full thickness necrosis of coral tissue associated with marine algae with some depletion of zooxanthella (Fig. 3).

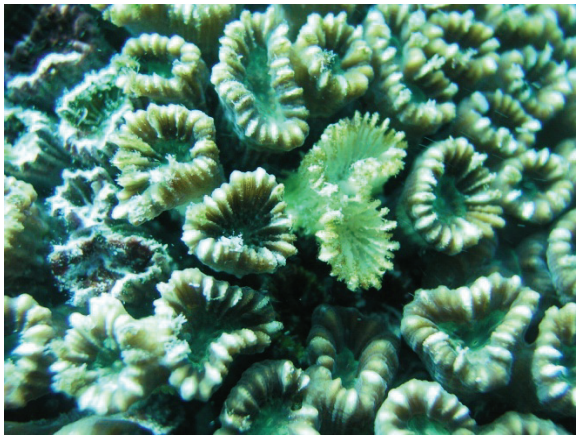
Fig. 3. A) Massive *Porites*. B) *Acropora cytherea*. A-B) note central area of coralline algae infiltration with exuberant skeletal growth surrounding lesion. C) Photomicrograph of E. Note, mass of algae and sponges and clumps of necrotic coral tissue.





Fish bites: This was manifested by well-circumscribed localized loss of tissue accompanied by bare eroded skeleton (Fig. 4). On histology, these lesions were characterized by fragmentation of coral tissue with depletion of zooxanthella from gastrodermis.

Figure 4. *Montipora* sp. fish bite. Note characteristic erosion of skeleton and loss of tissue.



Subacute tissue loss: This was manifested by complete tissue loss of single polyps revealing bare intact skeleton completely covered with algae. Only *Lobophyllia* sp. was sampled with this lesion (Figure 5).

Figure 5. *Lobophyllia* tissue loss

Acute tissue loss: This was manifested by a well-circumscribed diffuse area of tissue loss revealing intact white skeleton. The tissue loss usually encompassed the edge of the colony. On histology, the major changes seen included no lesions, tissue fragmentation, or hypertrophy of calicoblastic epithelium. In some cases, this tissue loss was associated with presence of corallivorous snails. Tissue loss in *P. rus* was attributed, on microscopy, to infestation with sponges associated with necrosis. (Fig. 6).

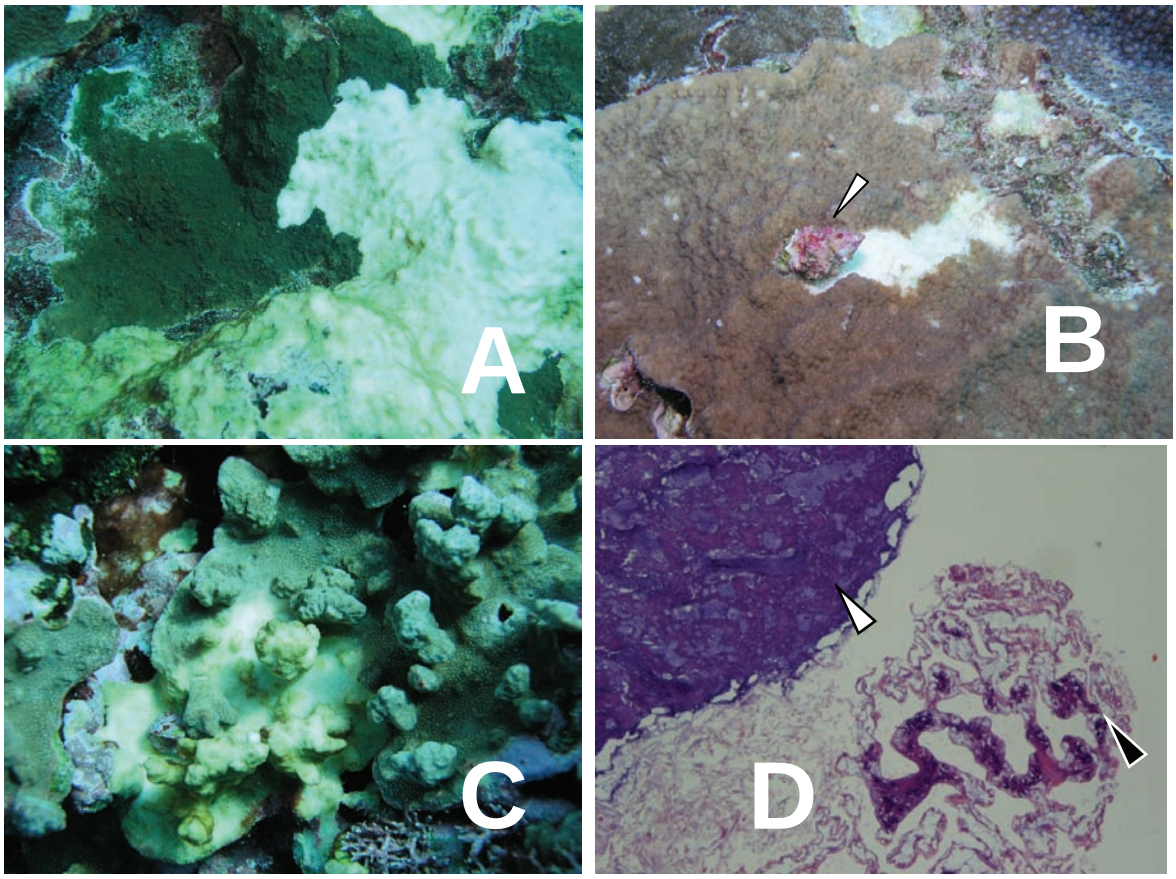


Figure 6. A-B) Acute tissue loss in encrusting *Montipora* sp., note bare white skeleton bereft of tissue and lesion predominantly on the edge of the colony. B) Snail (arrowhead) associated with acute tissue loss in *Montipora* sp. C) *P. rus* acute tissue loss. D) Photomicrograph of D. Note invasion of coral tissue with sponge (white arrowhead) and necrosis of tissue (black arrowhead).

Acute to subacute tissue loss: This was manifested by well circumscribed diffuse areas of tissue loss revealing intact skeleton with presence of recent algal growth in the center and bare intact white skeleton at the interface between tissue and algae. This was found mainly in plating *Acropora*. On microscopy, findings ranged from simple uncomplicated tissue loss to infection with microparasites (ciliates) (Fig. 7).

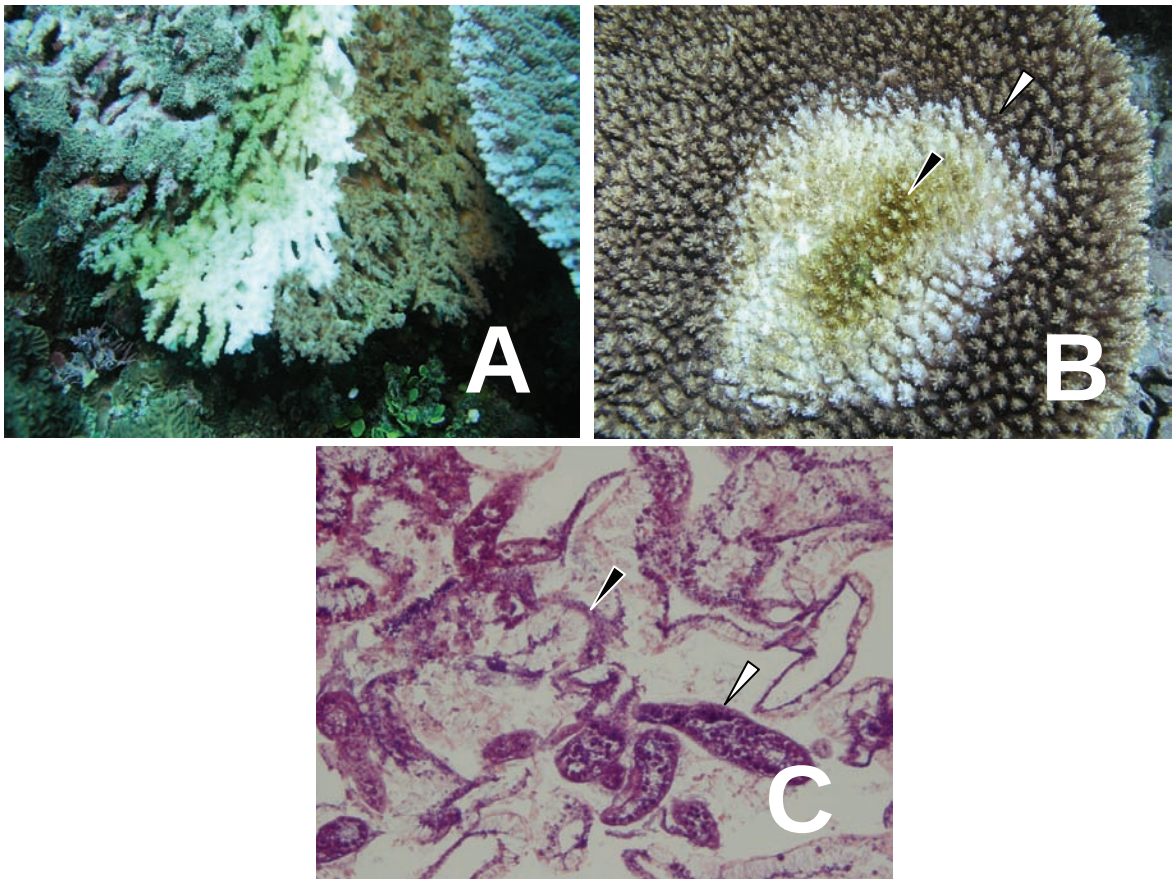


Figure 7. A-B) Plating *Acropora* sp. with subacute tissue loss. A) note distinct band of white skeleton bereft of algae separting intact tissue and skeleton covered by algae. B) Note presence of algae covered intact skeleton (black arrowhead) separated from intact tissue by intact skeleton bereft of algae (white arrowhead). C) Photomicrograph of B, *Acropora* with ciliate infection. Note invasion of ciliates (white arrowhead) associated with necrotic tissue (black arrowhead).

Discoloration:

Bleaching: We saw 10 cases of bleaching in 7 genera of corals. Grossly, bleaching was characterized by a diffuse white discoloration. Bleaching in corals is typically attributed to loss of symbiotic zooxanthellae from coral tissues, and on microscopic examination, this was seen in all cases. In 3 cases, additional microscopic lesions were seen including necrosis of tissue associated with infiltrates of sponges or algae. In those cases, the sponge/algae complex was seen invading tissue from below and overlying epidermis and gastrodermis were intact but atrophied and bereft of zooxanthella (Fig 8).

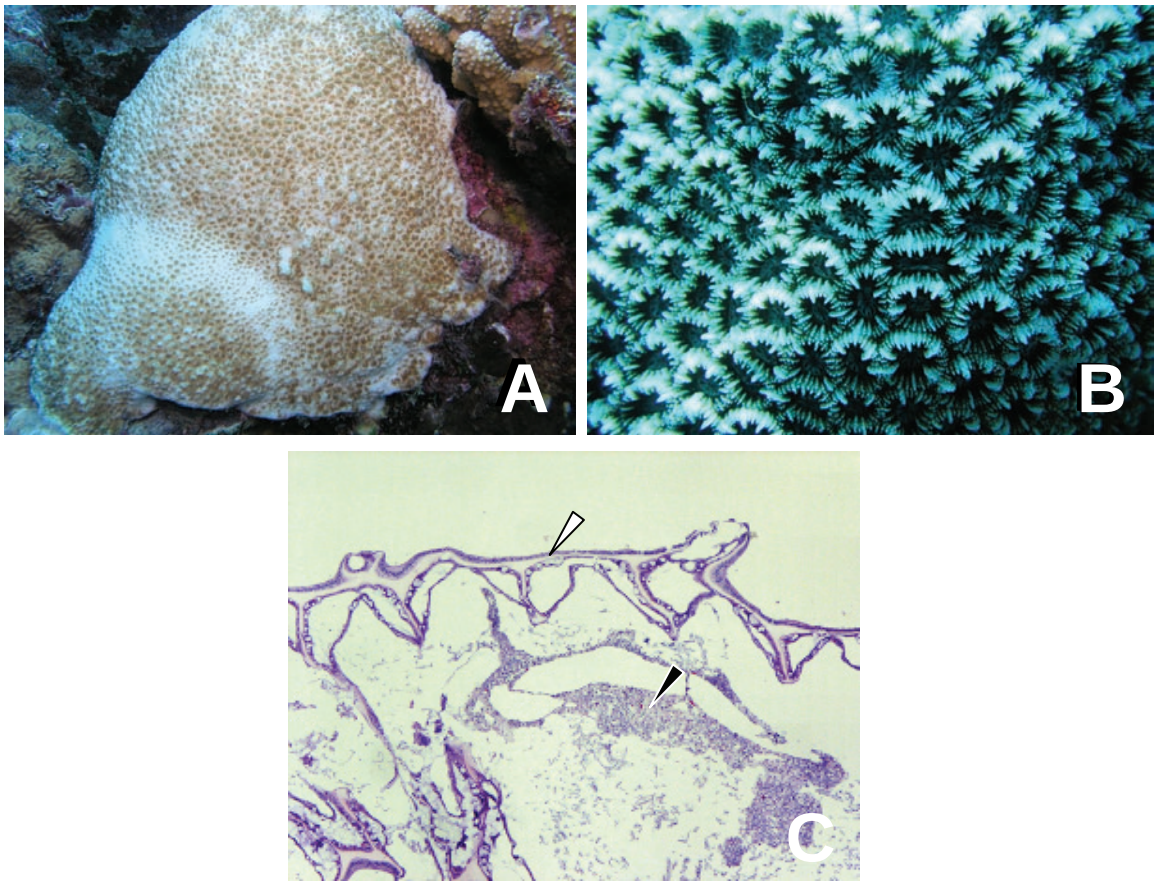


Figure 8. A) *Astreopora* sp. with diffuse bleaching. B) *Favia* sp. with diffuse bleaching. C) Photomicrograph of B. Note infiltration of sponges and algae (black arrow) below the intact epidermis (white arrowhead) with gastrodermis bereft of zooxanthella.

Non-bleaching discoloration: Other cases of discoloration did not fit the bleaching pattern. Discoloration in *Favia/Favites* was, on microscopy, attributed to mucus sheathing and not considered abnormal. No microscopic lesions associated with discoloration were seen in *Lobophyllia* (Fig. 9).

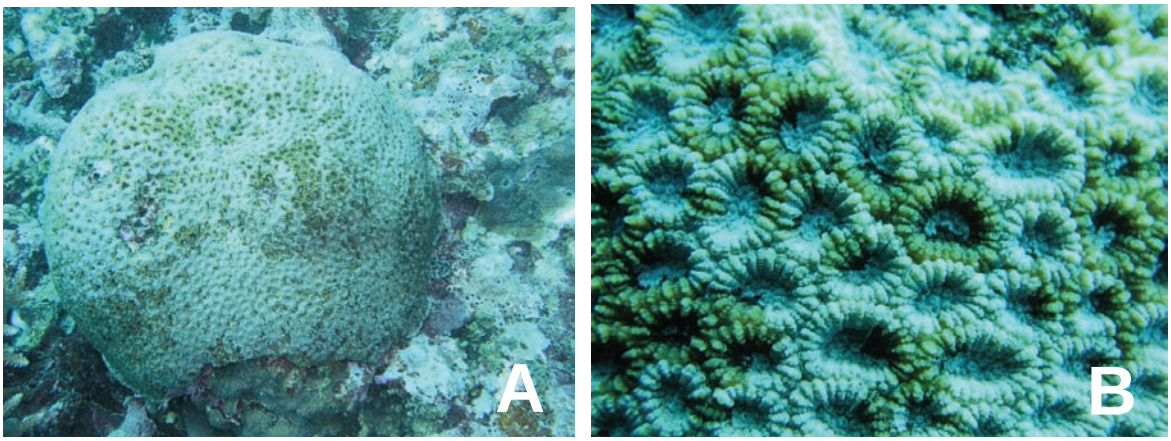
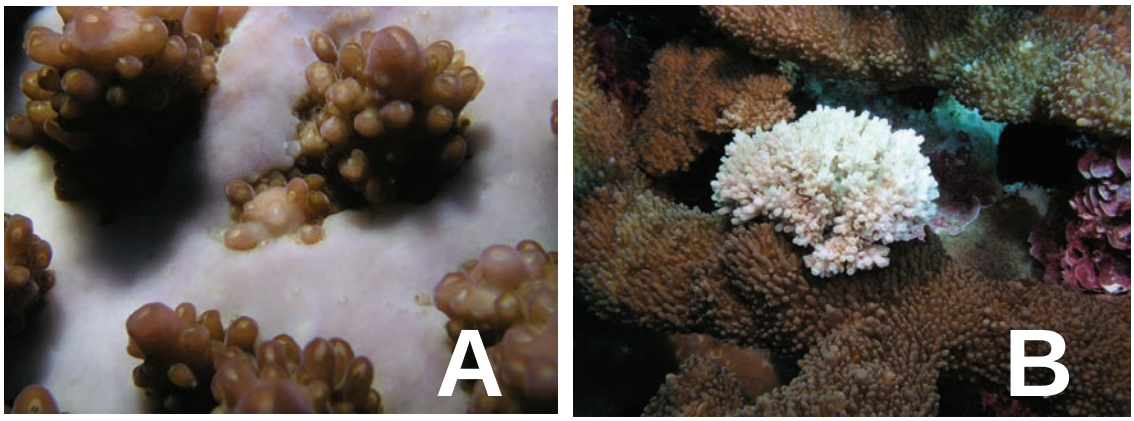


Figure 9. A-B) Mucus sheathing in *Favia* sp., note well-defined areas of discoloration.

Growth Anomaly

Acropora spp. were the most common genera see with this lesion although *Montipora* spp., also had growth anomalies. These ranged from smooth to more rugose and cauliflower shapes and was found in branching, corymbose, and plating *Acropora (*Figure 10*)*. On microscopy, the most common finding was hyperplasia of gastrovascular canals; however, there were not the classic hallmarks of cancer as seen in other animals making it difficult to conclude that these are true neoplasia *sensu stricto.* In two cases, growth anomalies were, on microscopy, associated with necrosis of tissues associated with marine algae.



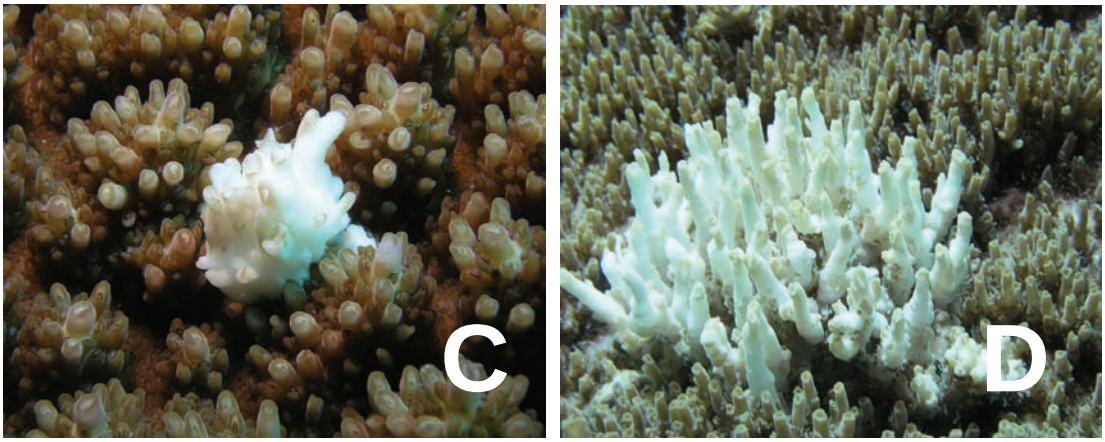


Figure 10. Growth anomalies in Acropora species. A) Corymbose colonies; B) Branching colonies; C-D) plating colonies.

Summary of Survey Findings

1. The overall prevalence of coral disease was found to be low (0.143%) as compared to other regions in the Indo-Pacific. Overall disease prevalence in the Northwestern Hawaiian Islands was found to be 0.5% (Aeby, in press). Willis et al. (2004) surveyed eight sites along the Great Barrier Reef (GBR) and found the prevalence of disease in hard corals to range from 7.2-10.7%. Raymundo et al. (in press) surveyed eight sites in the Philippines and reported an overall prevalence of disease of 14.2%.
2. Six coral disease states and one disease of CCA were documented from the reefs of Tutuila. Bleaching was found at low levels.
3. Five of the six coral disease states have been reported from other areas of the Indo-Pacific. *Porites* tissue loss syndrome is reported from the NWHI (Aeby, in press) Australia (Willis et al., 2004) and the Philippines (Raymundo et al., in press). *Acropora* white syndrome is reported from the NWHI (Aeby, in press) and Australia (Willis et al., 2004). Growth anomalies in both *Acropora* and *Montipora* have been recorded from Australia (Willis et al., 2004), Johnston Atoll (Work et al., 2001) and Okinawa (Yamashiro et al., 2000, 2001; Yamashiro 2004). *Lobophyllia* tissue loss syndrome has not yet been reported elsewhere. It must be noted that there are regional differences in names assigned each set of field disease signs but through the efforts of the Coral Disease and Health Consortium (www.coral.noaa.gov/coral_disease/cdhc.shtml) this nomenclature problem will eventually be resolved. It should also be noted that any similarities in field signs of disease between regions does not necessarily imply the diseases have the same etiology.
4. After histopathological analysis it was found that a number of coral lesions found during surveys were not associated with infectious agents or underlying pathologic process. These include lesions due to predation, barnacle infestation and mucous sheathing. This confirms the critical component histology plays in understanding disease processes.

5. There were differences in prevalence of disease among coral genera with *Acropora* having the highest prevalence. *Acropora* comprised only 10.1% of the overall coral community along the transects yet showed the highest overall prevalence of disease (1.2%). In contrast, *Montipora* comprised 40% of the coral community but had a disease prevalence of 0.031%. This suggests that there may differences in disease susceptibility among coral genera and that pathogens do not necessarily affect the most common or abundant corals. *Acropora* have also been found to have the highest levels of disease in the NWHI (Aeby, in press). The sites surveyed had differences in coral community which would be a factor in what diseases and what levels of disease would be found at a particular reef.
6. No pattern emerged suggesting that disease levels were directly related to anthropogenic watershed stress.

Summary of Histological Findings

1. Some cases of acute tissue loss are probably due to snail predation. We opted to be conservative and to include only those cases where snails were visible, however, this is probably an underestimate. Lesions presumably attributable to snails were generally peripheral on encrusting colonies and this pattern was more prevalent in certain sites versus others. Quantifying populations of corallivores in conjunction with measurement of lesions would be helpful in evaluating their effects on reefs.
2. Certain gross lesions have clear causes. For example, patchy tissue loss and discoloration with presence of a crustacean in the center of the lesion is indicative of barnacle infestations. Likewise, focal erosion of tissues and skeleton are indicative of fish bites. Determining the species of barnacles affected and the types of bites produced by particular fish may be useful in the future.
3. Not all discolorations are disease processes. In the case of faviids, mottled discoloration is probably indicative of mucus shedding.
4. Growth anomalies are more common in acroporids. Determining how fast these grow and what effect they have on the health of the corals should be the focus of future investigations.
5. There is a need to refine the description of gross lesions in corals in order to better understand whether certain gross lesions can be related to microscopic findings in a more consistent manner.

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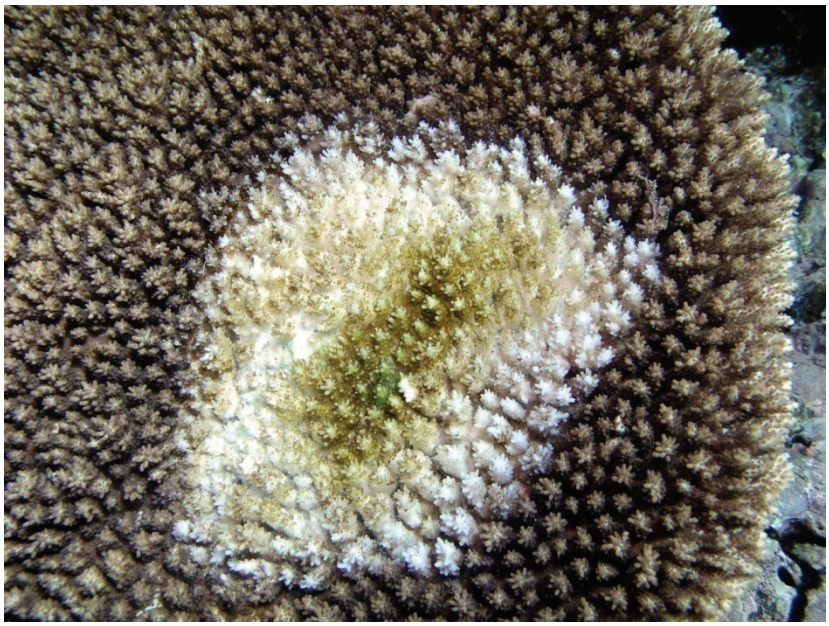
Appendix I. Summary of coral lesions found in American Samoa in June 2004.

A. CORAL DISEASES

Acropora ciliate disease

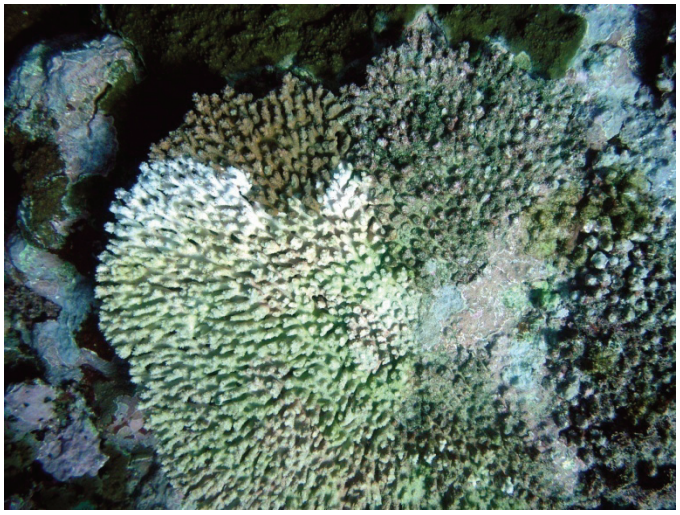


Histology: ciliates associated with necrotic tissue

Location: Vatia

Frequency of occurrence (# sites w/ disease/tot sites surveyed): 9.1%

Acropora white syndrome

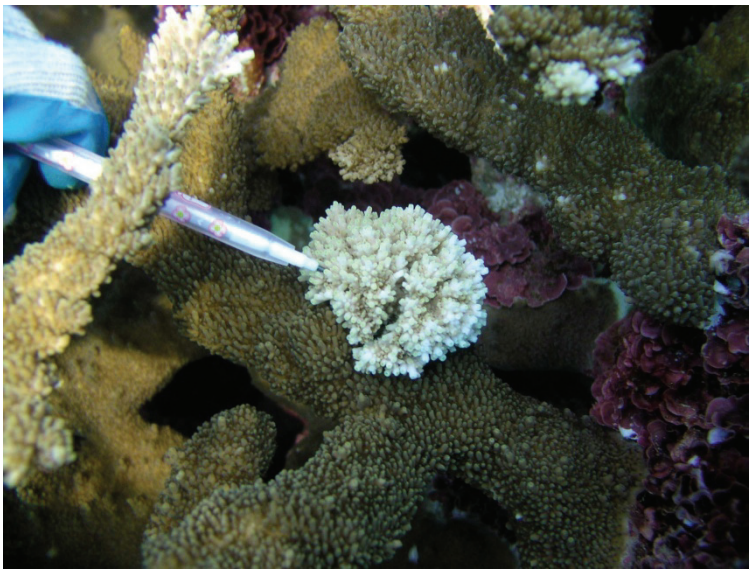


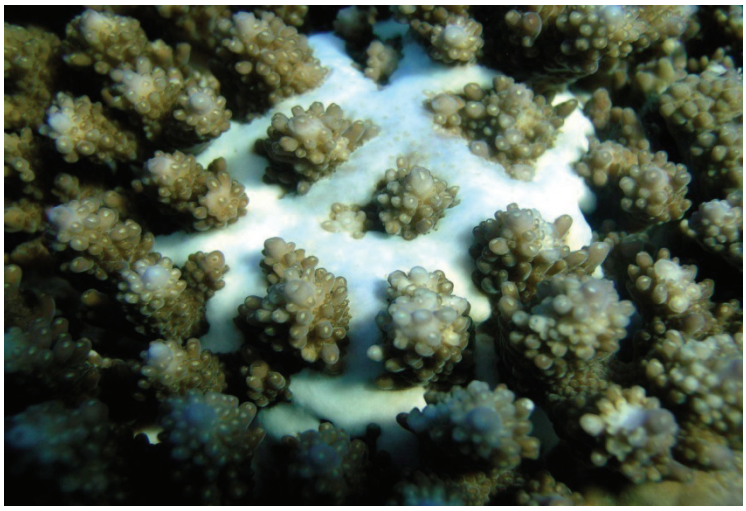
Histology: subacute tissue loss and necrotic tissue consistent with disease

Location: Fagatele, Tafeu, Vatia, Faga'itua, Faga'alu

Frequency of occurrence: 45.5%

Acropora Growth Anomalies





Histology: hyperplasia (overgrowth) of gastrovascular canals.

Location: Fagatele, Maloata, Vatia, Leone

Frequency of occurrence: 27.3%

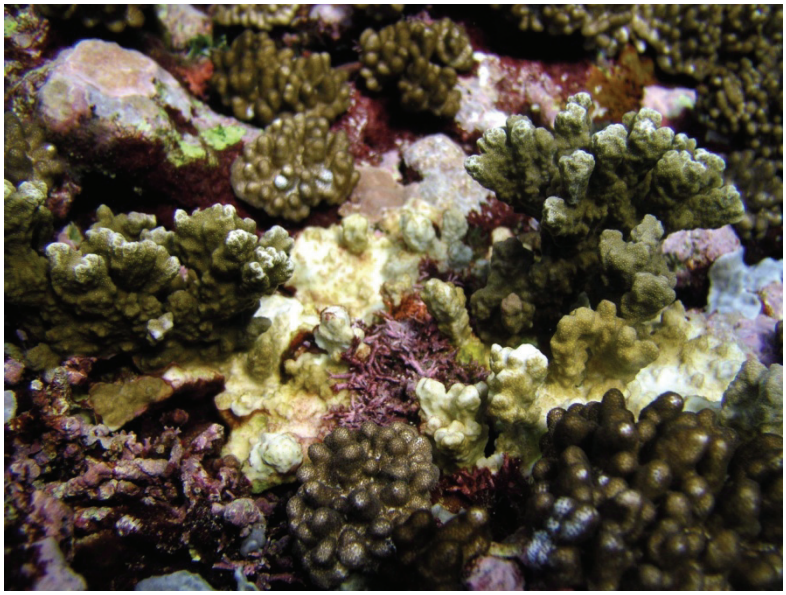
Montipora growth anomalies

Histology: hyperplasia (overgrowth) of gastrovascular canals.

Location: Maloata

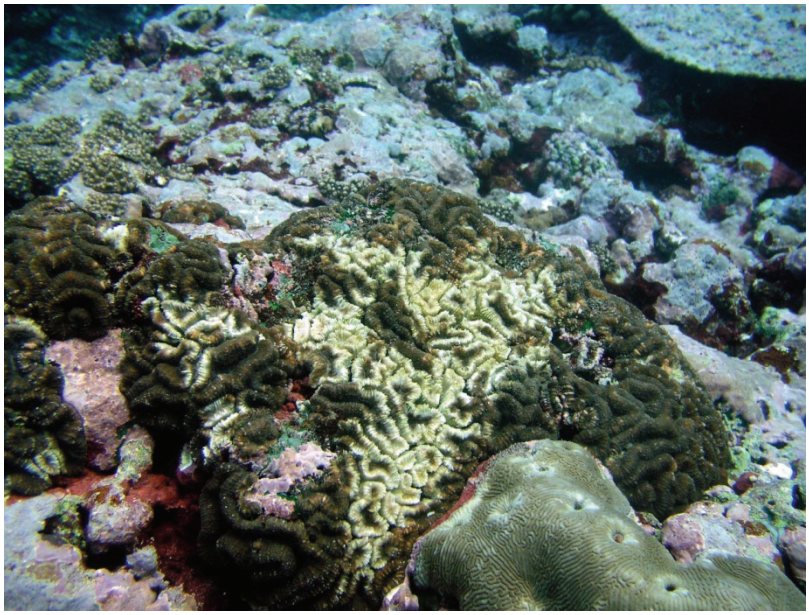
Frequency of occurrence: 9.1%

Porites tissue loss syndrome



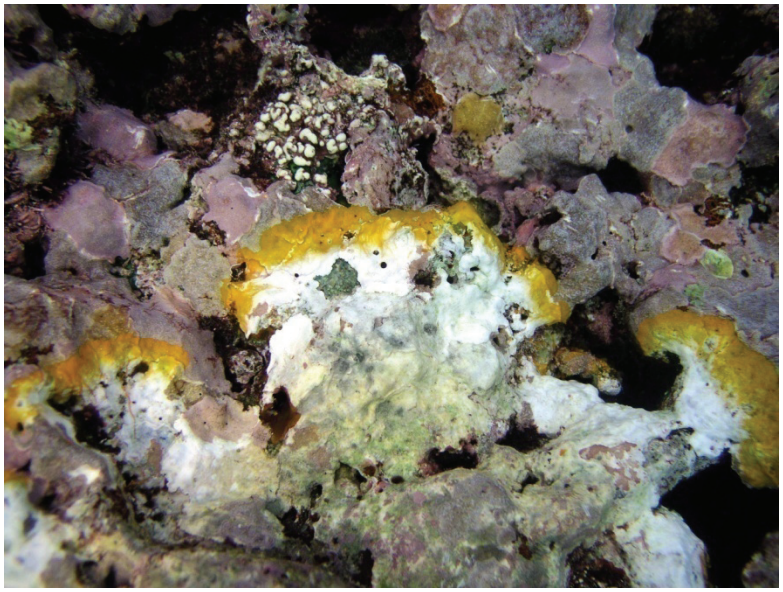
Histology: tissue necrosis and sponge invasion
Location: Fagatele
Frequency of occurrence: 9.1%

Lobophyllia tissue loss syndrome



Histology: chronic tissue loss
Location: Fagaitua
Frequency of occurrence: 9.1%

Coralline lethal orange disease (CLOD)



Location: Fagatele Bay, Maloata, Vatia, Leone

Frequency of occurrence: 45.5%

Report 2. JOHNSTON ATOLL REEF HEALTH SURVEY

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INTRODUCTION

Johnston Atoll is an overlay U. S. Fish and Wildlife Service (USFWS) National Wildlife Refuge located ~1300 km southwest of Honolulu, Hawaii. Johnston Atoll is not part of the Hawaiian Islands, but is more closely associated with a subsurface mountain range called the Marcus-Necker Rise (Amerson and Shelton, 1976). The atoll was discovered in 1796, used in the early 19th century for guano extraction, and during WWII, the US Navy used it for aerial and submarine operations. Subsequent activities included establishment of LORAN towers by the US Coast Guard, and use of the atoll for atomic weapons tests (Amerson and Shelton, 1976). Since 1976, the USFWS and the Defense Nuclear Agency have an agreement whereby USFWS manages the natural resources of the atoll. In 1985, a chemical weapons disposal plant (Johnston Atoll Chemical Agents Disposal System) was built on the Western end of the main island (Johnston). This plant is soon to be decommissioned, and Johnston Atoll will revert back to the USFWS in ~2003.

Johnston Atoll is composed of a fringing reef surrounding 4 islands, two of which (Haukau and Hikinau) are man made (Fig. 1). The lagoon becomes progressively deeper from northwest to southeast and supports a variety of corals, reef fish, sea turtles, and seabirds (see Coles et al., 2001, Appendix A for full listing of recent and previous reports). Compared to other atolls and reef environments, the marine fauna of Johston Atoll is depauperate by species, although coral coverage is high. The dominant species of corals include *Acropora cytherea, A. humilis, A. valida, Montipora patula, Pocillopora eydouxi* and *P. meandrina.* Maragos and Jokiel (1986) classified the lagoon into 4 area with the northwest portion harboring the greatest abundance of corals. During and after WWII, much of the coral in that area was destroyed during extensive dredging and filling of the reef and lagoon (Brock et al 1965, 1966).

Much work has been done to identify the identity and extent of organisms at Johnston Atoll (see Coles et al., 2001 for review). However, relatively little effort has been spent looking at health parameters of marine fauna. Cohen et al. (1997) observed bleaching of corals, and Coles et al. (2001) observed growth anomalies in *Acropora cytherea*. The USFWS sponsored the USGS National Wildlife Health Center to further investigate growth anomalies of corals in Johnston Atoll. From 29 March to 2 April, 2001, a team from the US Geological Survey Hawaii Field Station (Work, Rameyer) and the Bishop Museum (Coles) surveyed coral reefs at Johnston Atoll for lesions. The trip had the following objectives:

1) Conduct manta tow and spot surveys for lesions in dominant coral species in the northern lagoon and sites off Johnston and East islands.
2) Describe gross and microscopic anatomy of lesions in corals.
3) Obtain pigment profiles of the dominant coral (*Acropora cytherea*).

METHODS

Survey areas:

Broad-scale manta tow surveys were done using flat-bottom boats with an outboard motor focusing on the northwestern portion of the lagoon because it had the highest density of coral (Maragos and Jokiel, 1997), and because it encompassed the area where growth anomalies in *A. cytherea* were previously noted by Coles et al. (2001).

Six locations were selected for spot dives. Site 1 was where Coles et al. (2001) observed growth abnormalities in *Acropora* cytherea. Site 2 (Agent Orange) was immediately offshore the northwestern portion of Johnston Island where the herbicide Agent Orange had been stored during the Vietnam war. Site 3 (Mt. Pluto) was directly offshore the middle of north Johnston Island where an aborted launch of a nuclear warhead caused localized contamination with plutonium during the 1960s. Site 4 (Burn pit) was north of Eastern Island and was adjacent to an old burn pit where plastics and other materials were incinerated in open air. Sites 5 and 6 (Signal Tower and Donovan's reef, respectively) were "control" areas (Fig. 1).

Manta tows were done according to methods of English et al. (1994). Briefly, a diver was towed at low speeds ca. 30-40 feet behind a boat for 2 minute intervals at the end of which the diver recorded the bottom type (rubble, sand, coral), estimated percent coral cover by category (0-10%, 10-25%, 26-50%, >50%), and dominant coral genera (*Acropora* sp., *Montipora* sp. or *Pocillopora* sp.). For *Acropora* sp., the diver categorized lesions into 5 groups (see results). After each two-minute manta tow interval, the diver recorded the number of each type of lesion. Manta tows proceeded perpendicular to the long axis of Johnston Island and coordinates of way points were collected at the start and end of every 2 minute tow using a Garmin GPS 12 unit. Spot dives were done using SCUBA in Sites 1, 3, 5 and 6 and snorkel for the remaining sites. For areas 2 and 4, we snorkeled a network of parallel lines close to shore. Scuba surveys consisted of swimming in a haphazard pattern looking for lesions.

Corals were photographed using a Nikonos V underwater camera with a 20 mm lens and twin Ikelite 50 strobes or a digital camera in an underwater housing. Close-up photos were taken with a Nikonos V camera with a single Ikelite 50 strobe and a 2:1 extension tube. Coral samples were taken using bone shears, or hammer and chisel, and placed into labeled plastic bags in seawater. Corals were preserved in Helleys fixative (Barszcz and Yevich, 1975) with added salt and allowed to fix for 24 hr. The fixative was decanted and the coral rinsed with fresh water once every 12 hr for 24 hr. Subsequently coral was stored in 70% ethanol, decalcified with Cal-ex II (Fisher Scientific), placed in cassettes, processed for paraffin embedding, trimmed at 5 um, and stained with hematoxylin and eosin. Slides were examined using light microscopy at magnifications ranging from 20-1000X.

Twenty fragments of normal *A. cytherea* were collected for pigment analyses. Coral fragments were weighed (nearest 0.001 g), placed in 50 ml of 100% methanol (n=10) or 0.6M phosphate buffer pH 8 (n=10), and extracted in the dark at 4C for 24 hr. The supernatant was decanted, centrifuged (14000g) for 5 minutes, and stored at –190C. The extract was filtered using 0.45 μ syringe filters and scanned from 200-700 nm using a Spectronic Genesys 8 Spectrometer. Methanol extracts were for chlorophyll pigments, and phosphate buffer extracts for pigments insoluble in methanol.

RESULTS

Surveys

Coral cover seemed uniform over most of the lagoon but appeared denser away from the dredge channel (Fig. 2). Coral cover was relatively sparse east of Johnston Island. *Acropora* (*mostly A. cytherea*) was more dominant towards the reef edge, and *Montipora* sp. was more

dominant near the ship channel. *Pocillopora* sp. appeared more numerous east of Johnston Island (Fig. 3.).

Normal morphology

Acropora cytherea

Normal *A. cytherea* colonies formed wide flat tables (Fig. 4A) with small branches containing numerous tan-brown to tan-pink exsert axial corallites (Fig. 4B). On microscopy, the epithelium overlying was composed of an intact continuous layer of columnar cells with basally located nuclei. These were interspersed with occasional intracytoplasmic basophilic granular cells, mucus cells, and rare isolated spirocysts (a type of nematocyst) (Fig. 4C). Epithelium overlying tentacles contained numerous batteries of spirocysts mixed with barely visible holotrichous isorhizas. Spirocysts became less numerous and eventually absent in the stomodeum, the epithelium of which was lined by closely apposed ciliated columnar epithelium. Mesoglea was uniformly thin except near the base of tentacles where it thickened and contained prominent mesogleal pleats; within the gastrovascular canal, mesoglea was not discernable.

Coenosarc gastrodermis was composed of columnar and mucus cells mixed with focally aggregated zooxanthella. In tentacles, gastrodermis was thickened and contained clusters of zooxanthella. Within the gastrovascular canal, gastrodermis was focally hyperplastic and contained rare zooxanthella. The gastrodermis was either closely apposed to calicoblast or contained prominent mucus cells, giving it a foamy appearance. Mesenteric filaments were of two types. One consisted of densely packed columnar cells with closely apposed nuclei just below the cnidoglandular cap, giving the filament a distinct pattern (Fig. 4D). Other filaments contained prominent mesogleal pleats, scattered eosinophilic granular cells, and mucous cells giving them a moth-eaten appearance. Occasional filaments at the base of the branch contained ovarian tissue. Cnidoglandular caps of all filaments contained eosinophilic granular cells. The calicoblast was uniformly cuboidal to squamous, closely apposed to gastrodermis, and contained scattered desmoid processes.

Montipora patula

Colonies were plate-like to encrusting, with purple polyps interspersed among a network of yellow trabeculae (Fig. 4E). Epithelium overlying coenosarc was composed of a single layer of columnar cells with basal nuclei and interspersed with occasional mucus cells. Epithelium overlying tentacles contained localized batteries of spirocysts (Fig. 4F), which gave way to small clusters of eosinophilic granular cells in the stomodeum.

The mesoglea was barely visible in the coenosarc and tentacles, but became more prominent along with mesogleal pleats within gastrovascular canals at the base of polyps. Occasional eosinophilic granulocytes characterized by cells with an eccentric nucleus and distended by intracytoplasmic accumulations of brightly eosinophilic granules were seen within the mesoglea of the gastrovascular canal.

The coenosarc gastrodermis was cuboidal, contained numerous zooxanthella, and was closely apposed to squamous calicoblast layer. Within gastrovascular canals, gastrodermal cells were cuboidal or focally hyperplastic and apposed to squamous calicoblastic cells. The gastrodermis contained scattered zooxanthellae. Mesenteric filaments deep within canals contained batteries of macrobasic mastigophores (a type of

nematocyst). Other mesenteric filaments were composed of closely apposed columnar cells mixed with mucus cells and eosinophilic granular cells. Occasional ova were noted within filaments. The skeleton contained mats of gray to amphophilic branching filaments (probable endolithic algae).

Montipora capitata

Colonies were placoid to branching, brown, with a verrucose surface containing polyps interspersed among a network of trabeculae. Epithelium overlying coenosarc was simple columnar interspersed with occasional clear mucus cells. Epithelium overlying tentacles was pseudostratified columnar with numerous large glandular cells giving it a vacuolated appearance. Batteries of spirocysts were noted near the base of tentacles, and these disappeared in the stomodeum and were replaced by closely packed columnar cells with a ciliated surface. Deeper into the stomodeum, there were clumps of eosinophilic granular cells near the central lumen. Mesoglea was uniformly thin and bereft of cells except near base of polyps or near mesenteric filaments where mesogleal pleats were noted.

The coenosarc gastrodermis was simple to pseudostratified cuboidal and contained few zooxanthellae. Zooxanthellae were much more numerous within gastrodermis of tentacles. Within gastrovascular canals, gastrodermis was squamous and focally hyperplastic with few zooxanthella and occasional ovarian tissue. Mesenteric filaments were composed of closely apposed columnar cells interspersed with aggregates of red granular cells at cnidoglandular caps, and occasional mucus cells giving the filament a moth-eaten appearance. Batteries of macrobasic mastigophores were occasionally noted within the gastrodermis. Planulae, characterized by three layers of cells (ciliated columnar cells, mesoglea, and gastrodermis) surrounding a lumen were sometimes seen in the gastrovascular canal. Calicoblastic epithelium was uniformly squamous.

Pocillopora eydouxi

Colonies were composed of tan to cream upright flattened branches with a verrucous surface containing haphazardly arranged corallites (Fig. 7 C-D). Coenosarc epithelium was composed of a single layer of ciliated columnar cells. Over the tentacles, columnar cells were prominent and mixed with batteries of spirocysts, which disappeared within the stomodeum and were replaced by closely apposed ciliated columnar cells. Coenosarc mesoglea was thin and not discernable but widened and contained mesogleal pleats, particularly around polyps and within tentacles.

Gastrodermis was squamous to cuboidal and contained focally aggregated zooxanthellae, which were more numerous within tentacles. Gastrovascular canals formed a single layered parallel network below the external epithelium. Mesenteric filaments consisted of closely apposed columnar cells mixed with eosinophilic granular and mucus cells with rare zooxanthella. The calicoblast was squamous with focal hypertrophy where cells took on a columnar appearance.

Lesions:

Any anomaly in gross skeletal or tissue morphology was classified as a lesion. Lesions in *A. cytherea* were categorized into five groups (Fig. 5): purple bleaching, yellow bleaching, brown blotch, growth anomalies, and brown band. Purple bleaching (Fig. 5A-B) referred to areas of coral that were bleached with a raised light to dark purple margin, giving the entire lesion a bluish hue. This lesion usually surrounded a variably

sized central area of dead coral overgrown by algae. Polyps near the edge of these lesions appeared atrophied or entirely missing. Yellow bleachings (Fig. 5C-D) designated dwell-defined areas of bleached coral with a yellow hue and no distinct raised margin. These areas were separated from live coral and often had incipient algal growth on coral tissue. Brown blotch corresponded to single well-defined circular dark brown lesions on coral tables, usually near the middle of the plate, and was characterized by a film of brown mucoid material surrounded by apparently normal coral tissue. Growth anomalies (Figs. 5E, 6, 7A-B) included any abnormal growths of the coral skeleton. Brown bands (Fig. 5F) were slice-shaped areas of dead coral overgrown with algae demarcated from adjacent normal coral by a distinct band of bleached tissue

Growth anomalies in *A. cytherea* were of 3 types: Grossly, type 1 (Figs. 5E, 6A-B) growths were focal to coalescing, white to pink, and rugose, and tissue covering these masses was smooth and bereft of polyps. On histology (Fig. 6C), coenosarc epithelium was markedly hyperplastic and characterized by numerous pseudostratified columnar cells. Polyps were absent or manifested by rare batteries of spirocysts. The mesoglea was enlarged, and gastrodermal cells adjacent to epithelium were markedly hyperplastic to anaplastic, pleomorphic, and characterized by stellate nuclei, and widened intercellular spaces. Gastrodermis of gastrovascular canals was focally hyperplastic with clumps of proliferating cells free or projecting within the lumen of canals. Based on these characteristics, this lesion was considered neoplastic and classified as a gastrodermal neoplasm. Zooxanthellae were rarely seen. In some instances, calicoblast cells appeared enlarged and hypereosinophilic with swollen nuclei. Rare mesenteric filaments containing macrobasic mastigophores were noted.

Type 2 growths (Fig. 6D-E) were focal to coalescing, pink, with numerous cylindrical tubercles with an apical indentation. Tissue overlying these growths was generally bereft of polyps save for occasional single tentacles. On histology, (Fig. 6F) there was marked hyperplasia of coenosarc epithelium with cleft formation and thickening of mesoglea. Coenosarc gastrodermis was composed of simple columnar cells and that of gastrovascular canals was cuboidal and focally hyperplastic. Zooxanthella and mesenteric filaments were rarely seen. Calicoblast layer was uniformly squamous.

Type 3 growths (Fig. 7A) consisted of smooth, well-defined, sessile white firm growth arising from normal skeleton. Tissue overlying these growths lacked zooxanthella. On histology, tumor tissue was characterized by a markedly hyperplastic epithelium bereft of polyps. Coenosarc gastrodermis was largely bereft of zooxanthella and focally hyperplastic with stellate nuclei. Within gastrovascular canal, gastrodermal cells, particularly those associated with mesenteric filaments, were hyperplastic, fibroblast-like and pleomorphic forming papillary projections into the gastrodermal cavity. Myonemes within occasional mesenteric filaments were enlarged and occasionally diffusely necrotic. Diffusely, calicoblast was squamous and focally hyperplastic. Based on pleomorphic appearance of gastrodermal cells and cellular necrosis, these growths were classified as gastrodermal neoplasms.

Pocillopora eydouxi

Grossly, lesions were characterized by exuberant growth of skeleton overlaid by apparently normal tissue (Figs. 7D-E). These were seen in one location on the north lagoon and Site 3. On histology, no significant lesions were noted save for markedly

atrophied epithelium and absence of polyps and zooxanthella within coenosarc. One normal appearing colony from the northern lagoon that contained variably sized, well defined, round aggregates of deeply basophilic filamentous organisms (probable bacteria) within tentacular epithelium (Fig. 7F).

Montipora patula, Montipora capitata

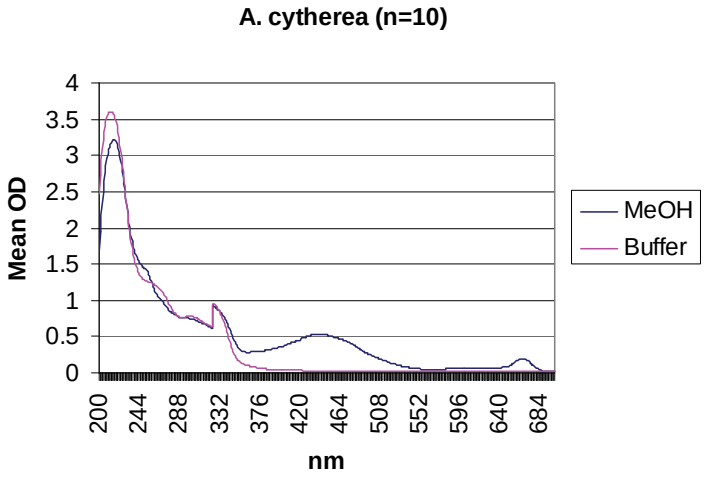
Grossly, lesions were characterized by single to coalescing well-defined firm smooth to rugose white nodules ranging in size from ca. 0.5-5 cm diameter. In some instances, the center of the lesion was ulcerated and colonized by filamentous algae. These lesions were seen sites 1-4 and did not appear particularly numerous at any one site (Fig. 8A-B, D-E).

On histology, large mats of variably sized eosinophilic filamentous organisms displaced coral tissue. Epithelium of adjacent coral was cuboidal to simple columnar, and gastrodermal cells were hyperchromatic with shrunken cytoplasm. The mixed population of filamentous branching structures was largely limited to the skeleton and ranged in invasiveness from localized penetration into gastrovascular canals to complete invasion and effacement of tissue architecture. In some cases, the filamentous material encompassed fragments of degenerating gastrodermis. In other cases, these nodules were composed of granular gray material overlaid by cells with cell walls (plant material) (Fig. 8C). The center of the nodule occasionally contained an invertebrate (metazoan) of unknown identity (Fig. 8F).

Distribution:

Lesions (all types) in *Acropora* cytherea were more numerous in the areas where this species was dominant (Fig. 9). Purple bleaching, yellow bleaching, brown band, growth anomalies, and brown blotch made up 57%, 38%, 2%, 2%, and 0.5%, respectively of the total (555) number of lesions seen. There did not appear to be a pattern to the distribution of the major types of lesions (Figs. 10-12). In addition to manta tow survey areas, purple and yellow bleaching was seen during spot dives in sites 1-5 and growth anomalies were noted in sites 1 and 2 (Fig. 1)

Pigment profiles



Peaks at ~230, 290, and 325 nm were seen for both methanol and buffer soluble pigments while peaks at 441 and 665 (Chlorophyll) were seen in methanol extracts only.

DISCUSSION

Distribution of corals and lesions

Distribution of corals in our survey area appeared similar to that observed by Maragos and Jokiel (1986). Although a variety of lesions were seen in different coral species, no distinct distributional pattern was recognized. The distribution and extent of lesions in *A. cytherea* was a function of coral abundance, and the most widespread lesion (purple bleaching), appeared to be a normal process ofsenescence, because this lesion was usually located adjacent to dead coral near the center of the plates. This area of dead coral seemed to expand as the plates became larger, whereupon they would collapse. Diffuse purple bleaching was noted on the underside of many collapsed colonies. Yellow bleaching was associated with incipient algal overgrowth and was noted wherever *A. cytherea* was present, including deep (80 ft.) habitats such as Donovan's reef. Contrary to expectations, skeletal growth anomalies in *A. cytherea* and other species of corals did not appear concentrated in areas contaminated with tumor-inducing compounds such as organohalogens (Site 2) or plutonium (Site 3). Although rare growth anomalies in *A. cytherea* were seen in Site 2, coral in the general area appeared particularly healthy.

Growth anomalies were noted in 4 species of corals (*A. cytherea, P. eydouxi, M. patula, and M. capitata*), however, in only two instance were these growth considered neoplastic (cancerous). Documented instances of neoplasms in corals are rare, and those that are documented mostly involve description of skeletal growth anomalies in absence of histology (Peters et al., 1986). In the most complete description of growth anomalies in a coral, Peters et al. (1986) concluded that growths in *A. palmata* were calicoblastic epitheliomas based on locally invasive skeletal protuberances with associated proliferation of calicoblast and associated tissues. In vertebrates, neoplasms are defined by uncontrolled proliferation of tissue with cells that exhibit abnormal morphology including nuclear or cytoplasmic polymorphism and, on occasion, mitotic figures and tissue necrosis (indicating rapid cell division) (Cheville, 1988). This is opposed to hyperplasia where cell growth is excessive but controlled, and where cell morphology is not abnormal. The definition of neoplasias in invertebrates is less clear, and some cases of neoplasia in invertebrates could also be interpreted as hyperplasia (Sparks, 1985).

In this study, growth anomalies needed to fulfill two criteria to warrant a diagnosis of neoplasia: 1) abnormal and excessive growth of skeleton and 2) cellular proliferation accompanied by cell anaplasia and polymorphism. In *A. cytherea*, only two growth anomalies (Type 1 and 3, Figs. 11 A-B) were neoplastic. The other growth anomalies (Type 2) were classified as hyperplasia. No organisms were seen in tissue associated with growth anomalies in *A. cytherea*, and the cause of such lesions remains unknown. Interestingly, tumors that appeared different in gross appearance (Types 1 and 3) were both considered neoplasm of the same tissue type (gastrodermis). Tumors similar in appearance to Type 3 growths were seen in a tabular *Acropora* from a shallow site in an embayment in Oman and were classified as calicoblastic neoplasms (Coles and

Seapy 1998). Given the simple anatomic plan of corals (4 tissue layers), it is perhaps not surprising that the same tissue type would give rise to different tumor morphs. There is, however, a clear need for continued refinement of classification of coral neoplasms. Future investigations might focus on molecular markers for different tissue types in corals to better differentiate the tissue origin of tumors. Given the uncertain state of classification of coral neoplasia, speculation as to potential causes of these lesions seems unwarranted. In higher organisms, causes of neoplasia typically include viruses, ongoing damage to DNA from chronic trauma or senescence, and certain environmental contaminants.

Skeletal growth anomalies in *Montipora* sp. were responses to foreign agents including fungi, algae, and intra-skeletal metazoans (invertebrates). The coral skeleton harbors a variety of metazoans, filamentous algae, and fungi, and in most cases, these do not invade coral tissue. Le Campion-Alsumard et al. (1995) demonstrated that invasion of tissue by these organism is kept in check by a continuous process of mineralization. In some cases, however, this process fails and algae and fungi colonize live tissue. Interestingly, tissue reaction (necrosis) was minimal in spite of invasion of the gastrovascular canal by algae. Colonies of bacteria were seen in tissue associated with skeletal anomalies in *P. eydouxi* , however, this was not consistent. Thus, the cause of such anomalies in this species remains unknown.

In the pigment profiles for A. cytherea, peaks in the methanol extracts at 441 and 665 nm corresponded to chlorophyll (Dustan, 1979). Various coral proteins (UV pigments)were probably responsible for the peaks below 400 nm (Dove et al. 1995). Fluorescent pigments have been shown to be protective against sunlight for several species of Australian corals (Salih et al. 1998; 2000) and determination of fluorescent pigment properties may prove useful in future investigations of coral health. This pigment profile should provide a baseline to investigate future events of bleaching or pigment abnormalities in *A. cytherea*.

RECOMMENDATIONS

1. There needs to be a better method to quantify lesions on reefs during broad scale surveys. Surveys of lesions are complicated by the three dimensional nature of coral reefs and the intermixing of species. Ideally, it would be useful to have percent coverage of different species. Surveys would be targeted in such a way as to calculate percent of area of coral x covered by lesion y. Possible tools to consider for this include quadrats or video transects.
2. Pigment profiles of corals with lesions, particularly growth anomalies, should be evaluated to further define whether pigments or their absence plays a role in genesis of these anomalies.
3. Consider measuring growth anomalies using manual methods or photogrametry and following growth through time (months, years) to determine whether or not they are growing uncontrollably or remain unchanged.
4. Additional criteria are needed to determine exactly what constitutes neoplasia in corals.

5. Most efforts on this trip focused on growth anomalies. It would be useful to examine other lesions in *Acropora* (brown blotch, yellow bleach, purple bleach, brown band) in more detail to evaluate potential causative factors.

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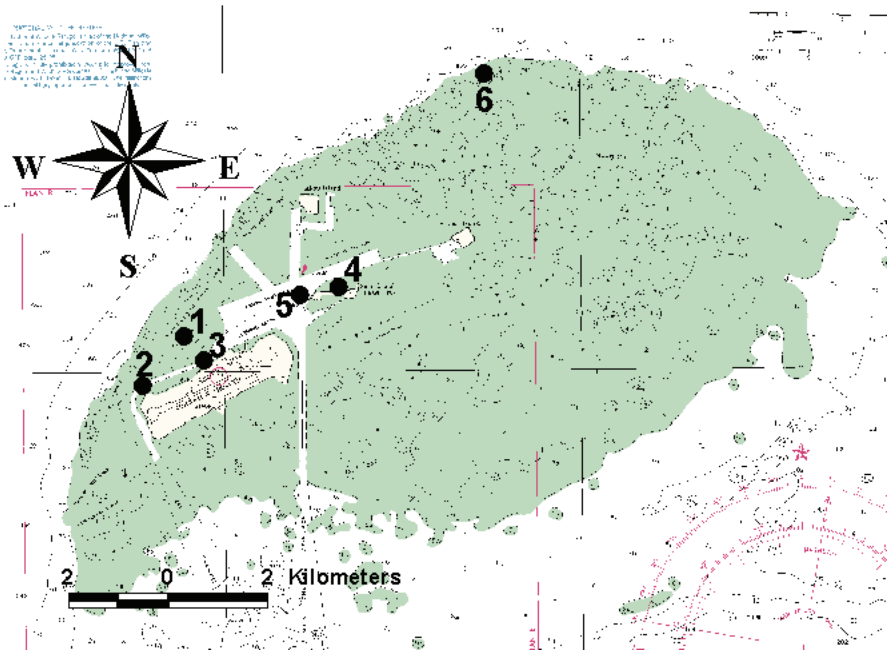

Figure 1. Johnston Atoll: Location of spot dives (see methods for details).

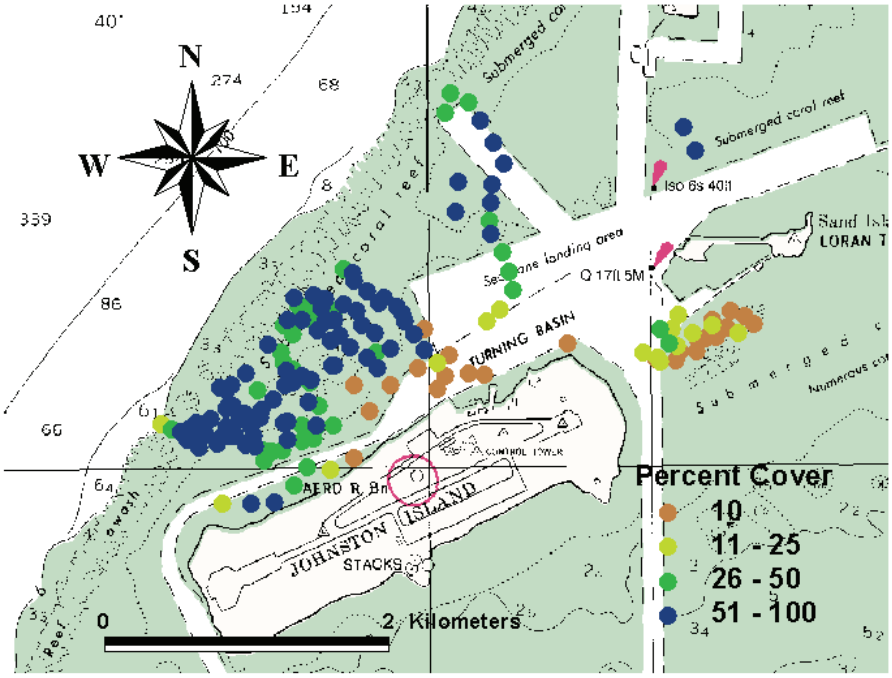

Figure 2. Percent coral cover observed during manta tows of North lagoon, Johnston Atoll.

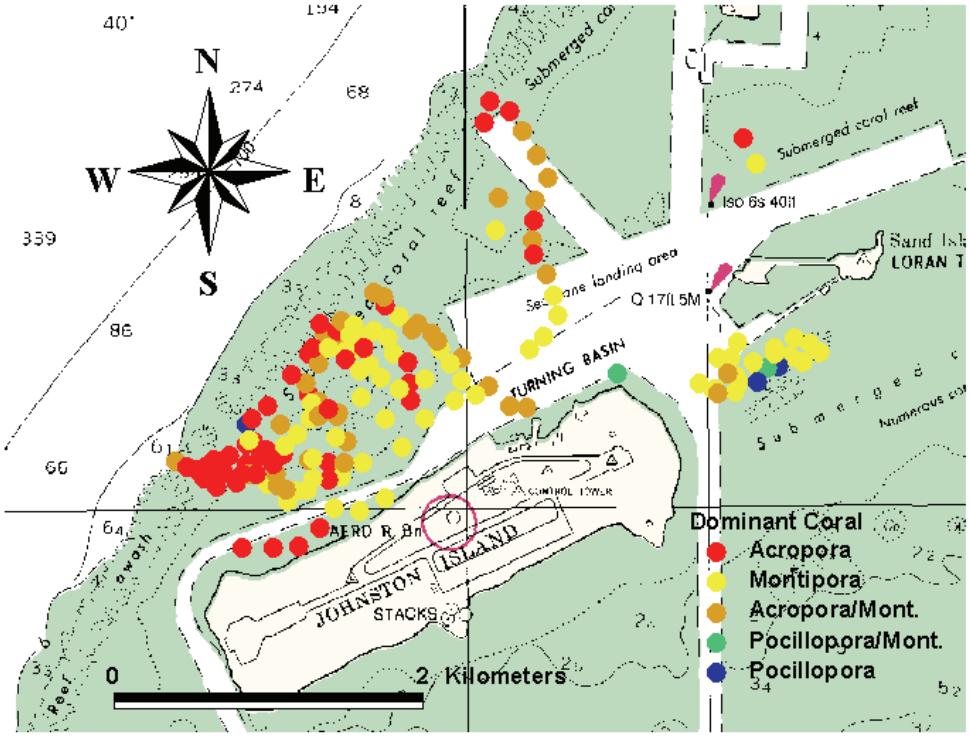

Figure 3: Dominant species of corals seen during manta tows of north lagoon.

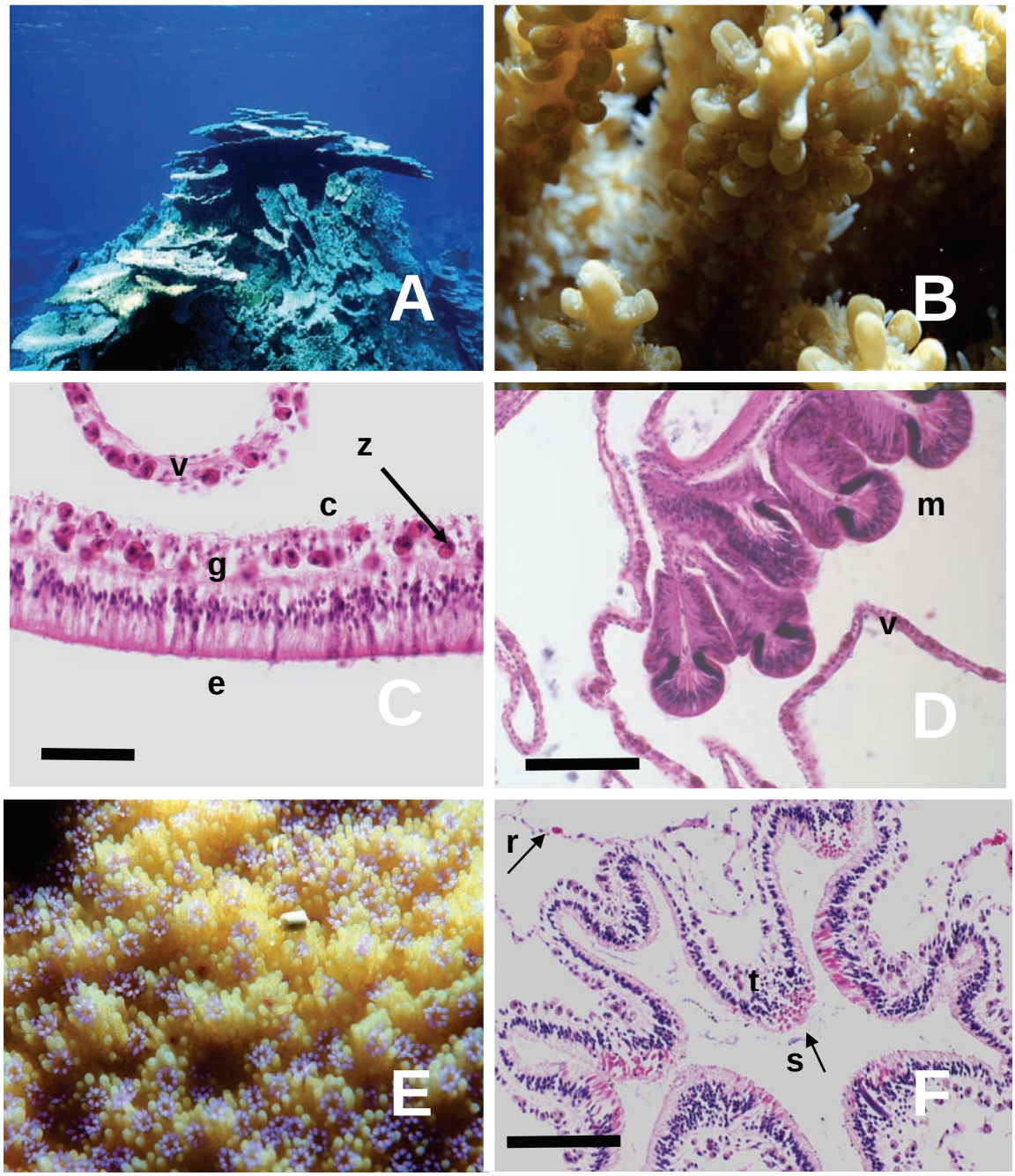

Figure 4. A-D) *A. Cytherea*; C) Coenosarc, bar= 50 µm; D) Gastrovascular canal (v) and mesenteric filaments (m), bar=100 µm; E-F) *M. Patula*; F) Polyp, bar= 100 µm. Epithelium (e), gastrodermis (g), calicoblast (c), zooxanthella (z), mesenteric filaments (m), tentacle (t), spirocysts (s), eosinophilic granulocyte (r).

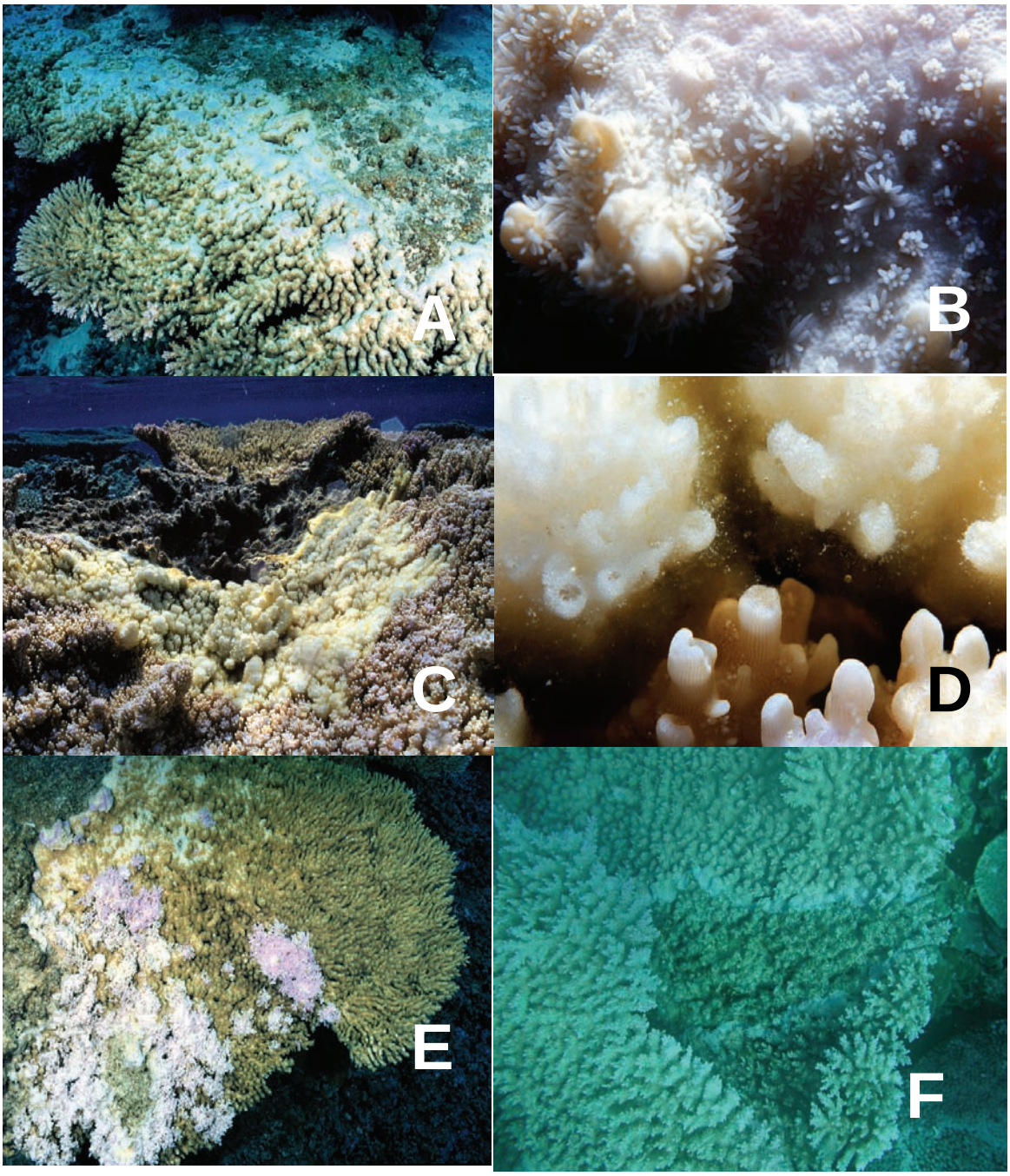

Figure 5. ***A. cytherea.*** **A) Purple bleaching; B); Purple bleaching-note atrophied polyps; C) Yellow bleaching; D) Yellow bleaching-note algal filaments; E) Growth anomalies; F) Brown band.**

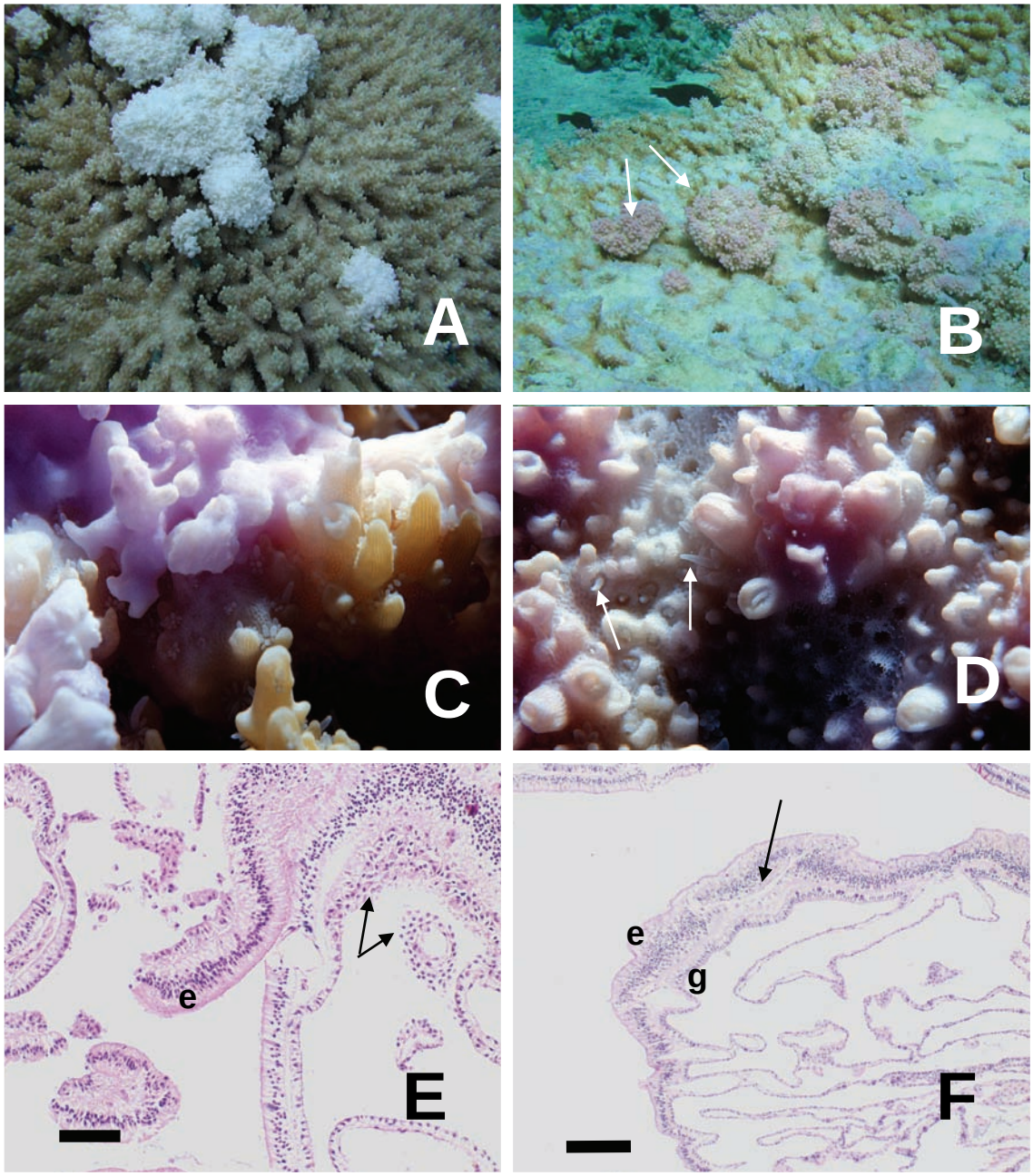

Figure 6. *A. cytherea.* **A-B) Type 1 growths, note absence of polyps; C) Note markedly hyperplastic epithelium and pleomorphic and hyperplastic gastrodermis (arrows), bar = 50 µm. D) type 2 growths (arrows); E) note malformed polyps with single tentacle (arrows); F) Note markedly hyperplastic epithelium with cleft formation (arrow) bar=200 µm. Epithelium (e), gastrodermis (g).**

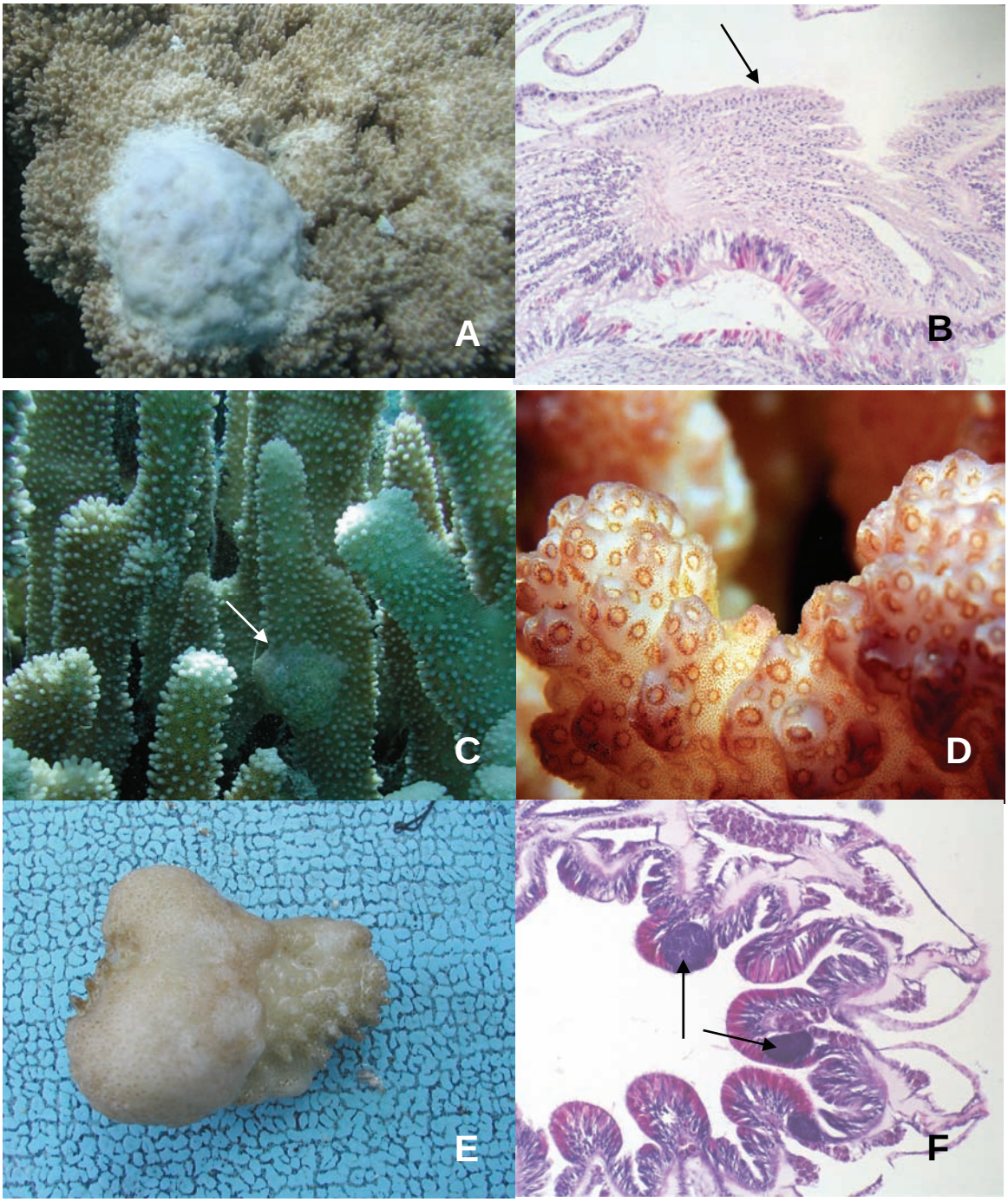

Figure 7. A-B) *A. cytherea*; D-F), *P. eydouxi*; A) Type 3 growth; B) Note marked pleomorphism of gastrodermal cells in tumor tissue (arrow), bar= 100 µm. C, E) growth anomalies (arrow) and normal coral (D); F) Polyp of normal appearing P. eydouxi, note bacterial colonies in epithelium (arrows) bar=50 µm.

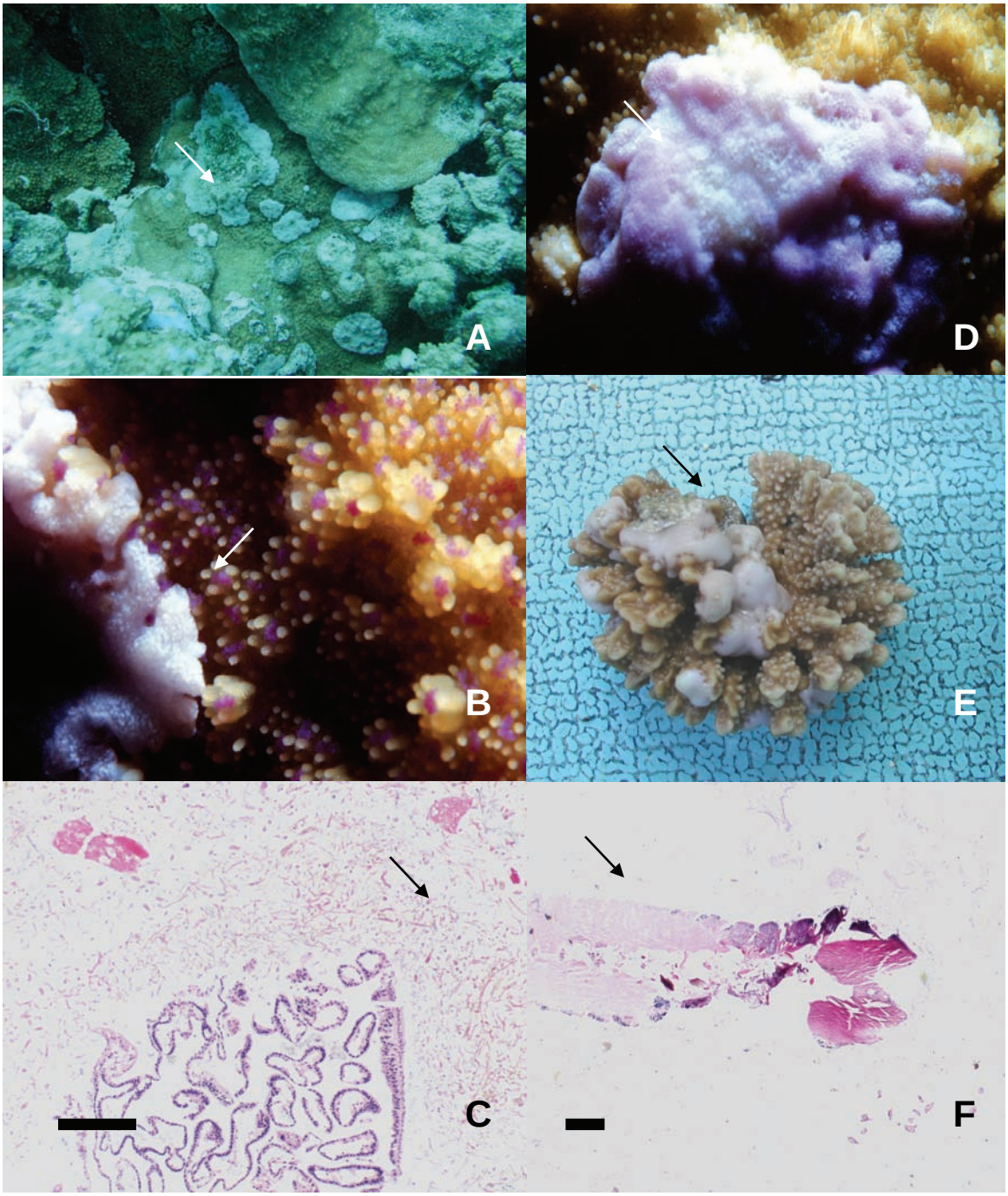

Figure 8. A-C) *M. patula*, D-F) *M. capitata*; A-B) growth anomalies (arrow); C) note invasion of skeleton and tissue with filamentous organisms (algae and fungi) arrow, bar= 200 µm; D-E) growth anomalies; F) note cross section of metazoan (arrow), bar=200 µm.

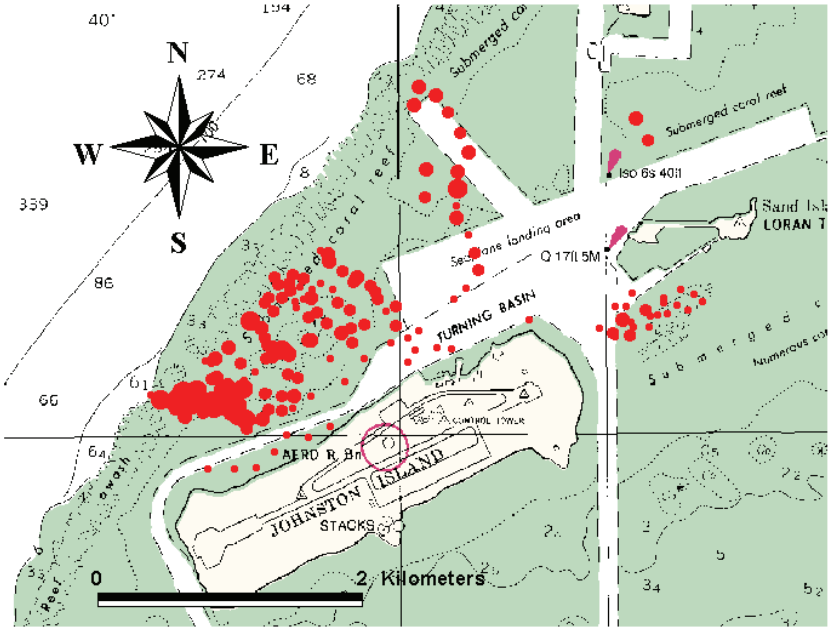

Figure 9. Number of lesions in *A. cytherea* observed during each manta tow interval.

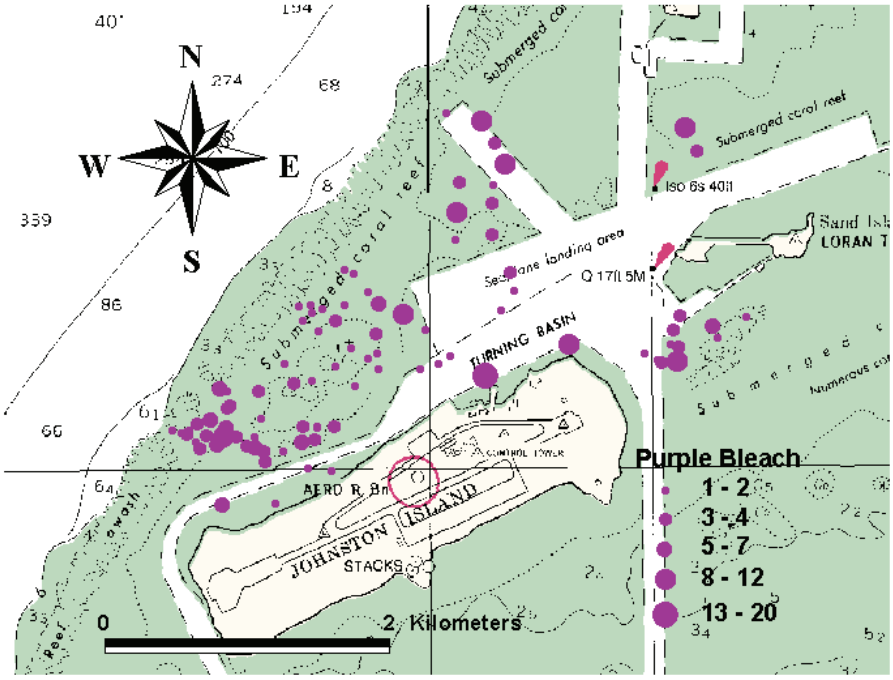

Figure 10. Number of instances of purple bleaching during each manta tow interval.

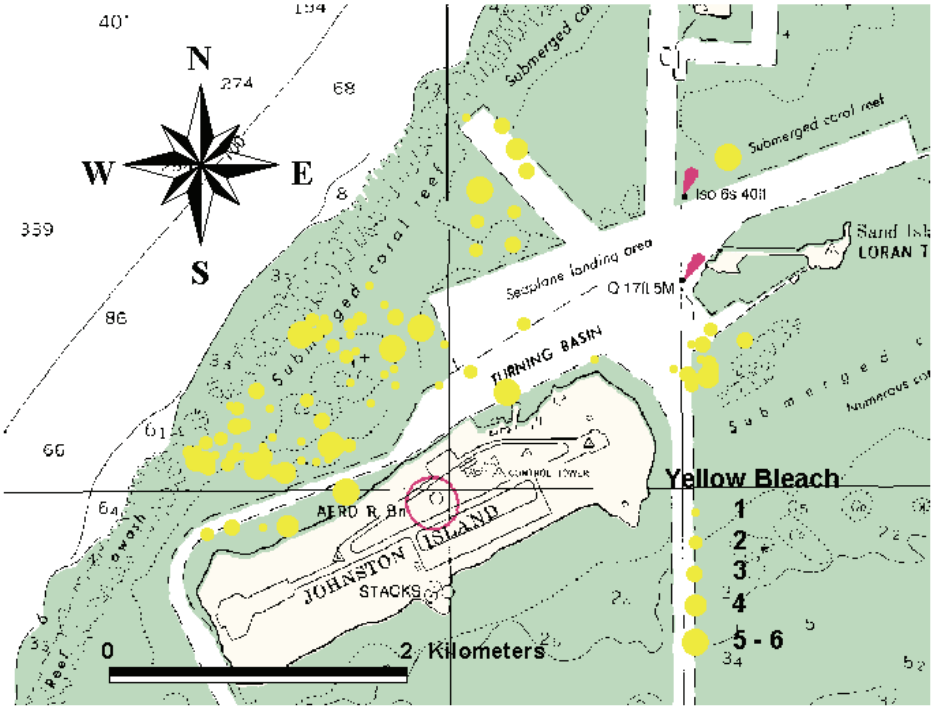

Figure 11. Number of instances of yellow bleaching seen during each manta tow interval

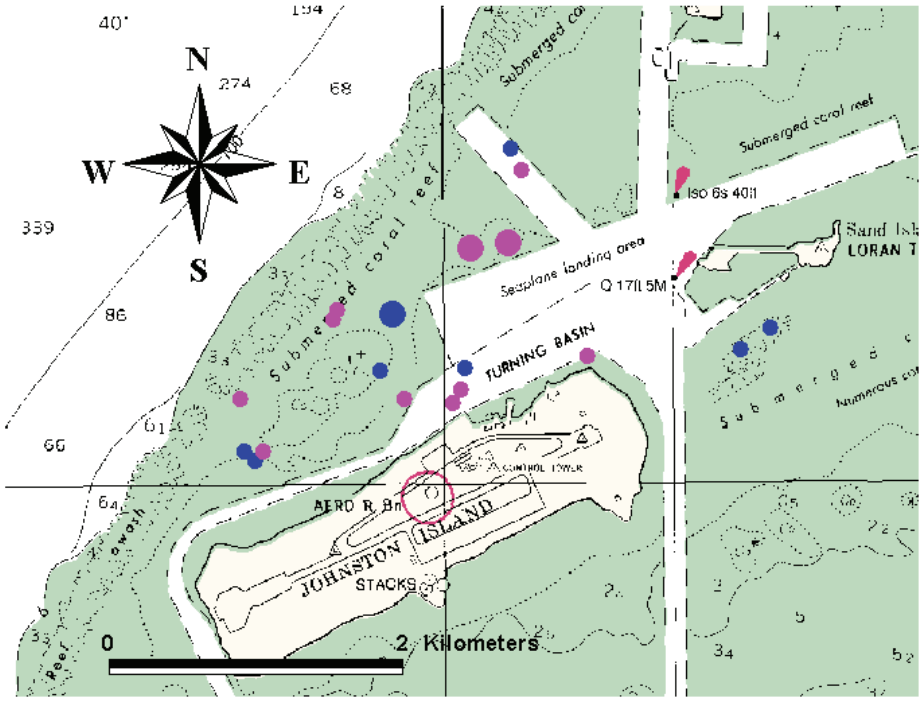

Fig 12. Number of instances of aberrant growth (blue) or brown band (violet) seen during each manta tow interval. Large dots=2, small dots=1.

CORAL DISEASE ON THE GREAT BARRIER REEF AND IN THE WESTERN PACIFIC

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Background

Until recently, it was assumed that disease has had little impact on the population dynamics or community structure of coral assemblages on the Great Barrier Reef (GBR). However, prior to 2000 there were only two studies of coral disease in the region, one focused on black band disease (BBD; Dinsdale 2002) and the other on skeletal eroding band (SEB; Antonius 1999; Antonius and Lipscomb 2001), both undertaken at Lizard Island in the northern sector of the GBR. Further anecdotal reports of BBD (Miller 1996) and a white disease (Baird 2000), plus increasing abundance of white syndrome (described below) detected in a Long Term Monitoring Program begun in the early 1990's by the Australian Institute of Marine Sciences (AIMS) (Willis et al. 2004), highlighted the need for a more in-depth study of coral disease on the GBR.

As an initiative of the GEF Coral Disease Working Group (DWG), surveys of coral disease were commenced in 2002 to more systematically assess the types and prevalence of coral disease on the GBR (Willis et al. 2004). Detection of some of the more common and infectious Caribbean diseases, in combination with discovery of diseases unique to the region (brown band disease: BrBD; Willis et al. 2004), suggested that coral disease occurs commonly on Indo-Pacific reefs and may have a greater role in structuring coral communities in the region than previously thought. Accordingly, a 7 year program to assess the ecological significance of coral disease on the GBR has been funded by the Australian Research Council (ARC). Results summarized below describe progress 2.5 years into the program. In collaboration with the GEF DWG, survey protocols and knowledge gained on the GBR have been applied to reefs throughout the Western Indo-Pacific, including Palau, the Philippines, Marshall Islands, Papua New Guinea and Indonesia, plus imminently Zanzibar, Tanzania, Kenya, Mauritius, Madagascar, Seychelles and the Comoros as a consequence of a recent GEF Workshop on Coral Disease for local reef scientists and marine park managers (Zanzibar, April 2006).

Survey design, transect protocols and targeted disease studies

Given the lack of baseline knowledge of coral disease on the GBR and the vastness of a reef system spanning north-south gradients more than 2000 km in length and cross-shelf (east-west) gradients in terrestrial influences up to 100 km across the

continental shelf, the region was divided into 3 sectors (northern, central and southern GBR) to optimize sampling a range of habitats and reef types. The sampling design comprises 3 belt and line intercept transects, at each of 2 sites, on each of 3 replicate reefs, in surveys at each of 3 cross-shelf positions (inner-, mid- and outer-reef positions) in the northern and central sectors, and at 1 cross-shelf (outer-reef) position in the southern sector. Transects are surveyed in summer, on the upper reef slope (typically 4-6 metres) where species diversity tends to be highest. In total, 19 reefs have been surveyed annually since 2004 and 21 reefs since the full design was inaugurated in 2005. Surveys of 2 reefs in the northern sector in both summer and winter since 2002-03 have shown a clear pattern of increased disease prevalence in summer (Willis et al. 2004).

The survey protocol developed to cope with species-rich, high-cover coral communities characteristic of Indo-Pacific reefs is based on a 20m x 2m belt transect combined with a 20m line intercept transect (LIT) to produce a concurrent estimate of percent coral cover. Belt transects of this size represent an efficient compromise between the need to survey a representative proportion of the species diversity and time constraints imposed by SCUBA surveys. Three replicate belt and LIT transects are typically completed within 1-2 dives (depending on percent cover) by a team of 2-3 experienced divers. All colonies within the belt are examined, recorded as healthy, diseased or showing signs of compromised health (see final section below) based on macroscopic field signs, and identified to genus (plus growth form for the genus *Acropora*) or family for less common groups. Diseased colonies are identified to species, photographed, and samples collected for microscopic examination and histological investigation as appropriate. In selected cases, typically when disease outbreaks are encountered, samples for microbiological and molecular studies are collected to isolate and identify pathogens. As evidence identifying the most common and virulent disease types has accumulated, more in-depth studies of selected disease types (SEB, WS, BBD, BrBD, AtN) have been initiated to quantify rates of progression across colonies, impacts on coral growth and reproduction, spread throughout populations and the effect of elevated temperature on these rates.

Broad ecological surveys inform detailed studies of lesions, pathogens and population impacts

The focus of the ARC-funded program is changing from its current emphasis on broad ecological surveys to more in-depth manipulative, microbiological and molecular studies of selected disease types as the research progresses. The objective is to use ecological surveys to provide the context for selection of disease types for more focused research. his overarching research plan recognizes the need to start with imperfect tools, i.e. observations of macroscopic disease signs underwater, as the first step in progressing research on under-studied Indo-Pacific coral diseases. Through extensive field surveys at diverse sites and through time, experienced coral biologists are well-placed to make informed decisions about the most prevalent and/or virulent disease types that should be targeted for further study. To carry this research to the next level, collaboration with biomedical histologists, microbiologists and molecular biologists have been developed. As knowledge of disease types is refined, diagnostic tools can be developed that will, in turn, refine ecological surveys. The importance of maintaining long-term monitoring programs cannot be over-stated, as these are the key to determining whether disease

incidence is changing through time and for developing hypotheses in relation to potential drivers.

In recognition of the early stage of Indo-Pacific coral disease research, we advocate the use of this integrated, collaborative approach to advance current understanding of coral diseases in the region. It is hoped that this meeting will be instrumental in developing further collaborations and a unified approach among research teams throughout the Indo-Pacific to strengthen and coherently build current understanding of coral disease in the region.

Recognizing and standardizing the naming of Indo-Pacific disease types

The issue of naming diseases when little is known apart from macroscopic and microscopic signs of disease is problematic. We support schemes under development to apply a structured approach to the description of gross lesions and the naming Indo-Pacific coral diseases (T. Work, G. Aeby, pers. comm.), with one additional pragmatic consideration. Whilst it would be ideal to incorporate the host species or genus of coral into each disease name, this approach becomes cumbersome when dealing with the more than 580 species and 200 genera of Indo-Pacific corals. An approach whereby disease names, at least initially, are applied to all corals exhibiting the same signs, avoids an unwieldy system for field surveys and researcher training purposes. Recording the names of coral species plus detailed descriptions for all records of disease retains species-level information in the event that diseases are later found to be specific to coral species. Refinement of disease names as histological, microbiological and molecular studies link pathogens to macroscopic field signs provides the foundation for an iterative approach to the development of definitive disease names. Figure 1 shows results for surveys of disease prevalence in the central and northern GBR using the above naming protocol. It is based on recognition of the following disease types in surveys on the GBR. All disease types have been detected in surveys on other western Indo-Pacific reefs.

Black Band Disease **(BBD):** BBD is widespread throughout the GBR, occurring on more than 70% of reefs surveyed (n=19) and in all 3 sectors, although its prevalence is typically low (~0.1% of scleractinian corals) (Page and Willis 2006). It has been recorded on at least 32 coral species in 10 families, with branching pocilloporid and acroporid corals being important hosts on the GBR (Willis et al. 2004). On reefs in Palau, a reddish band of cyanobacteria on *Pachyseris speciosa* and *Porites* sp. has been identified as having the same ribotype as cyanobacteria producing macroscopic signs of BBD on *Montipora* sp. (Sussman et al. 2006). Further evidence is required before the potential status of red band as a separate syndrome can be assessed (Sussman et al. 2006).

Skeletal Eroding Band **(SEB):** SEB, caused by the protozoan, *Halofolliculina corallasia*, erodes the tissue and skeleton of corals as it produces a black lorica or test (Antonius 1999). Clusters of ciliates along the tissue-skeleton interface produce a black band similar in appearance to black band disease, but, unlike the uniformly white skeleton exposed as BBD advances, the skeleton behind the advancing SEB is speckled with the remains of empty black loricae (Antonius and Lipscomb 2001). Progression of SEB can be relatively slow, approximately 1 mm per week, further distinguishing it from BBD, but it may also advance at rates up to 1 mm per day, comparable to BBD (Antonius

and Lipscomb 2001). SEB affects at least 31 species of corals in 6 families on the GBR. Recent records of a different species of *Halofolliculina* causing similar signs on 25 corals in 6 families from the Caribbean suggest that halofolliculinid infections affect corals on reefs globally (Croquer et al. 2006).

White Syndrome **(WS):** WS is a collective term for conditions producing white signs on corals from the GBR and Indo-Pacific reefs (Willis et al. 2004). Given the difficulty of consistently identifying features such as the variable zone of bleached tissue that distinguishes white band II (WBII) from white band I (WBI) or differences in the rates of movement that distinguish the faster moving white plague II (WPII) from white plague I (WPI; reviewed in Richardson 1998), we have elected to use the term white syndrome to describe conditions resulting in progressive loss of tissues to expose skeleton in white bands behind a moving front of tissue loss (Willis et al. 2004). A band of white bleached tissue may be present at the tissue-skeleton interface. The role of potentially secondary pathogens, like ciliates (see brown band description below), in possibly obscuring bleached zones as rates of tissue loss escalate, requires further investigation. ^{14}C studies near lesion boundaries on tabular *Acropora's* in the southern GBR suggest that photoassimilates are preferentially translocated away from lesions in an apparent shut-down reaction, potentially as a result of abiotic factors or pathogens triggering an apoptotic reaction in the host (Roff et al. 2006).

In addition to WBI/II and WPI/II, white syndrome could potentially encompass white pox (Patterson et al. 2002) and even shut down reaction (Antonius 1977). However, WS is distinguished from feeding scars by the narrow width of the zone of recently exposed, white skeleton and the relatively regular appearance of the tissue front. These features are in contrast to the wide zone of white skeleton commonly exposed following *Acanthaster planci* predation and the scalloped or wavy tissue front produced by *Drupella* spp. Determining the relationship(s) between the Caribbean white diseases and WS and applying the appropriate name(s) will not be possible until potential pathogens are isolated and compared to those producing white symptoms in Caribbean corals (Sussman et al. in prep.).

WS has been recorded for 17 species of corals in 4 families on the GBR, with species of *Acropora* being important hosts (Willis et al. 2004). Dramatic increases in abundance of WS on the GBR, by up to 20-fold on some outer-shelf reefs in the northern and southern sectors in 2002/03, suggest that the prevalence of WS may be increased by elevated temperatures when host densities are high (Selig et al. in press). Reports of a more than 50% increase in the prevalence of WS (from 8.55% to 13.58%) at a sub-tropical reef south of the GBR in 3 months following the summer of 2003 (Dalton and Smith 2006) provide further evidence of a correlation between aggregated distributions and high densities of hosts and WS prevalence.

Brown Band Disease **(BrBD):** BrBD is a new syndrome and has been recorded on corals in all 3 sectors of the GBR (Willis et al. 2004, unpubl. data). The distinctive macroscopic field symptom is a brown zone of variable width, flanked by healthy tissue at the advancing front and exposed white skeleton at the trailing edge as the band progresses over the surface of the colony. There is often a white zone between the healthy tissue and brown band, which may comprise bleached tissue and/or denuded skeleton. Dense

populations of ciliates, packed with zooxanthellae from engulfed coral tissue, cause the brown coloration of the band. As densities of ciliates decrease, the zone becomes lighter and may appear white at very low ciliate densities. In these latter cases, the condition would be assigned to the WS category based solely on field observations. The possibility that BrBD ciliates represent a secondary infection following tissue necrosis induced by a primary pathogen requires further investigation. At high densities, however, the ciliates become the primary agent of tissue loss. BrBD has been reported on 16 species from 3 families on the GBR, with acroporid corals being important hosts (Willis et al. 2004).

Note that an earlier report of a brown band on a colony of *Acropora formosa* (Dinsdale 1994) referred to a different, but unknown syndrome, and has subsequently been mistakenly quoted as affecting 20 coral species on the GBR (Santavy and Peters 1997; Borneman 2001). While it is possible that the unknown syndrome was caused by a cyanobacterium similar to the one causing red-band disease in the Caribbean as suggested by Santavy and Peters (1997), in the absence of the specimen it is not useful to speculate further about this isolated observation; it is not to be considered a record of BrB as described here.

Coral Tumors: Hyperplasia's, manifesting as raised masses projecting about 4.5 cm above the surface of the colony, were reported to affect 18-24% of populations of *Platygyra pini* and *P. sinensis* on Magnetic Island, central GBR (Loya et al. 1984). Tumors were associated with increased growth rates of polyps and a general proliferation of all cell types, some atrophied and others normal, but in all cases macroscopic polyp structures were discernible and tissues remained pigmented (Loya et al. 1984). Bleached neoplasms, manifesting as white, globular masses of skeleton raised above the surface of the colony with few discernible polyp structures, are most common on acroporid corals on the GBR (Willis et al. 2004). Bleached neoplasms, mainly on corals in the family Acroporidae, have been reported from throughout the Indo-Pacific, i.e. from Guam and Enewetak (Cheney 1975), French Polynesia (Le Champion-Alsumard et al. 1995), Japan (Yamashiro et al. 2001) and the Gulf of Oman (Coles and Seapy 1998).

Porites ulcerative white spots* (PUWS)*: PUWS is characterized by discrete bleached round foci, 3-5mm in diameter, that may either regress or progress to full tissue ulcerations that coalesce, occasionally resulting in colony mortality (Raymundo et al. 2003). Definitive cases have not yet been identified from the GBR.

***Atramentous Necrosis* (AtN):** AtN is characterized by spreading lesions of blackened, dead tissues and has primarily been recorded on a *Montipora* species in the central GBR (Jones et al. 2004). Further observations indicate that there are 4 stages in the progression of the disease (Anthony et al. in prep). The first stage involves multi-focal areas of bleached tissue, 1-2 cm in diameter, often in depressions on the colony surface. In phase 2, the bleached tissue degenerates, leaving an area of bare, white skeleton. In phase 3, areas of bare skeleton are covered with a white, anoxic bacterial film. In phase 4, a black deposit accumulates under the white film, giving the lesion a grayish appearance (the stage described by Jones et al. 2004).

Cyanobacterial Syndromes (other than BBD): These include cases of unidentified cyanobacteria that appear distinct, both in colour and morphological dimensions, from BBD-associated cyanobacterial filaments. Cyanobacteria aggregate along fronts at the interface between exposed skeleton and tissue and are associated with tissue mortality (Willis et al. 20004).

Gorgonian Black Necrosing Syndrome: Little is known about gorgonian diseases on the GBR. The only study of GBR gorgonians to date reports that 10% of populations of *Isis hippuris* on Davies Reef were infected with a fungal disease that manifested as black necrotic areas and led to loss of both tissues and skeleton (Morrison-Gardiner 2001). Although two species of *Penicillium* isolated from infected gorgonians were able to infect healthy colonies of *I. hippuris* and *Pinnigorgia* sp., and could be re-isolated, they did not produce the typical symptoms of the disease (Morrison-Gardiner 2001). Black necrotic patches have also been observed on gorgonians at Lizard Island and have been referred to as black necrosing syndrome (Willis et al. 2004).

Indicators of Compromised Health

Pigmentation responses: Species of *Porites*, in particular, appear to respond to a variety of competitive, invasive and parasitic challenges by producing pink or purple pigmentation in coenosarc and polyps adjacent to sites of competitive interactions and lesions. Hence pink lines, rings, patches or spots are often visible in coral tissue bordering the margins of competing or boring organisms (Willis et al. 2004). The pigmentation appears to be part of a generalized response mounted by the coral to contain invading or competing organisms such as cyanobacteria (Ravindran and Raghukumar 2002), polychaetes, molluscs, and the intermediate metacercariae stage of the digenetic trematode, *Podocotyloides stenometra* (Aeby 1998). Although most commonly observed on *Porites*, pigmentation responses have been observed on most genera.

Algal overgrowth*:* Algal filaments growing directly on live coral tissue may result in small areas of bleaching and subsequent coral mortality or sediment may accumulate under the algae leading to small areas of bleaching and subsequent tissue loss (Willis et al. 2004). Such cases are differentiated from coral-algal competitive interactions where only occasional contact is made between the coral and algae. On reefs in the central GBR, examples that appear to cross the boundary between competitive interactions and disease include overgrowth of coral by (1) the filamentous algae, *Coralliophila hurysmansii* causing tissue swelling, and (2) by *Anotrichium tenue*, which traps mucus, sediments and possibly microbes damaging the underlying tissues (McCook et al. 2001). It is unclear at this stage whether some other stress or pathogen has previously weakened the corals' resistance allowing algae to invade their tissues; hence such cases are categorized as an indication of compromised health (Willis et al. 2004).

***Unusual bleaching patterns**:* Distinct and unusual patches, spots, stripes etc. of bleached tissue differ from typical patterns of whole colony bleaching or paling seen during thermal anomalies. The causes of these unusual bleaching patterns are unknown and it is unclear whether they are caused by specific stressors or pathogens, or if they represent more generalized stress responses. At present they are recorded as another indicator of compromised health.

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