<u>Signalment/History</u>: *Montastraea annularis* (EPA FLK9503B2) collected from Sand Key, Florida in March 1995 by Debbie Santavy.

Field Diagnosis: Presumed yellow blotch.



Figure 15 A & A'. Presumed yellow blotch observed on *Montastraea annularis*: **A**- example of gross disease signs on an affected colony, field photo, **A'**- closeup example of lesion (Esther Peters); fixed specimen photo unavailable.

Histopathology Description (Fig. 16):

(Comment: The quality of preparation is poor.)

Coenenchyme: Clumps of eosinophilic debris mixed with <u>pyknotic</u> nuclei are within the lumen of gastrovascular canals. This observation may be necrosis or <u>autolysis</u>, but was not determined.

Polyp: Gastrodermis of some mesenterial filaments contains large numbers of eosinophilic granular cells. Multiple foci of gastrodermal cells are dissociating and sloughing into the lumen of gastrovascular canals (autolysis, Fig. 16C). Within the gastric cavity are accumulations of golden-brown granules. Within cnidoglandular bands are intracellular accumulations of golden-brown pigment (normal).

Skeleton: There are clumps of golden-brown debris (Fig. 16A, \uparrow) adjacent to a loose mat of thick non-septate, irregular walled fungal hyphae (Fig. 16B, \uparrow). These appear to be in the skeleton.

Morphologic Diagnosis: Undetermined.

Recommendation: None.



Figure 16 A-C. Photomicrographs of diseased *Montastraea annularis* tissue fixed in Helly's and stained with MHE: A- 10x, golden brown debris in the gastrovascular canal region that has lost its integrity (\uparrow); B- 40x, loose mat of non-septate, irregular walled organisms within skeleton (\uparrow); C- 20x, golden brown debris in the gastrovascular canal lumen (\uparrow).

Signalment/History: Acropora palmata (RTLA 5091) collected from Tague Bay, US Virgin Islands on 28 July 1981 by Esther Peters.

Field Diagnosis: Colony not showing disease, but located in the vicinity of colonies with external signs of presumed white band disease.



Figure 17 A. Acropora palmata exhibiting signs presumed to be white band disease (Esther Peters): both field and fixed specimen photos unavailable for this sample.

Histopathology Description (Fig. 18):

Coenenchyme: There is multifocal full thickness fragmentation of surface body wall on one end of the section accompanied by sloughing of gastrodermis. Multifocally, within deep basal body wall epithelia and surface body wall mesoglea is denuded (\uparrow). Occasional bacterial aggregates (basophilic rods appearing eosinophilic) are within mesoglea of calicodermis. Zooxanthellae were diffusely interspersed in the gastrodermis, but this may suggest species variation. There are focally extensive areas where no zooxanthellae were seen in gastrodermis of surface body wall. There are free zooxanthellae within gastrovascular canals (\triangleright).

Polyp: Cnidoglandular band cells are focally dissociated. Ova and spermaries were present.

Skeleton: No remarkable lesions seen.

Morphologic Diagnosis:

- Moderate decrease of zooxanthellae in the gastrodermis of the coenenchyme.
- Mild multifocal bacterial aggregates in the basal body wall of the coenenchyme.
- Mild multifocal fragmentation of surface body wall of the coenenchyme.

- Mild multifocal loss of the surface body wall and calicodermis of the coenenchyme.
- Mild focal dissociation of the cells in the cnidoglandular band of the polyp.

Recommendation: None.



Figure 18. Photomicrograph of *Acropora palmata* tissue fixed in Helly's and stained with MHE: A- 20x, showing denuded mesoglea (\uparrow) and free zooxanthellae in the gastrovascular canals (\triangleright).

<u>Signalment/History</u>: *Montastraea faveolata* (IRCP 144B) collected from Weimburg, Puerto Rico in April 2004 by Ernesto Weil.

Field Diagnosis: Presumed white plague.



Figure 19 A & B. Presumed white plague observed on *Montastraea faveolata*: A- similar field photo (Robert Jonas); B- fixed specimen (Kathy Price).

Histopathology Description (Fig. 20):

Coenenchyme: Clumps of gastrodermal cells are within the lumena of gastrovascular canals. Diffusely, zooxanthellae appear to have a granular eosinophilic cytoplasm, the <u>pyrenoid body</u> is often difficult to visualize, and <u>tinctorial contrast</u> is lacking (loss of viability).

Polyp: Within one polyp, there is diffuse necrosis of gastrodermis characterized by small fragments of cytoplasm and nuclear debris free in the lumen accompanied by free zooxanthellae; this is more prevalent in the mesenteries but also found in surface body wall (Fig. 20B&C, \blacktriangleright). Within areas of sloughing gastrodermis, zooxanthellae appear to have a granular eosinophilic cytoplasm, the pyrenoid body is often difficult to visualize, and a tinctorial contrast is lacking (loss of viability). There is an unstructured fibrillar <u>amphophilic</u> mass that appears to be mesoglea and which is surrounded by extracellular zooxanthellae (Fig. 20C, \uparrow). Epidermis and gastrodermis (Fig. 20D, \uparrow). There is a focus of amorphous eosinophilic debris with free zooxanthellae within a single cnidoglandular band. Spermaries noted.

Skeleton: There are mats of mixed fine eosinophilic filaments and thick irregular septate amphophilic structures (endolithic organisms) (Fig. 20A, ↑).

Other: At the periphery of the entire section, tissue lysis is suggestive of collection artifacts (\uparrow) .

Morphologic Diagnosis:

• Moderate, diffuse, necrosis, gastrodermis, mesentery and surface body wall, one polyp. <u>Addendum</u>: Upon review of the gross lesion, it appears that the lesion

described above as peripheral collection artifact was actually part of the gross margin of tissue loss (the grossly observed lesion).

Amended Morphologic Diagnosis:

• Diffuse gastrodermal necrosis, circumferential polyps.

Recommendation: Perform a silver stain for demonstration of fungi.



<u>Signalment/History</u>: *Acropora cytherea* (USGS 15440-006B) collected from Johnston Atoll, Central Pacific on 30 March 2001 by Thierry Work.

Field Diagnosis: Growth anomaly.



Figure 21 A. Malformed *Acropora cytherea*: **A**-field photo (Thierry Work); fixed specimen photo unavailable.

Histopathology Description (Fig. 22):

Coenenchyme: Multifocal, poorly demarcated, unencapsulated regions of proliferation of haphazardly folded calicodermis with decreased space separating them (increased soft tissue density relative to skeletal density) are present (Fig. 22A, \uparrow). These regions are composed of well-differentiated gastrodermis and calicoblastic epithelium (+/- increased cell density) and fewer polyps. Gastrodermal cells within the regions of proliferation are cuboidal and well-differentiated with round <u>euchromatic</u> nuclei and vacuolated cytoplasm. Calicoblastic epithelial cells are also well differentiated with euchromatic nuclei (Fig. 22B, \uparrow). Mitoses are not observed. This region is covered by <u>surface body</u> wall; multifocally, the gastrodermis has few to no zooxanthellae and the epidermis lacks mucocytes. Mesoglea in the deep calicodermis is focally denuded.

Polyp: Within the regions of proliferation, there is general absence of polyps. Existing polyps are haphazardly arranged within the peripheral regions of the masses (Fig. 22C, \uparrow).

Skeleton: No remarkable lesions seen.

Morphologic Diagnosis:

• Multifocal extensive hyperplasia of gastrovascular canals with an absence of zooxanthellae in the coenenchyme:

- chaotic arrangement of scant polyps and,
- overlying surface body wall lacks mucocytes and spirocysts.

Recommendation: Conduct immunohistochemical stains for calicoblastic epithelium.



Figure 22 A-D. Photomicrographs of diseased *Acropora cytherea* tissue fixed in Helly's and stained with MHE: **A-** 10x, haphazardly folded basal body wall (\uparrow); **B-** 10x, high density of well-differentiated calicoblastic epithelial cells (\uparrow); **C-** 4x, chaotic arrangement of polyps; **D-** 10x, showing metazoan organism (\uparrow).

Signalment/History: *Siderastrea siderea* (IRCP 97-1) collected from Maryland Shoals, Florida, on 15 July 2003 by Esther Peters.

Field Diagnosis: Presumed dark spots.



Figure 23 A & B. *Siderastrea siderea* observed with presumed dark spots disease: **A-** similar field photo (Esther Peters); **B-** fixed specimen (Kathy Price).

Histopathology Description (Fig. 24):

Polyp: There are two pieces of tissue on this histoslide. One was embedded so that the section is through the upper polyp region and there are nice cross-sections through the polyps above the oral disc, showing tentacular and septal covering tissues. There is no evidence of skeletal endolithic organisms in most of this piece. At one margin, there is one polyp that is cut through a slightly deeper plane on one side so that two mesenteries with oocytes are shown. Endolithic organisms are present in the septa adjacent to the gonad bearing mesenteries in this polyp (Fig. 24A, \uparrow). The other piece of tissue was embedded as a longitudinal section of polyps from the colony margin, which had appeared "dark" when collected. The lesion edge is colonized by mixed plants and animals (Fig. 24B, \uparrow). There are few zooxanthellae within gastrodermis, and those present were often shrunken with pale granular cytoplasm with indistinct cell walls. Large variably sized vacuoles distort the epidermis and gastrodermis of surface body wall and gastrodermis of mesenteries (species variation).

Coenenchyme: No remarkable lesions seen.

Skeleton: The skeleton shows an increased density of endolithic organisms aborally. The calicoblastic epidermis adjacent to, and sometimes being touched by the endoliths, has swollen and vacuolated mucocytes and the contents of the acidophilic granulocytes are dispersed, or the epidermis is necrotic (Fig. 24A, \uparrow).

Morphologic Diagnosis:

• Severe diffuse degeneration of zooxanthellae.

- Endolithic mycosis.
- Full thickness disruption of the basal body wall at the leading edge of the endolithic fungi.

Recommendations:

- Stain with PAS and GMS to identify fungi.
- Examine histology of dark spot in species other than *Siderastrea*.
- Perform TEM on zooxanthellae.





Figure 24 A-C. Photomicrographs of diseased *Siderastrea siderea* tissue fixed in seawater:Z-Fix and stained with MHE: A- 40x, high density of endolithic organisms with cell lysis and granules (\uparrow); B- 10x, colonization of the lesion edge by epibionts (e.g., filamentous macroalgae and arthropods) (\uparrow); C- 10x, endolithic organisms.