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An experimental histopathological rating scale of sedimentation stress in the Caribbean coral *Montastraea cavernosa*

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Abstract Colonies of *Montastraea cavernosa* were exposed to daily applications of approx. 200–225 mg cm^{-2} sediments, during a four-week period, in order to investigate coral responses to increased sedimentation. Effects were assessed based on the histopathological condition of specimen tissues. Mild stress responses were noted as early as week one, including tissue swelling and polyp retraction, as well as changes in size and appearance of mucous secretory cells. As stress progressed, further inflammation of the mucous secretory cells was observed. Severe effects were observed in weeks 3 and 4, including atrophy of the outer epidermis, increased granularity and debris in gastrodermal cells of the middle and lower polyp region, as well as swelling and granularity of the calicoblastic epithelium. A semi-quantitative rating scale was developed to score tissue condition based on the histopathological changes observed in control and treated corals. Although some signs of stress were also present in some control colonies, statistical analyses indicated significant differences in histopathological condition between control and treated corals. Our results confirm previous research that sub-lethal effects of sedimentation on corals are complex, involving both localized and systemic cell injury. Our results offer insight with regards to the diagnosis of healthy vs. unhealthy condition in reef corals, and provide a framework to survey for cellular reactions to environmental stress in coral reefs.

Keywords: *Montastraea cavernosa*, sedimentation stress, histopathology

Introduction

Excessive sedimentation from dredging, drilling, agricultural runoff, as well as coastal and upstream deforestation constitutes one of the most persistent and insidious sources of human-induced stress to corals and coral reefs (Brown 1997; review by Rogers 1990). Increased sedimentation and turbidity can interfere with coral autotrophy and heterotrophy by 1) decreasing light quality and quantity which may lead to a decrease in photosynthetic productivity (see Phillip and Fabricius 2003), and 2) the sediment overload on the corals causes polyps to retract and thus interferes with the prey capturing apparatus (Anthony and Fabricius 2000). This may result in the re-direction of energy expenditures for clearing excess sediments, at the expense of other vital functions, such as calcification, reproduction, and immune response (Riegl and Branch 1995; Anthony and Lacombe 2001). This energetic imbalance may eventually reduce the fitness of individual colonies, leading to increased susceptibility to disease, partial death, and thus in alterations to community structure and function (Dodge and Vaisnys 1977; Rogers 1990; Hodgson 1993; Brown et al. 2002).

The studies by Peters and Pilson (1985) and Riegl and Bloomer (1995) have provided direct evidence of tissue atrophy and damage associated with experimental sedimentation stress. Coral tissues and cellular elements readily exhibit histological changes that lie outside the normal range that may indicate cellular dysfunction and/or damage. Based on this notion, the objective of this study was to investigate the stress response of *Montastraea cavernosa* to increased sediment exposure under controlled laboratory conditions. In particular, our goals were: 1) to use histopathological techniques to examine the morphological and structural changes associated with sedimentation stress; and 2) to develop a rating scale to rank tissue condition with potential application as a tool for assessing stress in the field associated with increased sedimentation.

Materials and methods

Thirty-two healthy-looking colonies of *Montastraea cavernosa* (~5–7 cm diameter) were obtained from the reefs off Fort Lauderdale, Florida. Four coral specimens were randomly placed in each of eight 20-liter tanks, filled with seawater, and receiving artificial illumination (8, 175-Watt, 10000 K metal halide bulbs, supplemented with 8, 40-Watt, 10000 K fluorescent bulbs, and 8, 50/50 actinic fluorescent bulbs). Water temperature ranged between 25 and 27 °C (mean ± SD = 26.1 ± 0.5 °C) and salinity between 33 and 35.5‰ (mean ± SD = 34.5 ± 0.7‰). Tank maintenance included feeding (every other day) with a commercial zooplankton mix, as well as 50%

water changes once a week. Prolific algal growth was removed during water changes and trace elements (Ca, Sr, Mb, I) were also added once weekly. Water quality conditions (pH, nitrate, nitrite, and ammonia) were monitored weekly, and nutrient sponges (Renew®) were placed in the filtration system to remove excess phosphates. Corals were acclimated under the above conditions for a 19-day period. After acclimation, four tanks were assigned as treatment tanks and the other four as control tanks.

Locally collected sand from potential dredge sites was freshwater-rinsed, oven-dried at 65 °C, and sieved through a 500 μm mesh to remove coral and shell fragments. The sand used was roughly composed as follows: 250–500 μm: 55.1 ± 0.3%; 150–250 μm: 33.6 ± 0.4%; 63–150 μm; 9.6 ± 0.4%; and <63 μm: 1.7 ± 0.1% (mean ± SD % of dry weight). The clean, dry sand was applied to the treatment tanks at a rate of ~100–112.5 mg cm^{-2} twice per day for 4 weeks (29 days), by evenly sprinkling it over the corals, similar to Rogers (1983) and Peters and Pilson (1985). Excess sand on the bottom of the tanks was siphoned once weekly.

Tank maintenance and illumination during the experimental phase were similar to acclimation conditions; mean water temperature in control and treatment tanks was 25.4 (± 1.0 °C SD) and 25.6 (± 1.3 °C SD) respectively (p >0.05, t-test) and mean salinity was 34.7 (± 0.5‰ SD) and 34.3 (± 0.8‰ SD) respectively (p >0.05, Mann-Whitney Rank Sum). Differences in mean temperature and salinity during the experimental and acclimation phases were not statistically significant (p >0.05, t-test; p >0.05 Mann-Whitney Rank Sum, respectively).

Prior to daily sand applications, all specimens were visually inspected for signs of stress, including, but were not limited to: 1) polyp extension/retraction, 2) polyp swelling, 3) unusual appearance of oral disk (enlargement, contraction, or protrusion), 4) changes in coloration (intensification, and/or bleaching), 5) increased mucus production, 6) active/inactive sediment removal; 7) loss of natural texture lines (apparent smoothing-out of tissue), 8) extrusion of mesenterial filaments, algal overgrowth, and 9) appearance of lesions and tissue necrosis.

At the end of each week, one coral was removed from each tank and fixed in commercial Z-Fix® solution for 18–24 h. Decalcified tissues were embedded, sectioned longitudinally (6–8 μm), and stained with Harris's Hematoxylin and Eosin. Three to ten polyps per section were examined at 400× and 1000× magnification. Histopathological condition was assessed on selected tissues and cellular elements, based on the following criteria: 1) swelling of mucous secretory cells (MSC) and changes in the appearance and staining properties of the mucous secretions, 2) accumulation of cell debris and tissue granularity, 3) changes in zooxanthellae densities (increase or decrease) and/or zooxanthellae degeneration, 4) swelling and granularity of the calicoblastic epithelium, 5) degenerative changes in mesenteries and mesenterial filaments, 6) associated organisms; protozoa, algal and fungal infiltrates, and 7) necrosis.

Results

Gross changes

Minor signs of stress were observed within the first week of sand application. These included: slight swelling (especially polyps) and increased mucous production. With increasing stress, swelling became more pronounced, to the degree that natural texture lines of the polyps started to disappear. At this time, polyps also exhibited various levels of tentacle extension/retraction. Marked visual signs of stress included the magnification of the above condition, as well as changes in coloration: intensification, dullness, or bleaching; only one case of bleaching was observed, affecting <20% of the colony. In some colonies, tissues exhibited a thinned-out and sunken-in appearance, which contrasted to the preceded swelling. Tentacles were tightly retracted and oral disks were closed, enlarged and sunken down, or projecting outward. Mucous sheets were also evident. Only one treated coral exhibited necrosis, which developed around an old lesion where sand tended to accumulate.

Histopathological condition

An increase in size and staining properties of the mucous secretory cells (MSC) primarily in the outer epidermis, coenosarc, oral disk, and the middle polyp region were conspicuously noticeable after the first week of sanding. In the middle polyp region slight tissue swelling and increased mucous secretions, particularly along the upper portions of the mesenteries, indicated mild levels of stress. In contrast, in the lower polyp region, no swelling was apparent, but mucous secretions became more abundant and stained more intensely.

With increasing stress, the outer epidermis showed a clear tendency for atrophy, which progressed from swelling of the MSC, to increased granularity of columnar cells, to thinning and decreased number and size of MSC (Fig. 1A–B). The consistency of the mucous also changed, becoming denser and intense-staining in some cases; mucus was completely absent in others. At this time, cilia were not clearly discernible at the top of the epithelial columnar cells. In the middle and lower polyp regions, the gastrodermal cells lining the gastrovascular cavity exhibited increased granularity and accumulation of debris (Fig. 1C–D). Mesenteries and mesenterial filaments also revealed changes, including reduced size and numbers of MSC, increased staining properties of MSC, increased granularity and debris, as well as increased numbers spirocysts, and eosinophilic and basophilic gland cells in the cnidoglandular bands. An augmentation in granularity and thickening of the calicoblastic epithelium were also conspicuously noticeable with increasing stress (Fig. 1E–F). Skeletal algal infiltrates also proliferated; they were observed in both control and treatment corals, however, more prevalent and abundant in treated corals.

Although external changes in coloration were apparent, no clear trend in zooxanthellae densities were

noticeable in the histological slides (except for one partially-bleached specimen; ~20%). However, degenerating zooxanthellae occurred mainly in the middle and lower gastrodermis. This was inferred from abnormal-looking zooxanthellae, as well as degraded chloroplasts in these polyp regions.

Based on our observations, the histopathological condition of tissues was ranked on a scale of 0 to 4, considering the types of tissue and cell alterations detected and their possible effect on the overall ability of corals to function normally. Specimens with clear structural integrity and no apparent swelling of MSC, or tissue granularity were rated as healthy (0.0); those with slight swelling and increased staining properties of the MSC were considered to be mildly stressed (1.0); those exhibiting widespread swelling of MSC, cell debris, granularity, and zooxanthellae degradation were described as moderately stressed (2.0); those exhibiting atrophy of the outer epidermis and/or calicoblastic epithelium, and augmented cell debris and granularity, particularly in mesenteries and middle and lower gastrodermis, were markedly stressed (3.0). Specimens exhibiting breakdown of normal cell architecture, thinned-out and granular mesoglea in the cnidoglandular band, pyknosis, and localized/ widespread necrosis, suggestive irreversible changes, were rated 4.0. A complete description of the range of tissue and cell alterations observed in histopathological examinations is presented in Table 1.

Summary of tissue condition scores for each week are indicated in Table 2. Although there was some variability in the histopathological condition within and between treatments, tissue alterations were consistently more severe in treated than control corals, and those differences were significant (p <0.05, Mann-Whitney Rank Sum) for weeks 1 through 3.

Discussion

In this study, daily sand applications of ~200 mg cm^{-2} resulted in localized to widespread degenerative tissue changes in specimens of *Montastraea cavernosa*. Only one case of necrosis was observed; this was limited to the periphery of an old lesion. No additional necroses were noticed. However, based on our results, we infer that a longer sediment exposure would have likely resulted in tissue death in several specimens.

In similar experiments, Peters and Pilson (1985) found mild/moderate stress levels after two-week sand applications in fed, zooxanthellate, *Astrangia danae* specimens. These levels of stress are comparable to our week 2 observations. In contrast, Rogers (1983) reported no effects in field sediment application experiments of ~200 mg cm^{-2} in specimens of *Montastrea annularis*, *Diploria strigosa*, and *Acropora cervicornis*. These differences are probably due to differing sediment tolerance levels among coral species (Rogers 1990), as well as to passive sediment removal by wave action in field conditions, as compared to the laboratory.

Table 1 Rating scale for tissue changes observed in histopathological examinations of experimental specimens of *Montastraea cavernosa*, based on the appearance of selected tissues and cellular elements (mucous secretory cells, mucoid material, nucleus, cytoplasm, and zooxanthellae), stained with Harris's Hematoxylin and Eosin (abbreviations: EP, epidermis; CE, calicoblastic epithelium; GD, gastrodermis; MS, mesenteries; MSC, mucous secretory cells; ZO, zooxanthellae).

Range	Epidermis and coenosarc	Middle polyp region	Lower polyp region
Normal 0	Good integrity and structure. Distinct nuclei, membranes, and nematocysts. No swelling. MSC more numerous around oral disk.	Abundant clear-staining MSC in MS. MSC in GD not swollen. MS and CE intact, clear structural integrity.	Scattered MSC. GD thick with clear structural integrity. CE intact, clear structural integrity.
Mild 1	Same + slight swelling of MSC.	Same + increase in MSC size and abundance of mucoid material.	Same + slight increase in mucous in GD, and changes in staining properties (darker).
Moderate 2	Widespread swelling of MSC. Change in staining properties (more dense-looking). Possible increase in ZO densities.	Increased number and of MSC, color intensifying. Increased mucoid material. Presence of granularity and cell debris in GD and CE.	Same + Presence of cell debris and biogenic accumulations in GD and CE. Increased mucoid material. Apparent degradation of ZO.
Marked 3	Cell atrophy, increased cell debris. Swollen and/or reduced number of MSC. EP appears flattened, cilia not discernible. Possible localized necrosis.	Cell atrophy. MSC begin to coalesce. Increased debris, and biogenic accumulations in GD and MS. Apparent degradation of ZO Atrophy of CE. Possible localized necrosis.	Increased cell debris, and biogenic accumulations in GD and MS. Reduced number of MSC in MS. Atrophy of the CE. Possible gamete resorption/degeneration. Possible localized necrosis.
Severe 4	Atrophy; possible loss of cells. Clear loss of tissue integrity. Localized to widespread necrosis.	Atrophy. Increased cell debris and clear loss of tissue integrity. Localized to widespread necrosis.	Atrophy. Increased cell debris and clear loss of tissue integrity. Localized to widespread necrosis.

Table 2 Results of the histopathological rating for the duration of the experimental manipulations: numbers are median of histopathological scores for all colonies in each experimental group and range of scores found in each group.

Week	Control	Treated
1	1.5 (1.5–2.0)	2.5 (1.5–3.0)
2	1.5 (0.5–2.0)	2.0 (1.5–3.0)
3	2.5 (1.5–3.0)	3.0 (2.5–4.0)
4	3.5 (1.0–3.5)	3.7 (2.5–4.0)

Coral behavioral responses for particle removal include hydrostatic inflation of polyps (swelling), ciliary activity, and mucus production (Hubbard and Pollock 1972; Lasker 1980; Peters and Pilson 1985; Stafford-Smith 1993). At the beginning of this study, when sand was applied, polyps contracted but streams of sand started to trickle down the side of colonies within minutes and colonies removed all sediment in approximately 1–2 h after application. In histological sections, the effects of sanding were observable as early as week 1, including mild to moderate polyp swelling, as well as increased size and number of MSC in the tentacles, coenosarc, and oral disk. Interestingly, during week 2, swelling of the MSC decreased a bit, suggesting an improved tissue condition compared to week 1.

After week 2, sediment removal efficacy diminished and it took corals longer to remove all the sand (4–6 h). Sediment accumulation generally occurred over the coenosarc, while tentacles and mouths remained clear. This prolonged exposure to increased sedimentation resulted in color changes (intensification, dullness, or bleaching), severe swelling or deflation of polyps, unusual appearance of the oral disk, as well as extrusion of mesenterial filaments. Histological slides of treated corals corroborated these gross changes, revealing variable degrees of atrophy of the outer epidermis. This condition was indicated by: 1) reduced staining properties of sub-cellular elements such as nucleus, membranes, and nematocysts; 2) decreased number and size of MSC; 3) decreased or seemingly absent mucous production; 4) increased granularity of the columnar cells; and 5) apparent reduction/loss of cilia along the outer membrane. Loss of cilia and reduced mucous production were most likely responsible for the diminished capacity to shed sediments exhibited by the treated corals. Our observations are supported by Schumacher (1979) who found that increasing sediment loads resulted in decreased number of functioning mucous cells, as well as a reduced ability to replace them.

Early manifestations of sub-lethal cell damage include swelling of membrane-bound organelles, particularly endoplasmic reticulum and mitochondria. Further insult can lead to vacuolation and eventually to destruction of mitochondrial inner membranes (cristae) (Peters 2001; Stevens et al. 2002). In our study, evidence of marked sedimentation stress also included increased granularity and debris of tissues in the middle and lower polyp regions, particularly gastrodermal cells, mesenteries (cnidoglandular band), and calicoblastic epithelium (see Table 1). In week 3, 50% of the treated corals exhibited this condition. It is possible that these structural changes resulted from the swelling of internal organelles in concert with degradation of zooxanthellae. By week 4, all treated corals presented the above structural changes, and 50% of these also exhibited early signs of irreversible damage in localized portions of the cnidoglandular bands (i.e., granular mesoglea and breakdown of normal cell architecture). These changes probably represent a critical threshold at which long-term survival is decreased.

In this experiment, colonies of *Montastraea cavernosa* appeared in adequate health condition during the three-week acclimation period. In a few spare specimens that were sacrificed after acclimation, only low levels of stress were observed in histological slides. These probably relate to closed-system tank conditions. Within the first two weeks, the health condition of treated corals decreased, but control corals remained comparable to acclimation conditions. In week 3 however, two of the control corals exhibited gamete resorption (Stage II spermaries), and in week 4, all control corals showed localized granularity in some mesenterial filaments; in particular one specimen exhibited localized breakdown of normal cell architecture in several mesenterial filaments. These anomalies resulted in higher tissue condition scores for weeks 3 and 4. It could be speculated that a functional atrophy associated with the mesenterial filaments may have occurred during the last two weeks, or that subtle changes in water conditions in the control tanks caused such tissue changes. Further research is necessary.

Our results show that sediment applications twice a day for 4 weeks were enough to cause sub-lethal cellular damage in treated specimens of *Montastraea cavernosa*. These changes probably resulted from a depletion of energy reserves through excess mucous production for sediment removal (Peters and Pilson 1985; Anthony and Lacombe 2001). This study confirms previous research that sub-lethal effects of sedimentation on corals are complex, involving both localized and systemic cell injury. The results of the present study advance our

understating with regards to the diagnosis of healthy vs. unhealthy condition in corals, and provide a framework to survey for cellular reactions to environmental stress in field conditions. Histopathology provides a powerful means to effectively study and assess the effects of environmental change on coral morphology, composition, and function (Peters 1984; Bythell et al. 2002). These techniques are relatively inexpensive and are standard in ecotoxicology and stress biology research. The use of these techniques is a desirable component of surveys and monitoring programs aimed at assessing coral and coral reef health status.

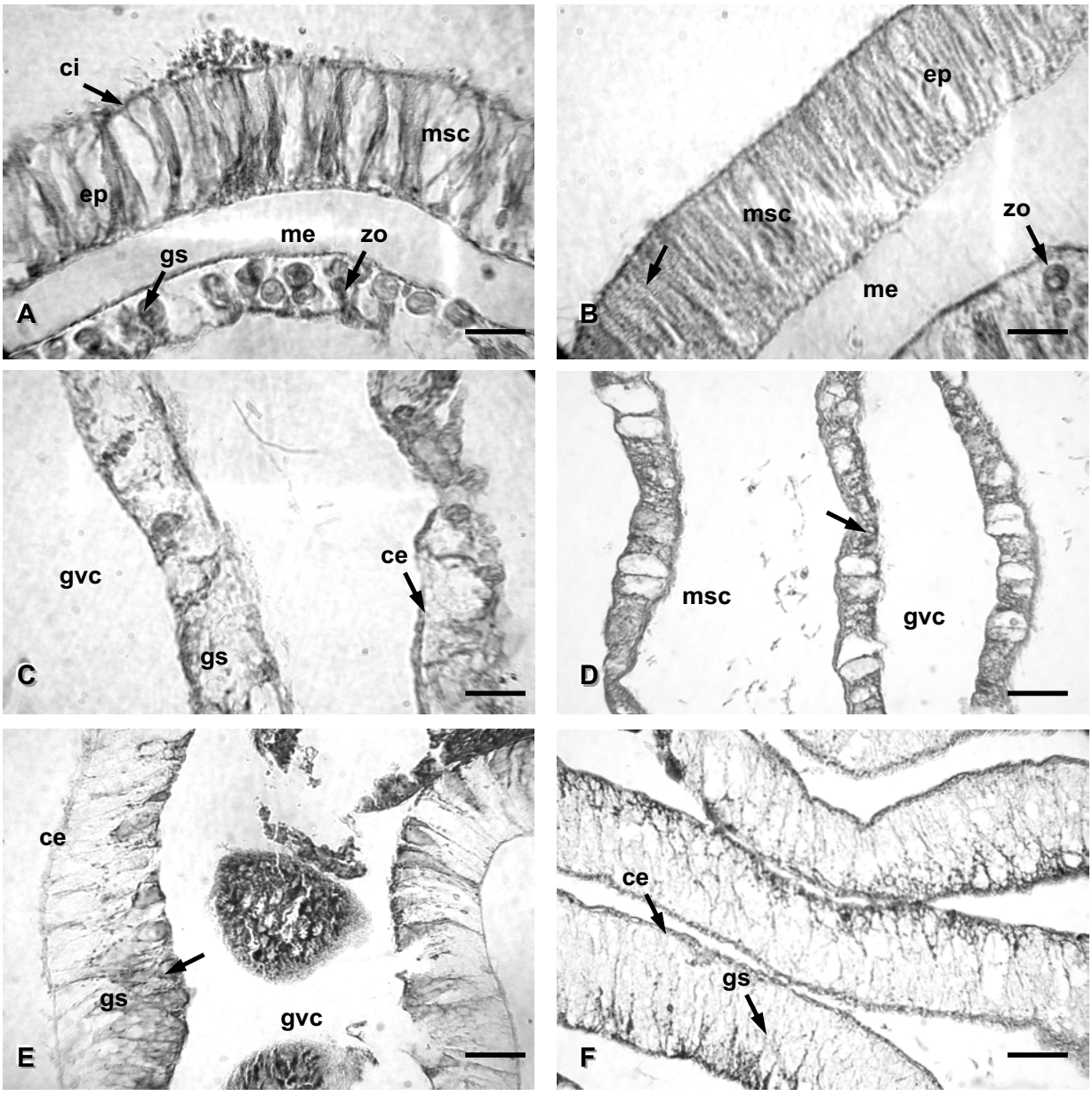

Fig 1 Photomicrographs of *Montastraea cavernosa* tissues stained with H&E, illustrating condition of tissues during the increased sediment exposure experiment. (**A**) Longitudinal section through the coenosarc of healthy polyp, control week 1. (**B**) Epidermal atrophy in treated coral, week 3; note tissue granularity (arrow), reduced size of mucous secretory cells, and inconspicuous cilia. (**C**) Longitudinal section through middle-polyp region of healthy polyp, control week 2. (**D**) Middle gastrodermis showing enlarged mucous secretory cells and debris-filled gastrodermal cells (arrow) in treated coral, week 3. (**E**) Section through the lower gastrodermis of healthy polyp, control week 1; note normal-looking mucous accumulations in gastrodermis (arrows). (**F**) Section through lower polyp region of treated coral, week 4, showing debris-filled gastrodermis lacking mucous secretions and swollen calicoblastic epithelium (arrow). Legend: ci: cilia; ce: calicoblastic epithelium; ep: epidermis; gs: gastrodermis; gvc: gastrovascular cavity; me: mesoglea; msc: mucous secretory cell; zo: zooxanthellae. Scale bars: A, B, and C = 10 µm; D, E, and F = 20 µm.

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