

## ABSTRACT

Title of Dissertation:

### THE ROLES OF NUTRIENTS AND HERBIVORY IN CONTROLLING THE MICROBIOEROSION OF TROPICAL REEFS

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Coral reefs worldwide are suffering degradation from increasing fishing pressure, pollution, diseases, and coral bleaching. One important ecological consequence of such degradation is an increase in biological erosion, or bioerosion, of the coral framework by boring and grazing organisms. Therefore, it has become essential to understand the factors that control the processes and agents of bioerosion. The aim of my dissertation is to understand how organic and inorganic nutrients and herbivory affect the bioerosion of carbonate substrates by microbial endolithic organisms (bacteria, fungi and algae), an often overlooked component of bioerosion processes in coral reefs.

Results of controlled experiments using herbivore-exclusion cages and fertilizers at Glovers Reef, Belize consistently showed significant effect of nutrients in stimulating microbial endoliths' substrate colonization and bioerosion rates of *Strombus gigas* shells. The addition of inorganic nutrients increased bioerosion rates by a factor of 8 to 15 in comparison to control treatments. Changes in nutrient ratios changed microbial endolithic

community structure. The addition of nitrogen alone or in combination with phosphorus stimulated green algae, the addition of phosphorus alone stimulated cyanobacteria, and the addition of organic matter alone stimulated fungi. The inclusion of herbivores reduced observed bioerosion rates by half, demonstrating the importance of herbivory in modifying bioerosion processes.

Field experiments on the relationship between water quality and the amount of microbioerosion in *Lambis chiragra* shells in nine coral reefs in East Africa demonstrated that other factors within reefs may interact with nutrients in determining bioerosion rates. Results suggested that epilithic algal cover, particularly crustose coralline algae, may decrease microborer colonization and bioerosion rates by reducing light conditions within substrates, so that no direct effects of nutrients on bioerosion rates are detected.

A critical review of the evidence for nutrients as a primary control of bioerosion by different bioeroder groups (microborers, macroborers, and grazers) suggests that macroborer abundances reflect increases in nutrient conditions and may therefore represent a useful indicator of eutrophication and coral reef “health”.

This dissertation contributes to a better understanding of the factors affecting bioerosion by microbial endolithic organisms, which are important but often overlooked agents of bioerosion in coral reefs.

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MICROBIOEROSION OF TROPICAL REEFS

By

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## Foreword

Marina Carreiro Silva made substantial contributions to the jointly authored work presented as Chapter 2 of this dissertation. This chapter was published in 2005 in the journal *Coral Reefs* with TR McClanahan and William E. Kiene.

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## Executive Summary

This dissertation investigates the roles of nutrients (organic and inorganic) and herbivory as major controlling factors of microbial endolithic communities (bacteria, fungi, and algae) and their bioerosion rates. This was achieved by conducting experimental studies using herbivore-exclusion cages and fertilizers at Glovers Reef, Belize, and field studies using herbivore-exclusion cages on nine reefs in East Africa. Study sites in East Africa were characterized by variable nutrient concentrations and number of herbivorous fishes and sea-urchins.

Chapter 1 is an introductory chapter, explaining the terminology used in the field of microbioerosion. It also includes descriptions and illustrations of the boring traces of organisms observed in experiments in Belize and East Africa.

The following three chapters report the results of experimental studies in Belize. Chapter 2 investigates the effects of inorganic nutrients (nitrogen + phosphorus) and herbivory on microbial endolithic communities and their rates of bioerosion of *Strombus gigas* shells. Results from this study indicated that bioerosion rates by microbial endoliths were enhanced ten times by fertilization but reduced by half with the inclusion of herbivores. This study provides the first direct experimental evidence of the influence of nutrients on microborer bioerosion rates and emphasizes the critical role of herbivory in influencing microborers. Herbivorous fish influence bioerosion rates by feeding on endolithic algae, thus decreasing measurable bioerosion rates, and potentially masking nutrient effects on microbioerosion. Results of this study have been published in the journal *Coral Reefs* (Carreiro-Silva et al. 2005 vol. 24: 214-221).

Chapter 3 examines the interaction between inorganic nutrients and organic matter fertilization on microbial endolithic community composition and bioerosion rates. Results revealed that the addition of organic matter alone increased the relative abundance of endolithic fungi, changing microbial endolithic community structure. Bioerosion rates were not affected by organic matter additions, but were increased by a factor of 8 to 9 with the addition of inorganic nutrients, suggesting that inorganic nutrients are a more important controlling factor of microbioerosion than organic nutrients.

Chapter 4 reports on the response of microbial endoliths to different proportions of nitrogen and phosphorus. Results suggest that variations in nutrient ratios can modify endolithic community composition. The addition of nitrogen alone or in combination with phosphorus (high N:P) stimulated green algae, whereas the addition of phosphorus alone (low N:P) stimulated cyanobacteria.

Chapter 5 describes spatial patterns of microbioerosion in eight reefs along the Kenyan coast and one reef in Northern Tanzania. The objective of this study was to determine the relative importance of nutrients and herbivory in controlling patterns and rates of microbioerosion, and to examine how these interact with other physical-chemical and ecological variables in reefs. Results demonstrated that epilithic algae cover, in particular crustose coralline algae, interacts with nutrients in determining bioerosion rates. Crustose coralline algae may decrease microborer colonization and bioerosion rates by reducing light conditions within substrates, so that no direct effects of nutrients on bioerosion rates are detected.

Finally, Chapter 6 reviews and synthesizes evidence for the effects of nutrients on bioerosion by different groups of bioeroders (microborers, macroborers, and grazers) within different geographic regions. It discusses the effect of bioerosion in the inorganic and organic carbon cycle of reefs, identifies the main factors controlling bioerosion rates, and discusses the usefulness of bioerosion rates as indicators of nutrient enrichment and coral reef “health”. Critical examination of nutrient effects on bioerosion shows that reef macroborers most directly reflect increases in nutrient conditions. While there is experimental evidence that microborers are also influenced by nutrient enrichment, field studies have produced variable results because of interactions with epilithic algae cover and grazing pressure. Sea-urchins appear to be more directly controlled by over-harvesting of their predators. However, they may also be influenced indirectly by increased nutrients, particularly in reef areas that have also been affected by bleaching, where increased algae growth on the newly-available substrate becomes a source of sea-urchin food. High bioerosion rates by herbivorous fish appear to be normal in healthy reefs. Evidence suggests that macroborers and sea-urchins are the groups of bioeroders that most directly reflect human pressures, such as nutrient enrichment and overfishing. Therefore, in reef monitoring programs, macroborer abundance may be a useful indicator of eutrophication, with sea-urchin numbers a reliable indicator of over-fishing of their predators. Because high bioerosion rates can also be obtained in “pristine” reefs (mainly due to high abundances of herbivorous fishes), bioerosion rates should be used as indicators of reef health only when used together with rates of carbonate accretion to determine if the reef is accreting or eroding.

## Chapter 1

# A description of microbial endoliths in mollusk shells from Belize and East Africa coral reefs

### Introduction

Microbial endoliths, or microborers, are a diverse group of specialized microorganisms (bacteria, fungi, and algae) that, through chemical dissolution, penetrate and live within hard calcareous substrates (Golubic et al. 1975; Golubic and Schneider 2003; Radtke and Golubic 2005). They are found in terrestrial, freshwater, and marine environments, and cope with an array of ecological conditions ranging from extremes of polar and alpine deserts to the most stable environments in tropical reefs (Golubic et al. 1975; Golubic and Schneider 2003). In coral reefs, microbial endoliths inhabit a multitude of carbonate substrates (Golubic and Schneider 2003; Tribollet et al. 2006), including skeletons of live and dead corals (Le Campion-Alsumard et al. 1995a), mollusk shells (Radtke 1993; Kiene et al. 1995; Mao Che et al. 1996; Vogel et al. 2000), limestone rocks (Schneider and Torunski 1983), and loose carbonate sediment grains (Tudhope and Risk 1985).

Microbial endoliths play important ecological and geological roles in reef environments. Recent experimental work by Tribollet et al. (2006) has demonstrated that endolithic phototrophs (cyanobacteria and algae) are one of the major primary producers in coral reef ecosystems, with rates of net photosynthesis of as much as  $2\text{g C m}^{-2}\text{ day}^{-1}$ . In addition, studies on endolithic algae living within live coral skeletons suggest that endolithic algae may provide an alternative source of energy to bleached corals, enabling

them to survive until the recruitment of new zooxanthellae (Fine and Loya 2002). Another potentially important, but not fully understood, role of microbial endoliths living within coral skeletons is the role of endolithic fungi as pathogens of live corals. Several authors suggest that endolithic fungi are part of the microbial community living in the skeletons of healthy corals, but under environmental stress conditions that affect the calcification ability of corals (e.g., increased atmospheric CO<sub>2</sub>, elevated sea surface temperature or coastal eutrophication), fungi may become opportunistic pathogens, penetrating the coral skeleton and entering the coral tissue (Le Campion-Alsumard et al. 1995b; Bennis et al. 2000).

Geologically, microbial endoliths participate in the erosive morphogenesis of coastal limestone and other carbonate substrates (Schneider and Torunski 1983; Radtke et al. 1996), in the production of fine grain sediments, and in the modification of sediment grains by micritization (Schneider and Torunski 1983).

Experimental studies have demonstrated that microbial endoliths are important but often overlooked agents of bioerosion involved in the breakdown of coral skeletons (Chazottes et al. 1995, 2002; Tribollet et al. 2002, Tribollet and Golubic 2005), representing a significant destructive force in a reef's calcium carbonate budget. The role of microbial endoliths as bioerosion agents is particularly important in reef areas experiencing eutrophication. Fertilization experiments in Belize, as part of this dissertation, have shown that bioerosion rates by microbial endoliths were enhanced 8 to 15 times by fertilization with inorganic nitrogen and phosphorus (Carreiro-Silva et al. 2005; Chapters 3 and 4). In addition to the erosion caused by their boring activity, microbial endoliths reinforce bioerosion of carbonate substrates by facilitating the

recruitment of macroborers (worms, sponges and mollusks) and by making such substrates attractive for grazers as a source of food (Chazottes et al. 1995; Pari et al. 1998).

Microbial bioerosive activity has a long geological history that has left recognizable and fossilized traces of the microbes' boreholes and boring patterns in the substrate (Golubic et al. 1975; Golubic and Schneider 2003). The oldest boring traces to be found correspond to cyanobacteria and are dated from the Proterozoic (1,700 Mya: Zhang and Golubic 1987). Microbial endoliths became more abundant and diversified during the course of the Phanerozoic, paralleling the evolution of skeleton-bearing animals that provided a variety of substrates for endolithic activity (Le Campion-Alsumard and Golubic 1985).

Through the preparation of resin casts that faithfully replicate the shapes of the boring tunnels within the substrate (resin casting technique: Golubic et al. 1970; 1975), microbial endolithic traces can be studied in ancient and modern substrates. Comparisons of modern borings that contain resident endoliths to ancient traces provide a biological interpretation of traces (Radtke and Golubic 2005).

The distribution of microbial endolithic trace communities has been shown to be strongly correlated to light availability and hence boring traces have been used as indicators of past environmental conditions and paleobathymetric estimates (Glaub 1999; Vogel et al. 1999). Based on this relationship, a set of bathymetric indexes of trace fossil assemblages has been established; trace communities characterizing the upper photic zone are dominated by chlorophytes and cyanobacteria, the lower photic zone is characterized by chlorophytes and rodophytes, and fungi characterize aphotic settings

(Glaub 1999). Studies on ancient and modern trace assemblages in tropical (Caribbean, Pacific Ocean and Red Sea) and temperate (Mediterranean Sea and NE-Atlantic) settings showed consistency in trace communities' zonation patterns (Wisshak et al. 2005; Gektidis et al. 2007).

Within this context, the relationship of microbial endoliths and nutrient conditions in reef waters that I have investigated in this dissertation has a potential application in understanding paleo-environmental indicators. Hence, microbial endolithic community composition, or specific species, could be used as indicators of enrichment by nitrogen, phosphorus and particulate organic matter in the fossil record.

### **Nomenclature used in the characterization of microbial endoliths**

Because trace fossils are not actual organisms or parts of organisms, they cannot be given Linnaean names recognized by the ICBN (International Code of Botanical Nomenclature) or ICZN (International Code of Zoological Nomenclature) (Bertling et al. 2006). Therefore they are named and classified separately from the organisms that made them (Vogel et al. 1999; Golubic and Schneider 2003; Bertling et al. 2006).

The study of trace fossils is the discipline known as ichnology (from the Greek *ichnos*, footprint: Bertling et al. 2006). Morphologically distinctive trace fossils are given genus (ichno-genus) and species names (ichno-species), while boring trace communities are referred to as ichnoceonoses.

The study of microbial endolithic traces has also enabled the quantitative assessment of the bioerosion caused by microbial endolithic organisms. Through measurements of density and depth of penetration of microbial endolithic traces in the



substrate, we can estimate the volume of calcium carbonate removed by different microbial endolithic organisms. Because this quantification is achieved through measurements of boring traces and not by measurements on the organisms themselves, a dual nomenclature is applied to microbial endoliths. Thus they are referred to by their ichno-species as a morphological classification of the traces and by the biological nomenclature that classifies the endolithic organisms that produced those traces (bio-species). For example, the boring trace *Eurygonum nodosum* is produced by the cyanobacterium *Mastigocoleus testarum*.

### **Characterization of microbial endoliths in mollusk shells from Belize and Kenya**

In this section I document 17 traces and their producers found in mollusk shells from Belize, Kenya, and one reef in Northern Tanzania. These traces include one trace corresponding to a species of coccoid bacterium\*, five corresponding to fungi, seven to cyanobacteria, and four to chlorophyta. I present the microbial endoliths and their traces according to their taxonomic classification and describe their morphology and ecology.

The following publications were used for the identification of microendoliths: Zebrowski (1936); Le Campion-Alsumard (1979), Lukas and Golubic (1981), Lukas and Golubic (1983), Lukas and Hoffman (1984), Le Campion-Alsumard and Golubic (1985), Porter and Lingle (1992), Radtke (1993), Radtke and Golubic (2005), and Wisshak et al. (2005).

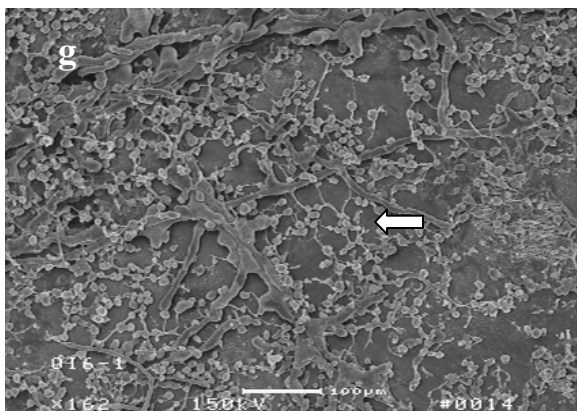
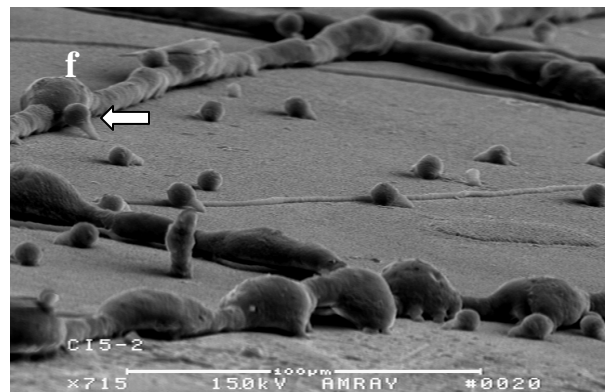
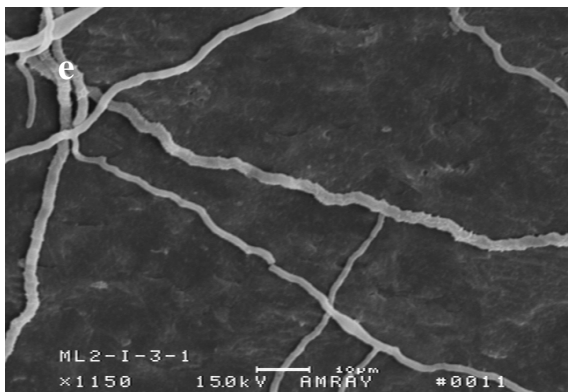
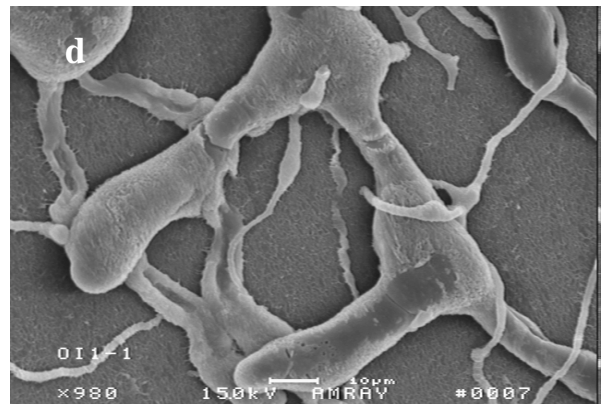
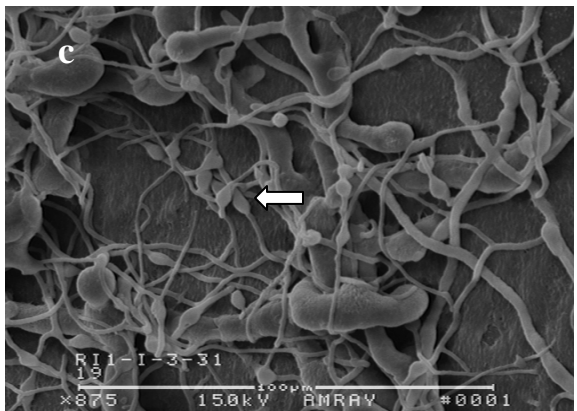
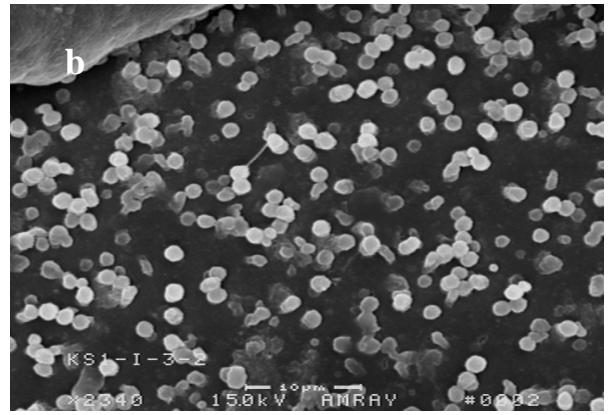
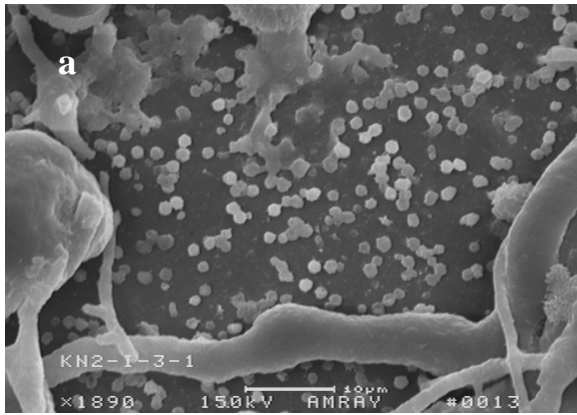
**Bacteria** \* Boring bacteria belong to the chemoorganotrophic groups (DiSalvo 1969).

Although the exact boring mechanism of endolithic bacteria remains unknown, it is

suspected that they bore into the substrate by secreting organic acids (e.g. oxalic, fluconic, citric, acetic: DiSalvo 1969). In this study, a “coccoid form” with diameters of 0.3-0.5  $\mu\text{m}$  was interpreted as a bacterium trace (Fig. 1.1a, b). However the specific species that produced this trace is unknown.

Traces by coccoid bacteria have been rarely described in the literature. The only reported observations correspond to traces in the skeleton of deep-sea coral *Lophelia pertusa* (Beuck and Freiwald 2005). In my experimental studies in Belize I found coccoid bacteria traces in only one sample in the organic matter treatment. In Kenya, coccoid bacterial traces were recorded in only three reefs (Kanamai, Ras Iwatine and Kisite) and occupied less than 2% of substrate cover.

**Figure 1.1** Scanning electron micrographs of traces produced by boring heterotrophic organisms. (a) Coccoid bacteria traces; (b) detail of (a); (c) boring trace *Orthogonum fusiferum* produced by the fungus *Ostracoblabe implexa* (arrow). Thicker filaments are the boring trace *Rhopalia catenata* produced by the green algae *Phaeophila* sp.; (d) *Orthogonum fusiferum* tunnel penetrating *Rhopalia catenata*'s tunnel; (e) boring trace *Orthogonum lineare* produced by an unidentified fungus; (f) boring trace *Saccomorpha clava* (arrow) produced by the fungus *Dodgella priscus*; (g) *Rhopalia catenata* and the boring trace *Saccomorpha spherula* (arrow) produced by the fungus *Lithopythium gangliiforme*; (h) Detail of (g).



**Fungi** Endolithic fungi that bore into mollusk shells are found in the orders Saprogeniales, Peronosporales (Oomycota) and Chytridiomycetes (Eumycota) (Bornet 1891; Zebrowski 1936; Porter and Lingle 1992; Golubic et al. 2005). Their penetration into the substrate is thought to be achieved through the secretion of acids (e.g., sulfuric or nitric acid) secreted apically by their hyphae (Porter and Lingle 1992), although this still needs confirmation (Golubic et al. 2005). Being heterotrophic, endolithic fungi are light-independent and can be found in all depths ranging from shallow waters to abyssal depths (Golubic and Schneider 2003; Golubic et al. 2005). Thus the presence of their traces, in the absence of phototrophic microbial endoliths, has been used as indicators of aphotic environments in the fossil record (particularly the traces *Saccomorha clava* and *Orthogonum lineare*: Glaub 1999). Because endolithic fungi are difficult to cultivate in the laboratory, taxonomic classifications are usually difficult (Beuck and Freiwald 2005). Fungi are distinguished by their size, shape, mode of ramification and sporangia (Beuck and Freiwald 2005). Based on their morphology, boring fungi can be divided into two main groups: (1) vegetative, undetermined, branched filaments (hyphae) and (2) homogeneous hyphae with local or central swellings that function as reproductive chambers (sporangia). The sporangia harbour a large number of zoospores (Zebrowsky 1936).

**Ichno-species:** *Orthogonum fusiferum* Radtke 1991 (Fig. 1.1c)

**Bio-species:** *Ostracoblabe implexa* Bornet and Flahault 1889

**Trace morphology:** Trace characterized by fine tunnels of 1-2  $\mu\text{m}$  diameter, with spindle-shaped swellings (4-5  $\mu\text{m}$  diameter). The boring pattern often comprises rectangular branching galleries parallel to the substrate (after Radtke 1993).

**Geographic distribution:** *Orthogonum fusiferum* has been recorded in tropical and cold-temperate environments, down to 300 m depth (Radtke 1993; Vogel et al. 2000; Wisshak et al. 2005). This trace was observed in all my experiments in Belize, and was particularly common in the organic matter treatment (Chapter 2), often observed parasitizing *Phaeophila* spp. (Fig 1.1d). In East Africa, *Orthogonum fusiferum* was recorded in all reefs studied, with abundances reaching 15% substrate cover in some reefs.

**Ichno-species:** *Orthogonum lineare* Glaub, 1994 (Fig. 1.1e)

**Bio-species:** The producer of this trace is still unknown. However, because this trace has been recorded from water depths deeper than 500 m (Glaub 1999), it is certainly a heterotrophic organism (most likely a fungus).

**Trace morphology:** Smooth tubular galleries of near-constant diameter (10-15  $\mu\text{m}$ ) without swellings, oriented parallel to the substrate surface, and exhibiting predominantly perpendicular branching.

**Comments:** This trace is very similar to traces of another ichnospecies, *Orthogonum tubulare*. However, the majority of the traces encountered were closest to the diagnosis for *O. lineare* as given by Glaub (1999), and were distinguished from *O. tubulare* by more constant tube diameters, the absence of swellings at branchings, and blunt instead of tapering gallery endings. The occurrence of this trace in association with *Saccomorpha*

*clava*, and in the absence of phototrophs, is an indicator of aphotic environments in the fossil record (Glaub 1999).

**Geographic distribution:** *Orthogonum lineare* has been recorded in tropical and cold-water environments, down to 2350 m depth (Zeff and Perkins 1979; Radtke 1993; Beuck and Freiwald 2005; Golubic et al. 2005; Wisshak et al. 2005). This trace was recorded in both Belize and East Africa, but its abundance was always lower than 2% substrate cover.

**Ichno-species:** *Polyactina araneola* Radtke 1991 (not shown)

**Bio-species:** *Conchyliastrum enderi* Zebrowski 1937

**Trace morphology:** This trace has a general star-shape borehole, composed of a central chamber and radiating branches. The central chamber is variable in size (diameter 10 to 30  $\mu\text{m}$ ); can be spherical, hemispherical, or irregular; and can reach a depth of penetration of 350  $\mu\text{m}$ . The radiating branches (diameter 8-10  $\mu\text{m}$ ) are often constricted at their contact with the central chamber (after Radtke 1993).

**Geographic distribution:** *Polyactina araneola* has been recorded in tropical and cold-water environments, down to 800 m depth (Zeff and Perkins 1979; Budd and Perkins 1980; Radtke 1993; Beuck and Freiwald 2005; Golubic et al. 2005; Wisshak et al. 2005). This trace was very rare in Belize and was not recorded in East Africa.

**Ichno-species:** *Saccomorpha clava* Radtke, 1991 (Fig. 1.1f)

**Bio-species:** *Dodgella priscus* Zebrowsky (1936)

**Trace morphology:** This trace consists of sphere- to pear-shaped cavities measuring 10 to 30  $\mu\text{m}$  in diameter, which host the sporangia. These cavities are connected to the

substrate surface through narrow 4-6  $\mu\text{m}$  wide necks and are interconnected by narrow (1-2  $\mu\text{m}$  diameter) filaments (hyphae) protruding from the necks or sacs (after Radtke 1993).

**Comments:** Early stages of the borings may be missing the interconnecting filaments, and may resemble the cyanobacterium ichno-species *Planabola* isp. (Wisshak et al. 2005).

**Geographic distribution:** *Saccomorpha clava* has been recorded in tropical to cold-water environments, down to 2350 m depth (Zeff and Perkins 1979; Budd and Perkins 1980; Radtke 1993; Beuck and Freiwald 2005; Golubic et al. 2005; Wisshak et al. 2005). The occurrence of this trace in association with *Orthogonum lineare*, and in the absence of phototrophs, is an indicator of aphotic environments (Glaub 1999). This trace was rare in both Belize and East Africa.

**Ichno-species:** *Saccomorpha sphaerula* Radtke 1991 (Fig. 1.1g)

**Bio-species:** *Lithopythium gangliiforme* Bornet and Flahault 1889

**Trace morphology:** Trace characterized by spherical chambers (4 to 8  $\mu\text{m}$  diameter) interconnected with fine filaments (0.5  $\mu\text{m}$  in diameter). Filaments may be slightly curved or wavy and occasionally branched (after Radtke 1993).

**Geographic distribution:** *Lithopythium gangliiforme* has only been recorded in shallow tropical environments (Radtke 1993). This species was recorded in all experiments in Belize, and was particularly abundant in the organic matter treatment (Chapter 2). Here, dense layers of chamber clusters interconnected by a network of filaments were commonly found intermingled with *Phaeophyla* spp. filaments. The filaments of *L.*

*gangliiforme* seemed to penetrate *Phaeophila* spp. in some instances, suggesting that *L. gangliiforme* may parasitize *Phaeophila* spp. In East Africa, traces of *L. gangliiforme* were rare (Chapter 5).

**Cyanobacteria** Endolithic cyanobacteria are common inhabitants of carbonate substrates and have their highest abundance in the intertidal and shallow coastal waters less than 1 m deep (Le Campion-Alsumard 1979; Le Campion-Alsumard and Golubic 1985; Gektidis 1999; Glaub 1999). Species belonging to the genus *Hyella* constitute the majority of the species found in coastal environments (Le Campion-Alsumard and Golubic 1985). The boring traces *Fascichnus acinosus* produced by the cyanobacterium *Hyella balani* and the trace *Fascichnus dactylus* produced by the cyanobacterium *Hyella caespitosa* are the dominant traces in the intertidal zone and their abundance has been used as a paleobathymetric indicator of intertidal areas in the fossil record (Glaub 1999).

In zones of high light penetration, endolithic cyanobacteria tend to grow perpendicular to the substrate surface (Golubic et al. 1975), whereas in deeper photic zones, they predominantly grow parallel to the surface (Perkins and Tsentas 1976). Recently, Garcia-Pichel (2006) suggested three alternative mechanistic models for boring by cyanobacteria endoliths. The models are based on either the temporal or spatial separation of photosynthesis and respiration, and on the active extrusion of calcium ions through an active cellular uptake and transport process. Carbonate dissolution would be achieved by the production of CO<sub>2</sub> during endolithic respiration in the first two models, and by a calcium pump in cyanobacterial filaments in the third model. However, these models still need to be tested experimentally.



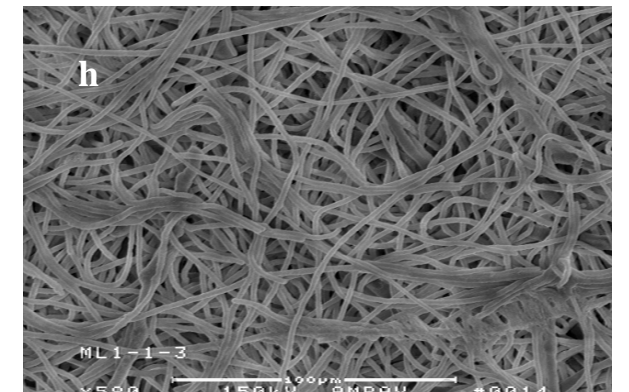
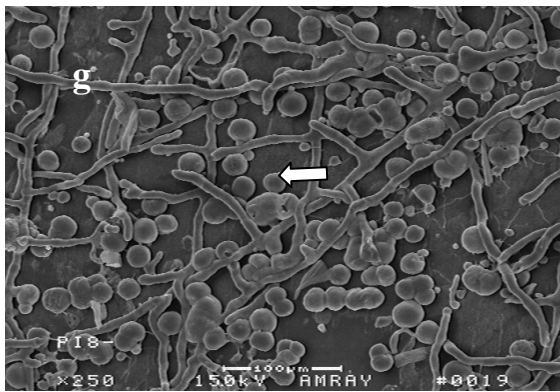
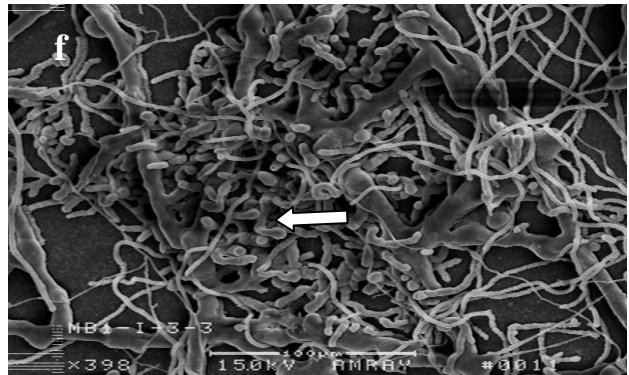
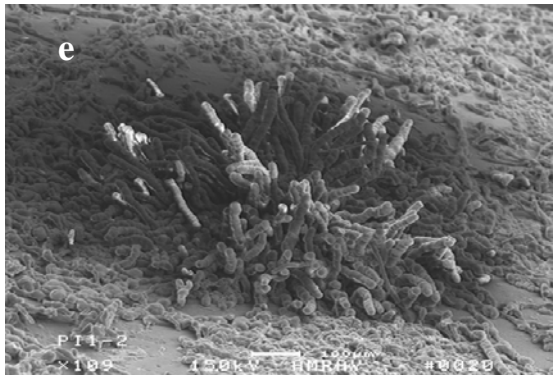
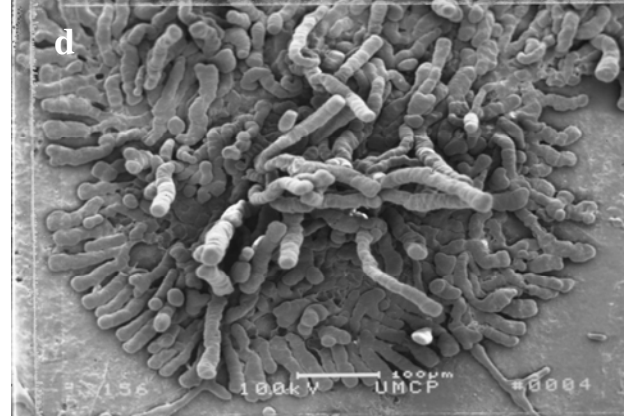
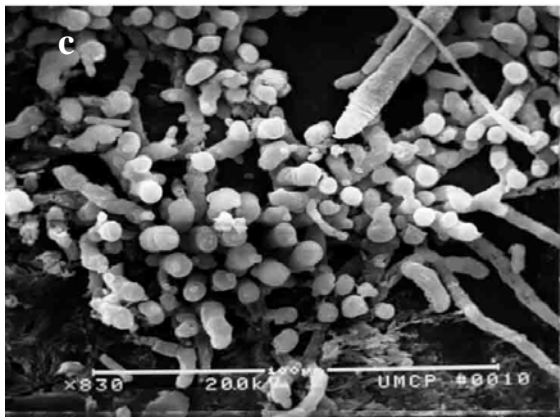
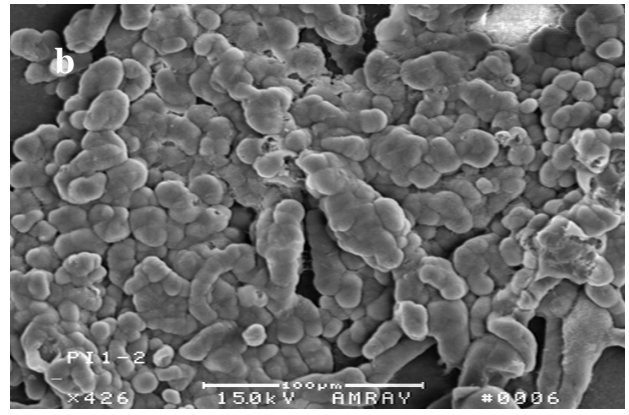
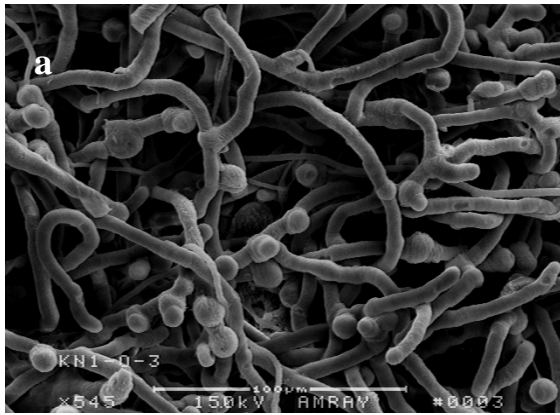
**Ichno-species:** *Eurygonum nodosum* Schmidt 1992 (Fig. 1.2a)

**Bio-species:** *Mastigocoleus testarum* Lagerheim 1886

**Trace morphology:** Boring pattern characterized by a shallow tunnel system with two types of branches: long branches straight or slightly curved (4 to 6  $\mu\text{m}$  in diameter); short lateral branches with terminal swellings (9 to 10  $\mu\text{m}$  in diameter) that contain heterocysts (after Radtke 1993).

**Geographic distribution:** *Masticoleus testarum* has a cosmopolitan distribution, and has been recorded down to 45 m (Perkins and Tsentas 1976; Radtke 1993; Wisshak et al. 2005; Gektidis et al. 2007). *Masticoleus testarum* traces were recorded in my experiments in Belize, increasing in abundance in shells fertilized with phosphorus alone (Chapter 4). In East Africa, *M. testarum* traces were recorded in all reefs studied, and were one of the dominant species, particularly during the first 3 months of exposure (Chapter 5)

**Figure 1.2** Scanning electron micrographs of traces produced by boring cyanobacteria. (a) Boring trace *Eurygonum nodosum* produced by the cyanobacterium *Mastigocoleus testarum*; (b) boring trace *Fascichnus acinosus* produced by the cyanobacterium *Hyella balani*; (c) boring trace *Fascichnus dactylus* produced by the cyanobacterium *Hyella caespitosa*; (d) boring trace *Fascichnus frutex* produced by the cyanobacterium *Hyella gigas*; (e) side view of *Fascichnus frutex*; notice the greater depth of penetration in comparison with other borings; (f) boring trace *Fascichnus parvus* (arrow) produced by the cyanobacterium *Hyella pyxis*; (g) boring trace *Planabola* isp. (spherical chambers) produced by the cyanobacterium *Cyanosaccus piriformis*; (h) boring trace *Scolecia filosa* produced by the cyanobacterium *Plectonema terebrans*.



**Ichno-species:** *Fascichnus acinosus* Glaub 1994 (Fig. 1.2b)

**Bio-species:** *Hyella balani* Lehmann 1903

**Trace morphology:** Colonies characterized by groups of tunnels, two or more, that are fused at the base of the colony (closer to the substrate surface) and separated at their distal parts. Tunnels have typical widths of 5 to 7  $\mu\text{m}$ . Colonies penetrate the substrate laterally or perpendicularly or both. Transversal constrictions along the tunnels are occasionally present (after Le Campion-Alsumard 1979; Le Campion-Alsumard and Golubic 1985; Wisshak et al. 2005).

**Comments:** Le Campion-Alsumard and Golubic (1985) described four morphological distinct ecotypes in response to gradients of humidity encountered across tidal ranges. In this study, I found only their status ‘dalmatella’ that is characteristic of lower intertidal ranges. This species is generally characteristic of intertidal zones and occurs less commonly subtidally (Le Campion-Alsumard 1979; Le Campion-Alsumard and Golubic 1985; Glaub 1999).

**Geographic distribution:** *Hyella balani* has been described in shallow (0-6 m depth) tropical and cold-temperate environments (Le Campion-Alsumard and Golubic 1985; Radtke and Golubic 2005; Wisshak et al. 2005; Gektidis et al. 2007). I recorded *Hyella balani* traces in only one experiment in Belize, where I tested the relative importance of nitrogen and phosphorus to microbial endolithic communities (Chapter 4). This species was the most abundant cyanobacterium in mollusk shells fertilized with phosphorus alone (Chapter 3), suggesting that addition of phosphorus may have released this species from P-limitation, increasing its recruitment and growth in subtidal areas where it is less

common. In East Africa, *Hyella balani* traces were moderately common (recorded in 5 out of 9 reefs studied), with abundances lower than 1% substrate cover (Chapter 5).

**Ichno-species:** *Fascichnus dactylus* Radtke 1991 (Fig. 1.2c)

**Bio-species:** *Hyella caespitosa* Bornet and Flahault 1889; *Solentia* sp. Ercegovic 1927

**Trace morphology:** This trace is characterized by a cluster of tunnels radiating deep in the substrate from a single point of entry. Each colony consists of a few up to 150 tubular borings uniform in diameter (4 to 9  $\mu\text{m}$ ) forming a hemispherically expanding bush. Transversal constrictions along the tunnels are common, possibly indicating positions of the cross walls between the cells of its producer. Sometimes the trace spreads predominately parallel to the substrate surface, forming large carpets (after Le Campion-Alsumard 1979; Le Campion-Alsumard and Golubic 1985; Radtke 1993).

**Comments:** Although the species *Hyella caespitosa* is the most common producer of the trace, another cyanobacterium genus *Solentia* produces very similar traces. Nevertheless, observations of microbial endoliths under light microscopy revealed that *Hyella caespitosa* was the most common species present in my study. *Hyella caespitosa* can be distinguished from *Solentia* species by its smaller cell widths (4-10  $\mu\text{m}$  compared to 5-20  $\mu\text{m}$ , respectively). This trace is distinguishable from other *Fascichnus* species by the tunnel diameter (Radtke and Golubic 2005; see descriptions below).

*Hyella caespitosa* is locally dominant intertidally, but occurs less commonly subtidally. It requires constant water supply but tolerates wide fluctuations in salinity. Very common in upper intertidal rock pools of Moorea, French Polynesia (Radtke et al. 1996).

**Geographic distribution:** *Hyella caespitosa* has a cosmopolitan distribution and has been recorded from 0 to 100 m (Lukas 1978; Le Campion-Alsumard and Golubic 1985; Radtke 1993). In my experiments in Belize, *Fascichnus dactylus* was more abundant in treatments with added phosphorus, although its abundance was always less than 5% of total percent cover. In East Africa, *Fascichnus dactylus* was present in all reefs studied and, although its abundance appeared to increase with time of exposure, there was no apparent relationship with nutrient conditions in reefs (Chapter 5).

**Ichno-species:** *Fascichnus frutex* Radtke 1991 (Fig. 1.2d, e)

**Bio-species:** *Hyella gigas* Lukas and Golubic 1983

**Trace morphology:** Colonies are formed by short and thick (11-25  $\mu\text{m}$ ) filaments arranged in radiating clusters. The individual tunnels have a club-shaped appearance and sometimes bifurcate in their distal part. Transversal constrictions along the tunnels are common, sometimes accompanied by longitudinal constrictions. (after Radtke 1993).

**Geographic distribution:** *Hyella gigas* and its traces have been recorded in tropical and cold-temperate settings at depths between 0 and 35 m (Budd and Perkins 1980, Lukas and Golubic 1983, Radtke 1993, Wisshak et al. 2005). *Fascichnus frutex* was recorded in all experiments in Belize, and was particularly abundant in the phosphorus treatment in the third experiment (Chapter 4). In East Africa, this trace was recorded in all reefs studied and increased in abundance with time of exposure (Chapter 5).

**Ichno-species:** *Fascichnus parvus* Radtke 1991 (Fig. 1.2f)

**Bio-species:** *Hyella pyxis* Lukas and Hoffman 1984

**Trace morphology:** Colonies characterized by small clusters of tunnels (1.5-3  $\mu\text{m}$ ) usually parallel to the substrate's surface and branched, although tunnels perpendicular to the substrate can also be observed (after Radtke 1993).

**Geographic distribution:** This species has been recorded in tropical and temperate settings at water depths between 5 and 22 m (Lukas and Hoffman 1984, Radtke and Golubic 2005). *Hyella pyxis* traces were present but rare in Belize and East African reefs.

**Ichno-species:** *Planabola* isp. Schmidt 1992 (Fig. 1.2g)

**Bio-species:** *Cyanosaccus piriformis* Lukas and Golubic 1981

**Trace morphology:** Solitary large, spherical and slightly compressed cavities, 15 to 45  $\mu\text{m}$  diameter, directly below the substrate surface. These cavities often occur together.

**Comments:** This ichno-species may resemble initial borings by the green algae *Gomontia polyrhiza*.

**Geographic distribution:** This species has been recorded between 0.5 to 75 m depths in tropical and cold-temperate settings (Lukas and Golubic 1981; Radtke 1993; Wisshak et al. 2005). *Planabola* isp. was present but rare in Belize and East African reefs.

**Ichno-species:** *Solecia filosa* Radtke 1991 (Fig. 1.2h)

**Bio-species:** *Plectonema terebrans* Bornet and Flahault 1889

**Trace morphology:** Long filigree filaments of 1 to 2  $\mu\text{m}$  curled in spaghetti-like networks. The trace is sparsely branched in rectangular angles, and is often collapsed to the cast surface (after Radtke 1993).

**Comments:** Because of its small size, *Solecia filosa* is sometimes difficult to distinguish from traces of fungal hyphae. However, the presence of swellings corresponding to fungal sporangia helps distinguish fungi from *S. filosa*.

**Geographic distribution:** *Plectonema terebrans* has a cosmopolitan distribution, and has been recorded at depths ranging from 0 to 370 m (Lukas 1978; Radtke 1993; Vogel et al. 2000; Wisshak et al. 2005). This species was very abundant (30 % substrate cover) in treatments with phosphorus addition in my first experiment in Belize, but was much less common in the following two experiments (<5%). In East Africa, this species was present in all reefs and increased in abundance with time of exposure, demonstrating its ability to grow under reduced light conditions associated with epilithic algae growth and in deeper parts of the substrate (Kiene et al. 1995; Gektidis 1999; Vogel et al. 2000).

**Chlorophyta** Several boring green algae are known from tropical shallow-water environments (Radtke 1993; Radtke et al. 1996). Algal species are restricted to water depths appropriate to the wavelength absorbed by their photosynthetically active pigments. For example, the algae *Phaeophila* sp. requires abundant light and generally restricted to depths shallower than 30 m, whereas *Ostreobium quekettii* occurs over wider bathymetric ranges of 0 to 300 m (Vogel et al. 2000). Green algae are one of the most abundant taxa colonizing carbonate substrates, and their ecological succession reflects changes in the amount of light available. *Phaeophila* sp. is generally an early colonizer,

with *Ostreobium quekettii* increasing in abundance with time and becoming a dominant species after 1 to 2 years of exposure. Its delayed growth is interpreted as a response to decreased light within the substrate because epilithic colonization develops with time and microbial endoliths have to bore deeper in the substrate (Kiene et al. 1995; Gektidis 1999; Vogel et al. 2000). The method of penetration in the substrate is probably the same as in cyanobacteria (Garcia-Pichel 2006).

**Ichno-species:** *Cavernula pediculata* Radtke 1991 (Fig. 1.3a, b)

**Bio-species:** *Codolium*-stage of *Gomontia polyrhiza* (Lagerheim) Bornet and Flahault 1889

**Trace morphology:** This trace is characterized by solitary, spherical- to pear-shaped cavities connected to the substrate by short, repeatedly ramified, rhizoidal appendages. The dimensions of the boring are 30-70  $\mu\text{m}$  deep and 25-45  $\mu\text{m}$  wide (after Radtke 1993).

**Comments:** This species was first described as *Codiolum polyrhizum* by Lagerheimm (1885). Subsequently the species was recognized as the *Codolium*-stage (i.e., the unicellular sporophyte generation of sexual life histories) of *Gomontia polyrhiza* by Bornet and Flahault (1889). However, more recently it has been documented that algae of similar morphology are part of the life history of several other species (Nielson 1987). Nevertheless, *Gomontia polyrhiza* has been referred in the literature as the most likely producer of the trace *Cavernula pediculata*. Immature specimens that exhibit only a few rhizoidal appendages may be confused with traces of *Planabola* isp.

**Geographic distribution:** *Cavernula pediculata* has been found in tropical and temperate to arctic settings, at depths between 0 to 50 m (Budd and Perkins 1980;



Nielson 1987; Radtke 1993; Wisshak et al. 2005). This trace was completely absent in my experiments in Belize, but was observed in all reefs studied in Kenya and Northern Tanzania, increasing in abundance with the length of substrate exposure.

**Ichno-species:** *Fascichnus grandis* Radtke 1991(Fig. 1.3c)

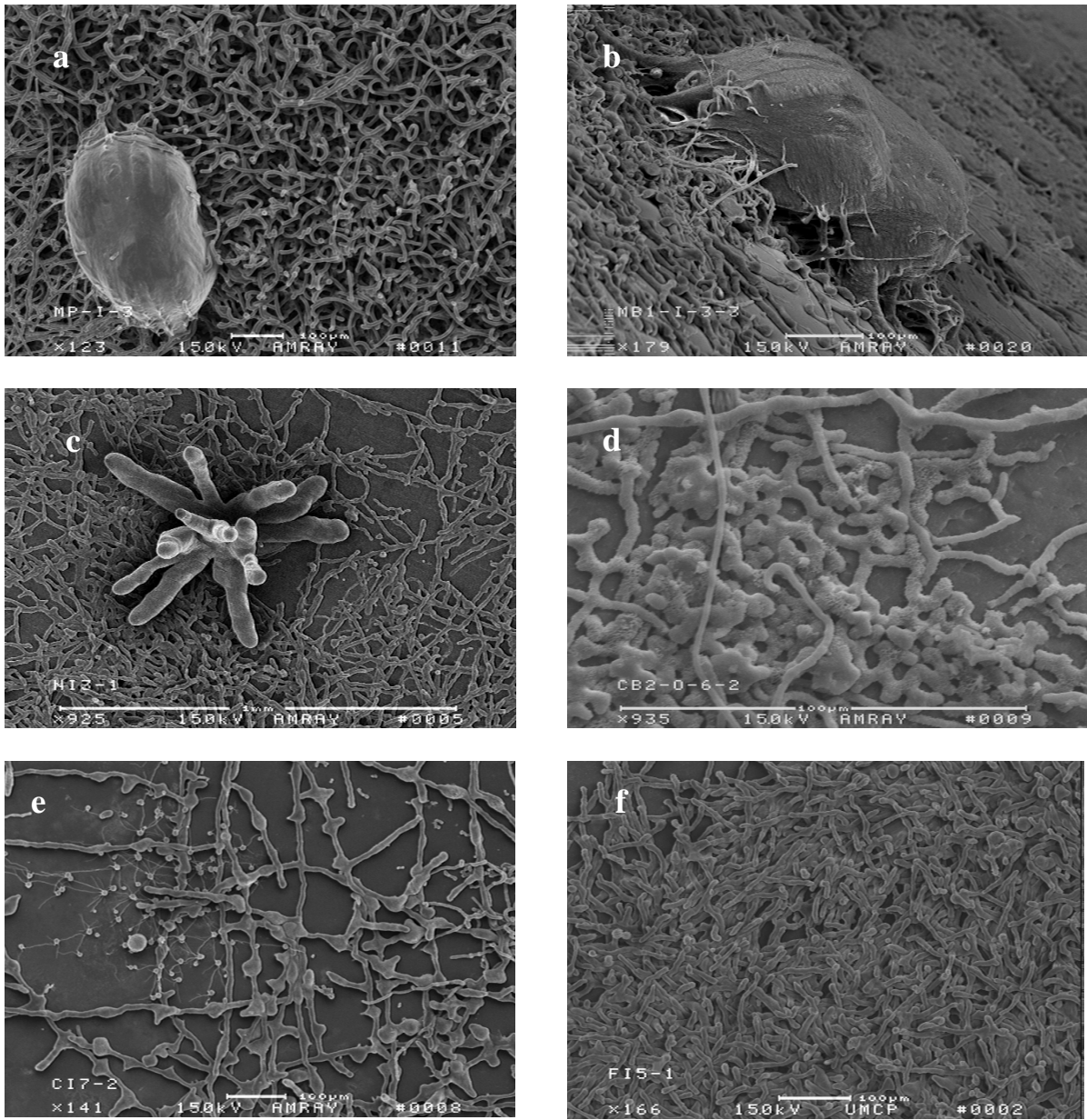
**Bio-species:** *Acetabularia* sp. rhizoid

**Trace morphology:** This boring is characterized by large loose clusters of dichotomously branched borings (10-30  $\mu\text{m}$  in diameter). This is the largest algal boring known, penetrating deep into the substrate to a maximum of 500  $\mu\text{m}$ . The tips of the traces may appear slightly swollen (after Radtke 1993).

**Comments:** The producer of this boring was initially classified as *Ostreobium barbanticum*, but more recent examination of the organism has attributed it to rhizoids of the green algae *Acetabularia* sp.

**Geographic distribution:** This species has been exclusively described from tropical environments. In the Caribbean, this species has been described from St Croix, West Indies (5-15 m depth: Perkins and Tsentas 1976), Belize (< 2 m depth: May et al. 1982), Puerto Rico (< 20 m depth: Budd and Perkins 1980) and Lee Stocking Island, Bahamas (<30 m depth: Radtke 1993; Vogel et al. 2000). In addition it has been described in shell substrates from One Tree Island, Great Barrier Reef (Vogel et al. 2000). More recent studies on Northern Atlantic sites (Glaub et al. 2002; Wisshak et al. 2005) have found *Fasciculus grandis* to be absent from studied materials, further supporting its restriction to tropical environments.

**Figure 1.3** Scanning electron micrographs of traces produced by boring chlorophytes. (a) Boring trace *Cavernula pedunculata* produced by codiolium-stage of the green algae *Gomontia polyrhiza*; (b) side view of *C. pedunculata*. Notice rhizoids and larger size compared with other borings; (c) boring trace of *Fascichnus grandis* produced by rhizoids of the green algae *Acetabularia* sp.; (d) boring trace *Ichnoreticulina elegans* produced by the green algae *Ostreobium quekettii*; (e) typical morphology of *Rhopalia catenata* produced by green algae *Phaeophila* sp. in less bored substrates; (f) typical morphology of *Rhopalia catenata* in more intensively bored substrates.



In my experiments in Belize, *Acetabularia* sp. rhizoid traces were only found in the last two experiments and with abundance < 1% of substrate cover. This trace was however more abundant in Kenya (5-10 %), particularly in ungrazed substrates after six months exposure.

**Ichno-species:** *Ichnoreticulina elegans* Radtke 1991 (Fig. 1.3d)

**Bio-species:** *Ostreobium quekettii* Bornet and Flahault 1889

**Trace morphology:** Spherical to flattened filaments measuring between 2 and 5  $\mu\text{m}$  in diameter and organized in an intricate and variable network of tunnels with occasional lobate swellings. These filaments ramify in preferred angles of  $90^\circ$  or  $120^\circ$ , creating a reticulated network of filaments, very often in a characteristic zig-zag pattern (after Radtke 1993; Radtke and Golubic 2005).

**Comments:** *Ostreobium quekettii* becomes abundant under low light levels, and is also found in sediments of the deep euphotic zone, suggesting a low rate of respiration (Budd and Perkins 1980). This species is the only green algae species recorded to grow inside live coral skeletons, probably because of its ability to grow under low light levels (Le Campion-Alsumard et al. 1995a; Priess et al. 2000).

**Geographic distribution:** *Ostreobium quekettii* has a cosmopolitan distribution and has been recorded at depths ranging from 0.5 m to 300 m (Lukas 1978; Radtke 1993; Vogel et al. 2000). This species was present in low abundance in my experiments in Belize (< 4%), probably because of the short time of substrate exposure (49-56 days).

*Ostreobium quekettii* was more common in Kenya, especially after six months exposure.

**Ichno-species:** *Rhopalia catenata* Radtke 1991 (Fig. 1.3 e,f)

**Bio-species:** *Phaeophila engleri* Reinke 1893, *Phaeophila dendroides* (Crouan) Batters 1902; *Eugomontia sacculata* Kornmann 1960

**Trace morphology:** This trace consists of chain-like connected chambers organized in a network of shallow tunnels oriented parallel to the substrate. Colonies often radiate from a central area, with ramifications in angles between 60 and 90°. Chambers are spherical to ellipsoidal and are linked to the substrate surface by thin rhizoidal connections (2 to 3 µm in diameter). Chambers have typical diameters of 10 to 25 µm and are connected by short narrower segments, 7 to 10 µm (after Radtke 1993; Radtke and Golubic 2005).

**Comments:** Although this trace can be produced by three species of chlorophytes, my observations under light microscopy revealed that *Phaeophila engleri* was the most commonly occurring species. *Phaeophila dendroides* was occasionally observed, but *Eugomontia sacculata* was not recorded. The distinction between *Phaeophila* spp. and *Eugomontia sacculata* is based in the presence of thin rhizoidal connections to the substrate surface in *Phaeophila* spp.; *P. engleri* is distinguished from *P. dendroides* by the larger diameter of chambers (15-25 µm and 10-20 µm respectively). However, because *Phaeophila* species present considerable morphological variability depending on their life cycle and physical conditions (Wilkinson 1975; Radtke and Golubic 2005), I decided not to distinguish between *P. engleri* and *P. dendroides* as the bio-species producing the trace *Rhopalia catenata*. I will refer to the bio-species as *Phaeophila* sp.

*Phaeophila* sp. is an early colonizer of denuded surfaces. The tops of the cells of *Phaeophila* sp. often protrude from shallow tunnels and groves, sending wavy tubular

bristles into the water column above (Radtke et al. 1997). Their life cycle appears to span a few weeks to months, so that most substrates contain only empty borings (Kiene et al. 1995). I observed different morphotypes of *Phaeophila* sp., with younger colonies presenting slender chambers and more mature colonies presenting larger chambers (Fig. 1.3e). In shells that were intensively bored, such as in the fertilized treatments, the species' tunnels are generally more slender and penetrate more deeply into the substrate (Fig. 1.3f). This type of morphology is attributed to avoidance behavior of tunnels at contact, with tunnels deflecting sideways or more frequently deeper into the substrate, underpassing earlier borings (Radtke and Golubic 2005).

**Geographic distribution:** *Phaeophila* spp. have been recorded from the tropics to sub-polar regions as endophytes, epiphytes, and endoliths in calcareous rocks and shells, and have been recorded at depths between 1 to 45 m (Perkins and Tsentas 1976; Budd and Perkins 1980; Radtke 1993; Voget et al. 2000; O'Kelly et al. 2004). In my experiments in Belize, *Phaeophila* sp. was the dominant cover in all treatments and responded strongly to the addition of inorganic nitrogen, suggesting that it was nitrogen-limited. When nitrogen and phosphorus were added in combination, *Phaeophila* sp. was still the dominant cover and appeared to be a better competitor for space and nutrients than other taxa (cyanobacteria, heterotrophs: Chapter 4). In East Africa, this species was particularly abundant during the first three months of the study, but was less apparent after six months of shell exposure (Chapter 5).

\* Information from Dr S. Golubic and Dr G. Radtke (personal communication) that I received after the dissertation defense date suggest that what I considered to be coccoid bacteria traces may be early stages of cyanobacteria in the order Pleurocapsales.

## Chapter 2

### The role of inorganic nutrients and herbivory in controlling microbioerosion of carbonate substratum<sup>1</sup>

**Abstract** The effect of herbivore abundance and nutrients on microborer communities and their rates of bioerosion of *Strombus gigas* shells was studied using herbivore-exclusion cages and inorganic fertilizers at Glovers Reef, Belize. Microborers colonizing shells in each treatment were identified and their colonization rates were calculated from scanning electron microscopy of the boring casts. In all treatments the dominant microborer was the green alga *Phaeophila* sp. Cyanobacteria were most abundant within fertilized and fungi in unfertilized treatments. The highest microbioerosion rates and most distinctive microborer community were found on the treatment with both reduced herbivores and fertilization. All fertilized treatments had significantly higher bioerosion rates than unfertilized treatments. Treatments with macrograzer access had microbioerosion rates less than half the fertilized cages. Bioerosion rates in unfertilized treatments were lowest and not different with and without macrograzers. Consequently, increased nutrient concentrations on reefs have the potential to increase rates of microbioerosion and macrograzers can modify the composition and density of the microborer community.

**Keywords:** algae, bioerosion, coral reefs, cyanobacteria, fungi, inorganic nutrients, herbivory, microborers

<sup>1</sup>Published in *Coral Reefs* (Carreiro-Silva M, McClanhan TR, Kiene WE 2005 vol. 24: 214-221).

## **Introduction**

High rates of bioerosion of carbonate substrata by endolithic organisms (borers) have been attributed to the effects of increased nutrients (Smith et al. 1981; Rose and Risk 1985; Holmes et al. 2000) and reduced herbivore abundance (Sammarco et al. 1987; Kiene and Hutchings 1994; Risk et al. 1995). These studies focused on bioerosion by macroborers (mainly sponges, bivalves, polychaete and sipunculan worms, and crustaceans), and have not considered bioerosion by microborers (bacteria, fungi and algae). Microbioerosion is the first bioeroding process to occur on newly exposed carbonate substrata (Golubic et al. 1975; Perkins and Tsentas 1976; Kobluk and Risk 1977; Tudhope and Risk 1985; Vogel et al. 2000). Studies on the Moorea Island coral reefs (French Polynesia) showed that the relative contribution of microborers to total bioerosion after two months was 60% of the total (Chazottes et al. 1995), demonstrating the importance of microborers in the initial stages of the bioerosion process. Rates of microbioerosion decreased, however, after two months, which they attributed to removal of substratum by grazing fish. Grazers and microborers effects are synergistic, microborers provide a renewable food source for excavating grazers and by weakening the superficial substratum layers they facilitate the process of grazing (Le Campion-Alsumard 1979; Chazottes et al. 1995, 2002). Conversely, the constant removal of substratum by grazers extends the depth to which algae can bore. Under conditions of intense grazing, however, the penetration of algae in the substratum is not rapid enough to compensate for the removal of substratum by grazers, resulting in an underestimate of microboring rates (Chazottes et al. 1995, 2002).

Several recent studies investigated microbioerosion rates in reefs exposed to different water chemistry conditions (Zubia and Peyrot-Clausade 2001; Chazottes et al. 2002; Tribollet et al. 2002). Two studies (Zubia and Peyrot-Clausade 2001; Chazottes et al. 2002) found higher bioerosion rates by microborers in reefs subjected to eutrophication compared to more oligotrophic reefs. Chazottes et al. (2002) recorded high microbioerosion rates in association with low grazing while Zubia and Peyrot-Clausade (2001) found higher rates in heavily grazed sites. Tribollet et al. (2002) study in the Great Barrier Reef found the lowest microbioerosion rates in inshore waters subjected to high terrigenous inputs and suggested that this resulted from low light levels that restricted colonization of microborers in the presence of high nutrients. The response of microborers to the experimental addition of nutrients has only been attempted as part of the ENCORE fertilization study (Kiene 1997; Koop et al. 2001) and produced inconclusive results. Kiene (1997) did not find significant differences in microbioerosion rates between treatments, but suggested that the microborers were unaffected by the nutrient treatments because the nutrient conditions at the surface of the reef were already sufficient for their support. Conflicting and inconclusive findings demonstrate the need for further experimentation.

To better understand the role of nutrients and herbivory in controlling microbioerosion of carbonate substrata, we exposed *Strombus gigas* shell fragments to two levels of herbivory and fertilization using a two-factor and -level interactive design using herbivore-exclusion cages and a slow-release fertilizer. The objective of our study was to investigate how inorganic nutrients and herbivory interact and affect microbioerosion of carbonate substrata by comparing species composition and bioerosion



rates between treatments. We hypothesized that elevated nutrients would increase bioerosion rates, and that the influence of nutrients on bioerosion rates would depend on whether carbonate substrata were exposed to grazing. Our findings support these hypotheses.

## **Materials and methods**

### Site description

The experiment was conducted at Glovers Reef, Belize during the summer months of June and July 2001. The reef is a coral-rimmed atoll 32-km long and 12-km wide, located approximately 45 km off mainland Belize (see map in McClanahan and Muthiga 1998). We placed experimental substrata at 2-m depth on the windward side of a patch reef in the Conservation Zone of the atoll's lagoon, where resource extraction is prohibited. The reef is remote and experiences no significant local organic pollution. The dominant herbivorous fish species were the surgeonfish *Acanthurus bahianus* and *A. coeruleus*, the damselfish *Stegastes* spp. and the parrotfish *Scarus croicensis*, *Sparisoma aurofrenatum*, and *S. viride* (McClanahan et al. 2001). Herbivory levels on these patch reefs are moderate with bite rates on experimental *Thalassia* assays at around 40% per day (McClanahan et al. 2001).

## Experimental design

We used a two-factor experimental design to test for the effects of herbivory and inorganic nutrients. We manipulated herbivory with two levels: closed-top cages, which excluded the larger herbivorous fishes, and open-top cages, which allowed all herbivorous fishes to graze on experimental substrata, and used two levels of nutrient enrichment: with and without fertilizer spread beneath the cages. We placed cages >1 m apart in a line aligned 90° to the dominant current direction such that neighboring cages would not slow the currents experienced by the cages and fertilizer would not influence the non-fertilized treatments.

Some small-bodied herbivorous fishes (*Scarus croicensis* and *Stegastes* spp.) entered both open and closed top cages and were seen feeding on the experimental substratum. To determine if there were differences in herbivory levels by small herbivores between open and closed-top cages, we counted the number of individuals of these two species and their bite rate per minute during two sampling periods in each of the 16 cages. A single individual of each species was selected at random, observed for 1 minute and the number of bites taken during that minute recorded. This resulted in 64 observations of herbivory, 32 replicates per treatment and species distributed evenly across the four treatments. The larger macrograzers were observed feeding in the open-top cages at rates that would resemble the natural substratum but no effort was made to quantify their bite rates as this has been done in some detail in previous studies of these patch reefs (McClanahan 1999; McClanahan et al. 2000, 2001).

The experiment used 16 cages (50 x 50 x 20 cm) constructed with PVC frames and 3-cm mesh plastic caging material. The cage mesh size allows for good water flow and light penetration and conditions in the cage are expected to resemble natural substrata but results of actual rates should be interpreted with caution due to possible caging effects. Two pieces (~12 x 6 cm) of *Strombus gigas* shell fragments were placed in each cage, making a total of eight replicate shell samples exposed to each of the four treatments. We used the interior of the shell as experimental substratum to avoid pre-existing microborings. Unsoaked shell fragments were collected and examined under electron microscope to determine if there was any evidence for borings in the samples prior to their experimental soaking. Samples were fixed to cages by drilling a hole in each of the conch shell pieces and attaching them to the bottom of the cages with black plastic cable ties such that the shell interiors were facing upward. Samples were retrieved after 49 days. We used wire brushes to clean all cages of algae and other settling organisms every 3 days to reduce caging artifacts such as decreased light and obstruction of local water flow associated with increased algal growth on the mesh of cages. We assessed the effectiveness of the herbivory treatments by observing whether fishes were inside closed-top and open-top cages nearly every day.

#### Nutrient enrichment and sampling

In the fertilized cages, 1.5-kg Scott's slow-release fertilizer was spread evenly beneath the cages on the first day and one-month after the start of the experiment. The fertilizer was a mix of 66% high phosphorus Osmocote and 34% high nitrogen Osmocote

fertilizer. The high P fertilizer contained 10% nitrogen in the form of ammonium and 50% phosphorus in the form of  $P_2O_5$ . The N fertilizer had 11.5% nitrate and 11.5% ammonium nitrogen and also contained 0.5% sulphate sulfur and 3.3% calcium. The fertilized cages, therefore, received a monthly dose of 500g $P_2O_5$ , 215g ammonium and 57.5g nitrates at the start of the experiment and again after one month. Fertilizer is tested to insure that it does not contain toxic chemicals such as heavy metals.

Scott's fertilizer company reports that the longevity of the high nitrogen fertilizer at 31°C is 1 month and 4 months for the high phosphorus fertilizer but do not report the environmental conditions under which this longevity was tested. Fertilizer pellets were, however, extant throughout the study period, with no indication of dissolution or migration from the cages. In order to determine the effectiveness of the fertilization treatment and the nutrient concentrations we collected water samples from each cage one week after the first fertilizer addition in the control and nutrient addition treatments, such that 16 water samples were taken balanced between the four treatments. Samples were taken from each cage by opening 50-ml acid-washed Nalgene bottles approximately 2-cm above the substrata surface. Concentrations of inorganic nitrate-nitrogen and phosphate-phosphorus were measured with a spectrophotometer at Glovers Marine Laboratory within three hours of sampling.

### Sample preparation

Immediately after collection from the cages, shell fragments were fixed in 4% formaldehyde solution. We cut and trimmed one 1 cm<sup>3</sup> cube from the middle portion of each shell fragment for subsequent analyses, bleached samples with sodium hypochlorite,

and made casts of boring traces by impregnating the dried samples under vacuum with epoxy resin (araldite) as described by Golubic et al. (1970). The carbonate matrix in samples was dissolved in hydrochloric acid (10%) to reveal the filled borings of the microborers. These casts and casts from unsoaked shells were investigated by scanning electron microscopy (SEM). Three samples of each treatment were lost or destroyed during the resin impregnation, leaving five from each treatment for analyses.

#### Identification of microborers and their boring traces

Microborers were identified using the morphology of casts of the borings produced by the organisms, rather than from the organisms themselves as described by Le Campion-Alsumard (1979) and Radtke (1993). The morphology of the borings produced by microborers is genus- and often species-specific for the organism that produces them (Golubic et al. 1975). The traces of borings left in substrata are referred to as "ichnotaxa" and the species that produced them as "bio-species" (Golubic et al. 1975). For example the boring trace *Scolecia filosa* is produced by the cyanobacterium *Plectonema terebrans* (see Table 2.2).

#### Bioerosion rates

Microboring traces can be classified into several basic types based on their morphology, density of colonization, and depth of penetration in the experimental substrata. Boring intensities on experimental samples were determined by comparing the SEM images to a key of different densities (measured as percentage surface area covered) and depth of

penetration by borings as described by Vogel et al. (2000). SEM photographs with several examples of boring intensities were prepared and the surface area, cross-sectional area, and depth of penetration of boring traces were carefully measured using a computer image analysis program. By comparing these key images to the small areas viewed with the SEM on each sample's upper surface, the areas could be rapidly classified as to their type, density and depth of boring without having to measure these parameters for every area observed. Although it is not an absolute measure of bioerosion, the results obtained from this procedure provide an adequate method for comparing bioerosion rates between samples and treatments (Vogel et al. 2000).

The abundance of different microborers and the rate of microboring was measured by classifying 20 1-mm<sup>2</sup> areas of the 1 cm<sup>2</sup> sample's upper surface using the keys above. The type of boring, density of the boring traces, and depth of boring penetration were recorded for each sample. The 20 1-mm<sup>2</sup> areas were selected systematically in each sample's upper surface following a predetermined pattern that was repeated in every sample. We selected four 1-mm<sup>2</sup> areas, one at each corner of the sample, four 1-mm<sup>2</sup> areas at the center, and four 1-mm<sup>2</sup> areas on transects between the mid-points of every two sides of the sample. This systematic sampling, as opposed to random sampling, minimizes the possibility of over-sampling areas of unusual high or low densities, increasing the sensitivity of the sampling and improving estimates of mean erosion rates. The type of boring and density of the boring traces were classified under x 30-100 magnification. The volume of calcium carbonate removed by the microborers in each sample was estimated by multiplying the estimates of the area covered by the boring traces by their depth of penetration. This figure was then multiplied by the density of the

substratum ( $2.65 \text{ g/cm}^3$ ) to estimate the rates of calcium carbonate loss by microborers and expressed in grams per square meter. Bioerosion rates over the 49-day experiment were converted to  $\text{g m}^{-2} \text{ year}^{-1}$  to present them in the form most commonly reported in previous studies

## Data Analysis

A two-way analysis of variance (ANOVA) was used to examine effects of herbivory, nutrient enrichment, and their interaction on bioerosion rates by all microborings (Mixed procedure, SAS statistical package). A one-way analysis of variance was used to examine differences in the water column nutrient concentrations and boring densities between treatments. Total fish bite rates per minute per cage (bite rates per individual x the number of individuals per cage) was calculated and tested for differences among treatments by a single-factor ANOVA. Examination of residuals indicated no violation of the ANOVA assumptions. We used the Tukey's T-test (Sokal and Rohlf 1995) to perform post-hoc means comparisons for significant effects. The bioerosion rate of one sample in the open-top cage non-fertilized treatment was 16 standard deviations away from the mean bioerosion rate for that treatment and was, therefore, considered an outlier and removed from the analysis.

## Results

### Effectiveness of treatments

Throughout the experiment, we observed several large-bodied parrot and surgeonfish species feeding in the open-top cages. The total bite rate per cage per minute for the species that were able to enter the closed-top cages, *Scarus croicensis* and *Stegastes* spp, was somewhat higher in the open cages but not statistically different ( $F = 2.6$ , NS, open-top =  $14 \pm 5$  and closed-top cages =  $5 \pm 3$ ,  $\pm = \text{sem}$ ). Concentrations of nitrate-nitrogen in the fertilized cages were doubled and of phosphate-phosphorus tripled just above the substratum one week after the fertilization (Table 2.1).

Table 2.1 Concentrations of nitrates and phosphates one week after the addition of fertilizer in the fertilized cages and control cages with no fertilizer addition, n=8 per treatment, and results of a single ANOVA.

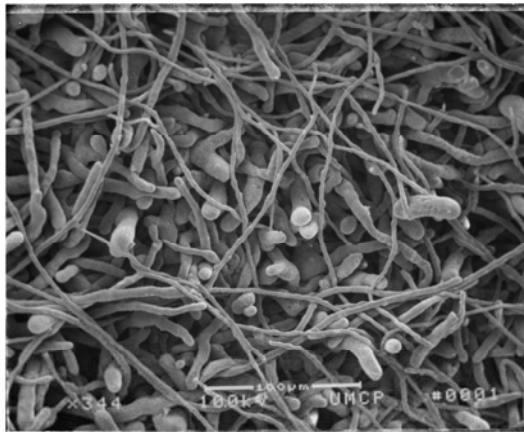
Nutrient	Control		Fertilized		F	p
	mean	S.D.	mean	S.D.		
Nitrates, $\mu\text{M}$	0.24	0.12	0.46	0.24	5.61	0.033
Phosphates, $\mu\text{M}$	0.34	0.41	1.11	0.97	4.33	0.056



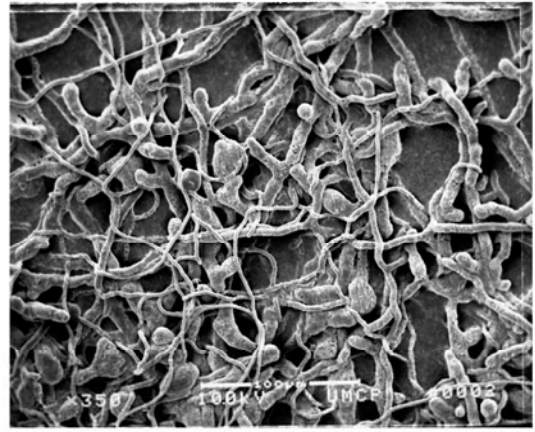
## Microborer composition

The conch substrata contained 9 different microborer traces corresponding to 4 species of cyanobacteria, 2 species of green algae, and 3 species of heterotrophs (Fig. 2.1, Table 2.2). The unsoaked conch substrata contained no microborer traces. Total percent cover by all microborers was two times greater in the closed-cage fertilized treatment than the open-cage fertilized treatment, and four times greater than the unfertilized treatments (Tukey test,  $p < 0.0001$ ). The unfertilized treatments did not differ in microborer's total percent cover (Tukey T-test,  $p = 0.98$ ).

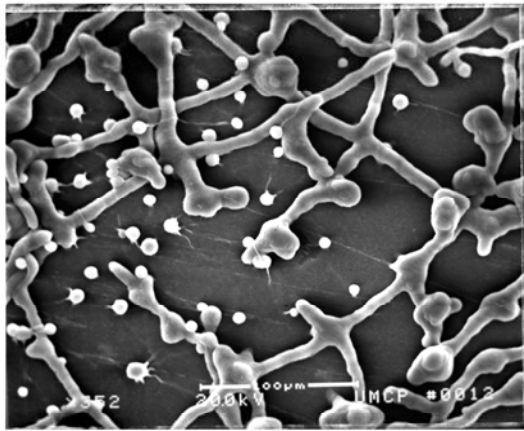
Figure 2.1 Scanning electron micrographs of microboring casts in experimental substrata made from *Strombus gigas* shell exposed at 2m depth, Glovers Reef, Belize. Typical intensity of boring by the green algae *Phaeophila* sp. (thick borings) and the cyanobacteria *Plectonema terebrans* (thin borings) in the (a) closed-cage fertilized and (b) open-cage fertilized treatments; (c) intensity of boring by *Phaeophila* sp. (thick borings) in the unfertilized treatments. Spherical chambers are the fungi *Lythopythium gangliiforme*. (d) Boring by *Ostreobium quekettii* in the closed-cage unfertilized treatment; (e) Boring by *Hyella caespitosa* in the closed-cage fertilized treatment; (f) boring by *Hyella gigas* in the open cage-fertilized treatment. SEM of shell fragments not soaked in cages showed no boring traces.



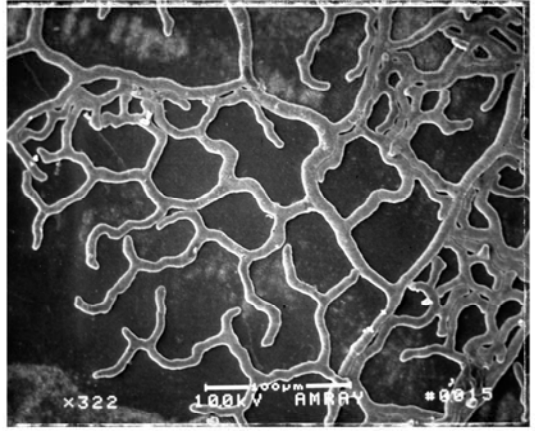
(a)



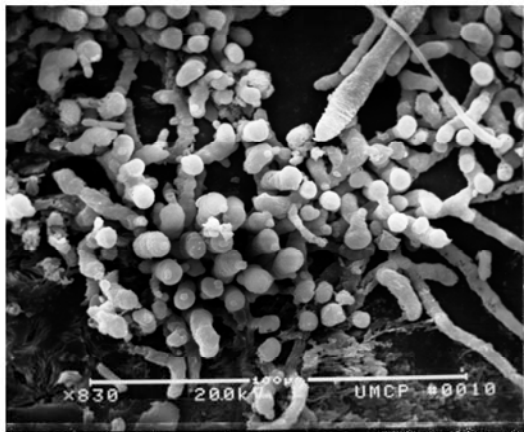
(b)



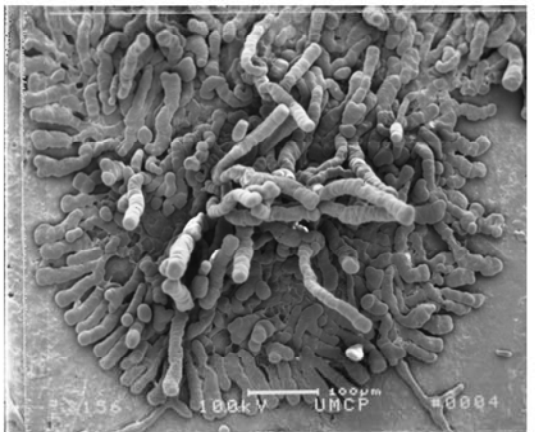
(c)



(d)



(e)



(f)

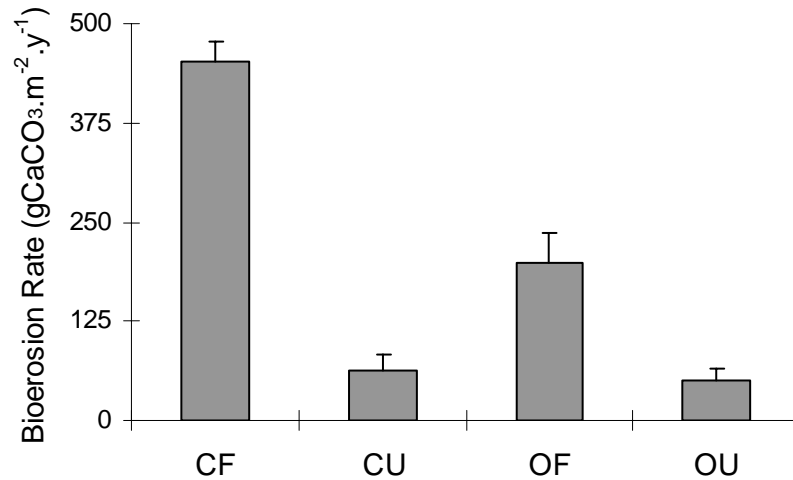
**Table 2.2** Percent cover of microboring traces (*ichnotaxa*) and their producers (*bio-species*) in experimental substrata made from *Strombus gigas* shell and exposed to different treatments for 49 days. Values are Mean (SD).

<i>Ichnotaxa = Bio-species</i>	Closed Cages		Open Cages	
	Fertilized	Unfertilized	Fertilized	Unfertilized
<b>Cyanobacteria</b>				
<i>Scolecia filosa = Plectonema terebrans</i>	31.7 (13.4)	3.6 (6.5)	5.8 (6.4)	0.9 (1.1)
<i>Fasciculus dactylus = Hyella caespitosa</i>	4.0 (5.1)	–	–	–
<i>Fasciculus frutex = Hyella gigas</i>	–	–	0.5 (1.0)	–
<i>Fasciculus parvus = Hyella pyxis</i>	0.5 (1.1)	–	–	–
<b>Green algae</b>				
<i>Reticulina elegans = Ostreobium quekettii</i>	–	1.8 (2.7)	3.5 (4.0)	2.0 (3.5)
<i>Rhopalia catenata = Phaeophila sp.</i>	46.7 (3.0)	11.6 (6.2)	24.1 (8.2)	15.2 (3.7)
<b>Fungi</b>				
<i>Saccomorpha sphaerula = Lithophythium gangliiforme</i>	0.8 (1.7)	1.8 (2.6)	2.3 (3.1)	2.0 (2.8)
<i>Saccomorpha clava = Dodgella priscus</i>	1.5 (3.4)	2.7 (3.0)	–	1.6 (2.4)
<i>Polyactina araneola = Conchyliastrum enderi</i>	–	0.0005 (0.001)	–	0.0005 (0.001)
<b>Total</b>	85.2 (11)	21.5 (10)	36.2 (11)	21.7 (7.3)

The chlorophyte *Phaeophila* sp. was the dominant cover in all treatments. The cyanobacterium *Plectonema terebrans* was present in all treatments and was particularly abundant in the closed-fertilized cages (31.7 (13.4) %). Surface cover by other microborer taxa was low (< 7%) and highly variable. The closed-cage fertilized community was most different, largely due to the presence of two cyanobacteria, *Hyella pyxis* and *H. caespitosa*. The open-cage fertilized treatment was also different, largely due the cyanobacteria *Hyella gigas*. Samples from the two unfertilized treatments were quite similar being colonized by the fungi *Lithophythium gangliiforme*, *Dodgella priscus*, and *Conchyliastrum enderi*.

#### Bioerosion rates

Bioerosion rates were most strongly influenced by fertilization and secondarily by herbivory with a significant interaction between nutrient enrichment and herbivory ( $F_{1,15} = 10.0$ ,  $p=0.006$ ). Pair-wise comparisons showed a significantly higher bioerosion rate in the closed- cage fertilized treatment ( $452 \pm 26$  g  $\text{CaCO}_3$   $\text{m}^{-2}$   $\text{y}^{-1}$ , Fig. 2.2) than in any other treatments (Tukey test  $p<0.0034$ ). Bioerosion rates in the open-cage fertilized treatment ( $200 \pm 38$  g  $\text{CaCO}_3$   $\text{m}^{-2}$   $\text{y}^{-1}$ ) were significantly higher than both unfertilized treatments ( $62 \pm 20$  for closed cages and  $49 \pm 16$  g $\text{CaCO}_3$   $\text{m}^{-2}$   $\text{y}^{-1}$  for open cages; Tukey test  $p< 0.02$ ). We found no significant differences between the closed -and open-cage unfertilized treatments (Tukey test  $p=0.75$ ).



**Figure 2.2** Mean rates and standard errors of bioerosion by all microborers in experimental substrata made from *Strombus gigas* shell and exposed to different treatments for 49 days: (i) Closed-cage fertilized (CF); (ii) Closed-cage unfertilized (CU); (iii) Open-cage fertilized (OF); (iv) Open-cage unfertilized (OU). Sample size = 5 for CF, CU and OF treatments and 4 for OU treatment.

## Discussion

Results support our hypotheses that elevated nutrients increase and herbivores decrease bioerosion rates by microborers. Inorganic nutrients were the strongest factor increasing microborer bioerosion rates by a factor of approximately 10 in the absence of herbivory by macrograzers. Nutrient concentrations in the control cages were very near averages reported for coral reefs (Kleypas et al. 1999) and we elevated concentrations in the fertilized cages to levels that are considered polluted (Lapointe 1999). Herbivory by macrograzers influenced microborers only in the fertilized treatments and decreased their bioerosion rates by one half, but these rates were still four

times higher than the unfertilized treatment. Herbivores are capable of removing the surface layers and the microborers that colonize fertilized substrata, but at the moderate herbivory levels found at Glover Reef, not to the level of unfertilized substrata. Grazers may reduce bioerosion rates by microborers but microborers may increase bioerosion rates by macrograzers by weakening the substratum and this complex interaction requires further investigation.

Increased bioerosion by microborers has been found in association with eutrophication and low herbivory in Reunion Island (Chazottes et al. 2002). These authors investigated the bioerosion processes in relation to changes in epilithic algae cover due to eutrophication and found that reefs with higher nutrient concentrations had the highest microboring rates and were associated with encrusting calcareous and macroalgae cover and the lowest grazing rates. In contrast to these findings, Zubia and Peyrot-Clausade (2001) found higher internal bioerosion by microborers at Reunion Island in dead branches of *Acropora formosa* that were outside damselfish territories compared to damselfish-defended branches. Branches in damselfish territories were expected to experience lower herbivory and the investigators suggested that deeper penetration by boring organisms in heavily grazed branches was responsible for the unexpected pattern. An alternative explanation is that the length of time the coral branches were dead and available for bioerosion influenced their results. *A. formosa* excluded from herbivory may have been dead for less time than those exposed to high herbivory and thus contain less boring organisms. Our use of fresh substrata created by recently dead *Strombus gigas* shells eliminated this possible confounding factor.

Our experiment indicates that the addition of nutrients can produce rapid and significant changes in the microborer community. Colonization of substrata by microborers is reported to occur within 4 to 9 days (Perkins and Tsentas 1976; Kobluk and Risk 1977; Tudhope and Risk 1985) and this rapid rate contributed to the fast response in our 49-day study. The pulsed additions of nitrogen and phosphorus to the water column at One Tree Island, Great Barrier Reef, Australia produced no changes in estimated rates of bioerosion by microborers after a 5-month exposure (Kiene 1997; Koop et al. 2001). The ENCORE study lacked a control for herbivory and this may have contributed to the difficulty in estimating the nutrient effect. Herbivory is still only at modest levels in Glovers Reef despite being remote and protected from fishing for ~5 y prior to this study (McClanahan et al. 2001) and it may be lower than One Tree Island. High herbivory could dampen the influence and ability to detect nutrient effects and this could explain the reported differences. Another factor potentially contributing to reported differences is that in the Glovers experiment the fertilizer was placed under the substrata while it was applied periodically to the water column above the experimental substrata in the ENCORE experiment. Slow-release fertilizer placed under the substrata is less likely to be quickly carried away by currents. It is likely that this continuous input produced higher nutrient concentrations around the substrata compared to the ENCORE experimental method and both factors contributed to the reported differences between the two studies. Bioerosion rates reported in the One Tree Island study on *Tridacna* shells of 20 to 30 g m<sup>-2</sup> y<sup>-1</sup> (Kiene 1997) are lower than the rates we obtained for both unfertilized treatments, which suggest a poor fertilization effect.

The Glovers experimental design relied on cages with possible artifacts and our methods to quantify bioerosion differed from other studies. Nonetheless, the microbioerosion rates we obtained in the low herbivory and fertilized treatment ( $452 \pm 26 \text{ g m}^{-2} \text{ y}^{-1}$ ) are similar to the rates of  $570 \text{ g m}^{-2} \text{ y}^{-1}$  obtained on reefs with low herbivory and the occasional elevation on nutrients at Moorea Island (Wolanski et al. 1993; Chazottes et al. 1995; Peyrot-Clausade et al. 1995). Bioerosion rates of the same order of magnitude or higher have been measured in the Great Barrier Reef, Australia. For example, Tudhope and Risk (1985) measured microboring rates of  $350 \text{ g m}^{-2} \text{ y}^{-1}$  in sediment particles exposed for 358 days at Davies Reef. Tribollet et al. (2002) measured microbioerosion rates between  $120\text{-}1340 \text{ g m}^{-2} \text{ y}^{-1}$  on coral substrata exposed for 1 year along a cross-shelf transect on the northern Great Barrier Reef. The highest bioerosion rates in this study were, however, measured in reef sites located on the outer barrier or oceanic reefs experiencing very little or no anthropogenic influence (Tribollet et al. 2002). Conversely, Chazottes et al. (2002) working in Reunion Island, recorded low bioerosion rates between  $57$  and  $67 \text{ g m}^{-2}$  in coral substrata exposed in reef areas experiencing nutrients input and low grazing, which are comparable to the rates we obtained in unfertilized treatments ( $62 \pm 20$  and  $49 \pm 16 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ). Differences observed between these bioerosion rates could be related to the use of different substratum, and the depth and length of exposure (Kiene et al. 1995; Vogel et al. 2000).

Differences in the species composition of microborers were greatest in the low-herbivory and high-nutrient treatment and fewer differences were observed among the other three treatments. Several past studies suggest that grazing fish may influence the species composition and succession of the macroborer community (Risk and Sammarco



1982; Sammarco et al. 1987; Kiene and Hutchings 1994; Risk et al. 1995). Grazing and associated removal of the surface substrata prevents the full ecological succession. Consequently, the newly-exposed substratum is colonized by early boring colonists but not larger and slower colonizing macroborers. Our results indicated that herbivory is also important in differentiating the microborer community. High nutrients appear to promote the relative importance of cyanobacteria over fungi and herbivory in the presence of nutrients appears to reduce cyanobacteria colonization. The 49-day period of our experiment may have been insufficient, however, to see the full succession of the microborer community. Species we reported are characteristic of early boring colonists and more than 90 days exposure may be required to document the full succession (Gektidis 1999; Vogel et al. 2000).

High densities of the cyanobacteria *Plectonema terebrans* in the low herbivory and fertilized treatments may result from the high phosphorus in the fertilizer. Cyanobacteria blooms in lakes are often associated with low N:P ratios caused by land-based pollution (Smith 1983). The response of *Plectonema terebrans* to phosphorus could represent a good indicator of reefs experiencing nutrient enrichment by phosphorus. This response was less evident, however, in the open-fertilized cages and the predictive power of this indicator may be limited when herbivory is high.

Our study provides the first direct experimental evidence of the influence of nutrients on microborer bioerosion rates and community structure and emphasizes the critical role of herbivory in influencing microborers in the presence of nitrification. Because of the rapid colonization of substrata by microborers, the response of these endoliths to elevated nutrients may represent a valuable early indicator of eutrophication in reef environments.

However, our study suggests that the use of bioerosion rates by microborers as an indicator of water quality may be most effective in reefs with low herbivory such as those exposed to heavy fishing or the loss of sea urchins.

## Chapter 3

### **Effects of inorganic nutrients and organic matter on microbial endolithic community composition and bioerosion rates**

#### **Abstract**

I used herbivore-exclusion cages in Glovers Atoll, Belize to test the effects of organic matter and inorganic nutrient additions on microbial endolithic communities (algae, bacteria and fungi) and their rates of bioerosion of *Strombus gigas* shells in a 49-day fertilization experiment. My hypothesis was that the addition of organic matter would release heterotrophic microborers from C-limitation and at the same time reduce light levels for endolithic microalgae, thus changing the microborers' community composition and their bioerosion rates. In agreement with my predictions, the addition of organic matter increased the abundance of heterotrophs (particularly fungi), but only when organic matter was added alone, not when it was combined with inorganic nutrients. By contrast, both green algae and cyanobacteria were stimulated by the addition of inorganic nutrients but were not affected by organic matter; these taxa were four times more abundant in treatments with inorganic fertilizers than other treatments. Lower fungi cover in treatment with added organic matter and inorganic fertilizers suggests that, when released from nutrient limitation, endolithic algae may be superior competitors for space and may have more efficient growth or nutrient uptake mechanisms than heterotrophs. Bioerosion rates in treatments with added inorganic fertilizer were eight to nine times

greater than bioerosion rates in the control and organic-matter-alone treatments, and were not affected by organic matter addition. I conclude that inorganic nutrients are an important factor controlling the bioerosion of carbonate environments. Microbial endoliths may be useful indicators of nutrient conditions in reef environments.

**Keywords** algae, bioerosion, coral reefs, cyanobacteria, fungi, microbial endoliths, nitrogen, organic matter, phosphorus

## **Introduction**

Degradation of coral reefs is often related to nutrient enrichment associated with increased agriculture activity and urbanization near coastal areas (McClanahan 2002; Szmant 2002; Fabricius 2005). Disturbances from nutrients and organic matter enrichment include shifts from coral- to algae-dominated reefs (Abram et al. 2003; Lapointe et al. 2004), decreased recruitment and growth of corals (Kinsey and Davies 1979; Tomascik 1991; Ferrier-Pagés et al. 2000; Ward and Harrison 2000), higher incidence of coral diseases (Harvell et al. 1999; Kuta and Richardson 2002; Bruno et al. 2003), and reduced reef accretion rates (Hallock 1988; Edinger et al. 2000). Elevated inorganic nutrients (e.g. phosphate, nitrate, nitrite, ammonia) have often been suggested to be the major cause of these disturbances, however, experimental support of this claim remains controversial (reviewed by Szmant 2002), and organic matter or other unmeasured toxins may often be responsible for some observed coral mortality (Jones and Kerswell 2003; Kuntz et al. 2005).

Although toxic pollutants are important locally, organic matter is a globally important constituent of pollution of near-shore coral reefs because most of the nutrients are discharged to the sea in particulate form (e.g., dead and decaying plants; human and animal waste) (Fabricius 2005). Furthermore, much of the dissolved inorganic nutrients can be taken up and converted into particulate forms within hours to days (Furnas et al. 2005). This issue prompted an experimental study of the interactive effect of organic matter and inorganic nutrients on fish, algae and coral condition at Glovers Atoll, Belize (McClanahan et al. 2005). Here, I report the results from investigations on nutrient effects on the microbial endolithic community composition and their bioerosion rates within the same fertilization experiment.

Microbial endoliths (mainly alga, bacteria, and fungi) are common inhabitants of carbonate substratum in temperate and tropical marine environments (Golubic et al. 1975; Perkins and Tsentas 1976; Budd and Perkins 1980; Golubic and Schneider 2003). Experimental work in tropical settings has demonstrated that microbial endoliths, or microborers, are important but often overlooked agents of bioerosion, involved in the breakdown of skeletal material (Chazottes et al. 1995, 2002; Tribollet et al. 2002; Carreiro-Silva et al. 2005), limestone coastal erosion (Schneider and Torunski 1983; Radtke et al. 1996) and erosion of loose carbonate sediment grains (Tudhope and Risk 1985). Microbial endolithic organisms colonize substrata more rapidly than any other group of bioeroders, representing the first bioerosion process to occur (within 4 to 9 days) on newly exposed carbonate substrata (Golubic et al. 1975; Perkins and Tsentas 1976; Kobluk and Risk 1977; Tudhope and Risk 1985; Vogel et al. 2000). Because of the rapid colonization of substrata by microborers, the response of these endoliths to elevated

nutrients have the potential to be valuable early indicator of declines in water quality in reef environments that result from eutrophication.

Under natural conditions, the early microborer community is dominated by the pioneer short-lived green algae *Phaeophila* sp. (Kiene et al. 1995; Gektidis 1999; Vogel et al., 2000; Carreiro-Silva et al. 2005). This community is then slowly replaced after 3 months by low light specialists such as the green algae *Ostreobium queketti* and the cyanobacterium *Plectonema terebrans*, which are able to grow under reduced light conditions caused by epilithic algal overgrowth of substrata, and in deeper parts of the substratum. Fungi and bacteria, as heterotrophs, depend upon a usable source of organic matter for food. They feed on the organic matrix of substrata, such as shells and skeletal bioclasts, and on algae (Golubic et al. 2005). Thus, heterotrophs are usually slow to colonize substrata and become more abundant as endolithic algal cover increases (Kiene et al. 1995; Gektidis 1999; Vogel et al. 2000).

Results from my previous fertilization-herbivory experiment in Belize indicated that bioerosion rates by microborers were enhanced nearly 10 times by fertilization but reduced by half with the inclusion of herbivores (Carreiro-Silva et al. 2005). This study indicates the potential for microborers as early indicators of changes in water quality but did not investigate the role of organic matter, another ubiquitous component of eutrophication. Here I examine the interaction between inorganic nutrients and organic matter fertilization and hypothesized that the addition of organic matter would change the microborer community structure from an autotrophic- to a heterotrophic-dominated community. Organic matter was hypothesized to interact with inorganic nutrients by

releasing heterotrophs from carbon limitation and by decreasing light available to endolithic autotrophs.

## **Materials and Methods**

### Site description

This study was conducted at Glovers Reef, Belize from June to August 2002. The reef is a coral-rimmed atoll 32 km long and 12 km wide, located approximately 45 km off mainland Belize (see map in McClanahan and Muthiga 1998). Experimental substrates were placed at 2-m depth on the windward side of a patch reef in the Conservation Zone of the atoll's lagoon, where resource extraction is prohibited. The reef is remote and experiences no significant local organic pollution. The waters in this area are calm with a small (<0.5 m) tidal range and slow currents (<1m s<sup>-1</sup>). No waves or other physical disturbances such as hurricanes were experienced during the study period.

### Experimental design

To test for the effects of organic matter and inorganic nutrients and their interactions, I used a two-factor experimental design with herbivore-exclusion cages and fertilizers over a 49-day period. I used two levels of inorganic nutrient enrichment - with and without inorganic fertilizer spread beneath the cages - and two levels of organic matter - with and without untreated fine wood dust placed in a mesh nylon bag (mosquito

netting) beneath the cages. In the combined organic and inorganic matter treatment, the fertilizer was added to the wood dust inside the same mesh bag. The experiment used 16 cages (50 x 50 x 20 cm) constructed with PVC frames and 3-cm mesh plastic caging material. Cages were tied to cement masonry blocks that kept them solidly on the reef bottom. The cage mesh size allowed for good water flow and light penetration and conditions in the cage were expected to resemble natural substratum. Nevertheless, results of actual bioerosion rates should be interpreted with caution due to possible caging effects.

Experimental substrates were made of *Strombus gigas* mollusk shells. Shells were used instead of coral blocks because their less porous structure produces better casts of boring organisms. This improves identification of boring traces, as well as measurements of surface cover and depth of penetration used for bioerosion rates estimates. In addition, blocks made of live coral often contain pre-existing traces of boring algae and fungi, which may compromise estimates of microborer surface cover and bioerosion rates due to treatment effects. By using the interior parts of shells in this study, pre-existing microborings were avoided. There are differences in substrate density between coral skeletons and mollusk shells, so bioerosion rate estimates for shells may not correspond to rates measured for corals. However, the objective of the study was to investigate how inorganic nutrients and organic matter interact and affect microbioerosion of carbonate substrates by comparing species composition and bioerosion rates between treatments, and not to determine absolute bioerosion rates that could be extrapolated to reefs in general.



Two pieces (~12 x 6 cm) of *Strombus gigas* shell fragments were placed in each cage, for a total of eight replicate shell samples exposed to each of the four treatments. Samples of unsoaked shell fragment were collected and examined under an electron microscope to determine if there was any evidence for borings in the fragments prior to their experimental soaking; there were none. Shell fragments were fixed to cages by drilling a hole in each of the conch shell pieces and attaching them to the bottom of the cages with black plastic cable ties such that the shell interiors were facing upward. Cages were placed >1 m apart in a line aligned 90° to the dominant current direction such that neighboring cages would not slow the currents experienced by other cages and fertilizer would not influence the non-fertilized treatments. Every other day, cages were cleaned with wire brushes to remove algae and other settling organisms so as to reduce caging artifacts such as decreased light and obstruction of local water flow associated with increased algal growth on the mesh of cages.

Cages excluded large herbivorous fishes and large predators but allowed small fishes such as damselfishes (*Stegastes* spp.), wrasses, and small parrotfish (*Sparisoma aurofrenatum* and *Scarus inserti*) to enter and forage (McClanahan et al. 2005). The number of damselfish, parrotfish, and wrasses that occupied each cage were counted three times during the study period over a 3-min period.

#### Nutrient enrichment and sampling

The inorganic fertilizer consisted of 1.5 kg Scott's slow-release fertilizer spread evenly beneath the cages (as described in Carreiro-Silva et al. 2005), such that each

fertilized cage received a dose of 500 g  $P_2O_5$ , 215 g ammonium and 57.5 g nitrates at the start of the experiment and again after one month. The organic matter treatment consisted of 5-kg of untreated and fine sawdust collected from a sander at a local carpenter's workshop. There was fertilizer remaining beneath the cages at the time of re-fertilization after 1 month, suggesting that the original fertilizer was still diffusing out when it was replenished.

Water samples from each cage were collected one week after the first fertilizer addition and one week before the end of the experiment, such that 32 water samples were taken balanced between the four treatments. Samples were taken from each cage by opening 100-ml and 500-ml acid-washed Nalgene bottles approximately 1-cm above the surface of the substratum. Concentrations of inorganic nitrate-nitrogen and phosphate-phosphorus and suspended solids were measured on the same day with a Hach DR/2500 spectrophotometer using the cadmium reduction method for nitrate and the ascorbic acid method for phosphorus (Parsons et al. 1984). Due to the high variability and uncertainty of ammonium measurements, only concentrations of nitrogen as nitrate and nitrite were used to determine nitrogen concentration. Variability in the ammonium results were attributed to problems with reduction packets used for the ammonium samples.

### Sample preparation

Immediately after collection from the cages, shell fragments were fixed in 4% formaldehyde in seawater solution. I used two approaches to document the composition and densities of microbial endoliths in each treatment: 1) Casts of the boring traces in the

experimental samples were observed under scanning electron microscopy (SEM) to provide a documentation of the microbial endoliths' community composition and percent cover of the substratum, allowing the quantification of rates of bioerosion by the endoliths; 2) observation of microbial endoliths under light microscopy for detailed identification and confirmation of organisms that produce the traces seen in the SEM casts.

For the cast's preparation, I cut and trimmed two 1 cm<sup>3</sup> cubes from the middle portion of each shell fragment using a diamond-blade rock saw. Organic remains in samples were dissolved with sodium hypochlorite for a period of 24 hours, then were rinsed with distilled water and dried overnight at 50 °C. Dried samples were impregnated under vacuum with epoxy resin (araldite) as described by Golubic et al. (1970). Embedded shell pieces were sawed along their longitudinal axes and placed in a solution of hydrochloric acid (5% HCl) to eliminate the shell carbonate matrix and expose the boring trace casts. These casts, and casts from unexposed shells, were investigated by scanning electron microscopy. I analyzed a total of eight shell samples per treatment and two 1-cm<sup>3</sup> sub-samples per shell.

For investigations by light microscopy, the soft epilithic overgrowth of shell pieces was removed under a dissecting microscope and diluted HCl was used to dissolve the remaining calcareous incrustation (coralline algae) and substratum. The emerging microbial endoliths were mounted on microscope slides and examined under a Zeiss Universal microscope (400x power).

## Identification of microbial endoliths and their boring traces

Because the identification of microbial endolithic organisms and quantification of bioerosion rates were based on the morphology of the boring traces, I apply a dual nomenclature to microbial endoliths and refer to them using their ichnotaxonomy as a morphological classification of the traces and using biological nomenclature for classifying the endolithic organisms that produced those traces. For example, the boring trace *Eurygonum nodosum* is produced by the cyanobacterium *Mastigocoleus testarum*. The names and identification of the microbial organisms and their boring casts followed descriptions in Le Campion-Alsumard (1979), Radtke (1993), Radtke and Golubic (2005) and Wissak et al. (2005). I adopted the proposed changes (Radtke and Golubic 2005) of the name for cyanobacterial traces *Hyella* and *Solentia* from *Fasciculus* Radtke 1991 to *Fascichnus*, and for traces of the green algae *Ostreobium* from *Reticulina* to *Ichnoreticulina*.

## Bioerosion rates

Microboring traces can be classified into several basic types based on their morphology, density of colonization, and depth of penetration in the experimental substrata. Boring intensities on experimental samples were determined by comparing the SEM images to a key of different densities (measured as percentage surface area covered) and depth of penetration by borings as described by Vogel et al. (2000). Scanning electron micrographs with several examples of boring intensities were prepared and the

surface area and cross-sectional area of boring traces were carefully measured using a computer image analysis program (ImageJ, available at the National Institute of Health website). By comparing these key images to the small areas viewed with the SEM on each sample's upper surface, the areas could be rapidly classified as to their type and density of boring without having to measure these variables for every area observed. The depth of boring was measured in each sample by observing vertical sections through selected parts of the boring cast. Although it is not an absolute measure of bioerosion, the results obtained from this procedure provide an adequate method for comparing relative bioerosion rates between samples and treatments (Vogel et al. 2000).

Abundance of different microborers and rate of microboring were measured by classifying 20 1-mm<sup>2</sup> areas of the 1 cm<sup>2</sup> sample's upper surface using the keys described above. The type of boring, density of boring traces, and depth of boring penetration were recorded for each sample. The 20 1-mm<sup>2</sup> areas were selected systematically in each sample's upper surface following a predetermined pattern that was repeated in every sample, as follows: I selected four 1-mm<sup>2</sup> areas, one at each corner of the sample, four 1-mm<sup>2</sup> areas at the center, and four 1-mm<sup>2</sup> areas on transects between mid-points of every two sides of the sample. This systematic sampling, as opposed to random sampling, minimizes the possibility of over-sampling areas of unusual high or low densities, thus increasing the sensitivity of the sampling and improving estimates of mean erosion rates. The type of boring and density of the boring traces were classified under 100-500x magnifications. The volume of calcium carbonate removed by the microborers in each sample was estimated by multiplying the estimates of the area covered by the boring traces by their depth of penetration. This figure was then multiplied by the substratum

density ( $2.65 \text{ g cm}^{-3}$ ) to estimate the rates of calcium carbonate loss by microborers expressed in  $\text{g m}^{-2}$ . Bioerosion rates over the 49-day experiment were converted to  $\text{g m}^{-2} \text{ year}^{-1}$  to present them in the form most commonly reported in previous studies.

## Data Analysis

I used a mixed model two-way nested analysis of variance (ANOVA) to test for the effects of inorganic nutrients and organic matter and their interaction on bioerosion rates by all microborings, and to examine the variation in bioerosion rates among shells within a treatment (Mixed procedure, SAS Institute, 2004). I treated inorganic nutrients and organic matter as fixed effects and shells within treatments as random effects. Fixed effects in the model were tested using the approximate F-tests of this procedure, and the random effect was tested using the variance component approach (Littell et al. 2006). Residual variance component was interpreted as variability among sub-samples within each shell (the basal unit of replication). The percent variation explained by the nested factor relative to total variation was estimated by dividing the variance component of the nested factor by the total variance (shells within treatments variance + residual variance). The analysis was performed on the log-transformed data to correct for lack of homogeneity of variance.

Treatment effects on percent substratum cover by different microboring taxa (green algae, cyanobacteria, and heterotrophs) were analyzed using Proc GLIMMIX in SAS, which fits a generalized linear mixed model to the data (SAS Inst. 2004; Littell et al. 2006). Predicted values of percent substratum cover were logit-transformed to

linearize data, and models were fit to the data using residual pseudo-likelihood. This generalized linear mixed model procedure assumed a pseudo-binomial error distribution because the data were recorded on a scale from 0 to 1, and a logit-link function (SAS Inst. 2004). Fixed and random effects in the model were the same as described above.

I used Tukey's test (Sokal and Rohlf 1995) to perform post-hoc means comparisons for significant effects. The percent cover and bioerosion rate of one sample in the treatment with inorganic fertilizer was 10 standard deviations away from the mean bioerosion rate for that treatment and was, therefore, considered an outlier and removed from the analysis.

## Results

### Microbial endolithic community composition

I identified 15 different microborer traces in *Strombus gigas* shells corresponding to six species of cyanobacteria, three species of green algae, and five species of fungi, and an unidentified bacterium (Table 3.1, Fig 3.1). Traces by the green algae *Phaeophila* sp. were the dominant traces in all treatments. The second most abundant trace corresponded to the fungus *Lithophythium gangliiforme* in the organic-matter-alone treatment ( $5 \pm 3\%$ , mean  $\pm$  SD).

Substratum cover by other microboring traces was very variable and generally less than 3%. Heterotrophic microboring traces were mainly composed of fungi traces,

with only two records of an unidentified trace corresponding to a coccoid bacterium\* in the organic matter + fertilizer treatment.

**Table 3.1** Percent cover of microboring traces (*ichnotaxa*) and their producers (*bio-species*) in experimental substrata made from *Strombus gigas* shell and exposed to different treatments for 49 days. Values are mean (standard deviation).

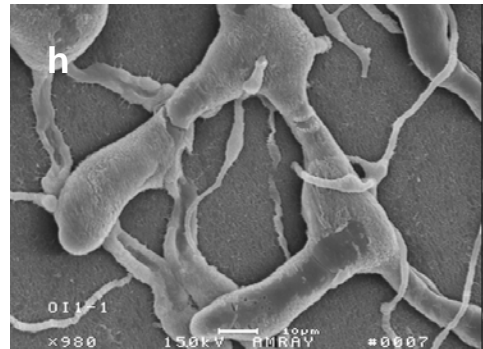
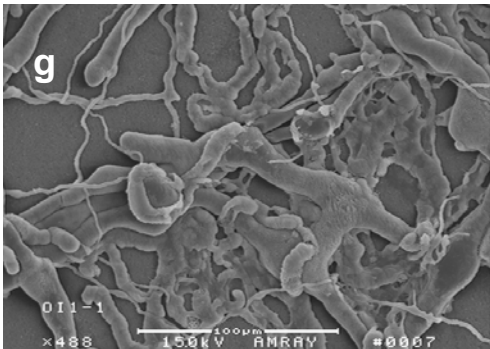
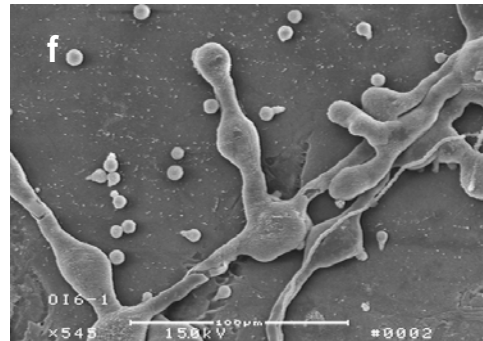
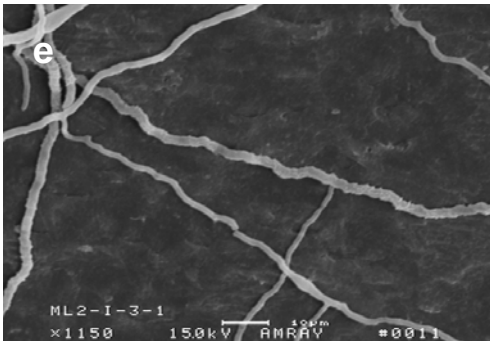
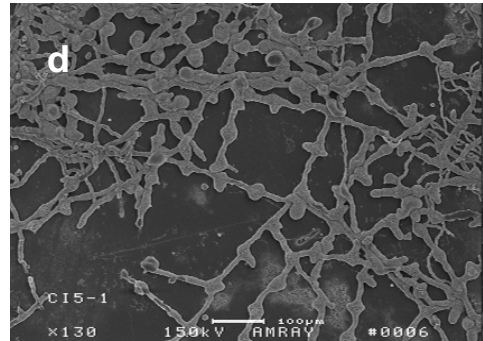
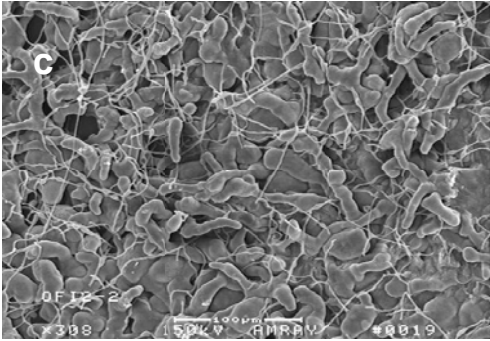
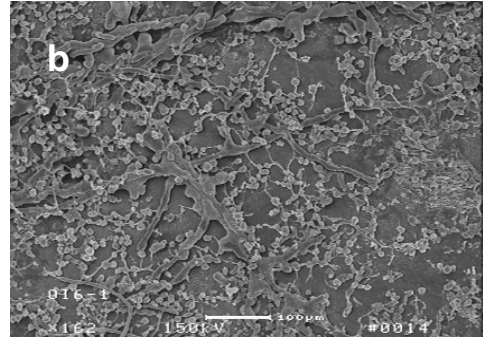
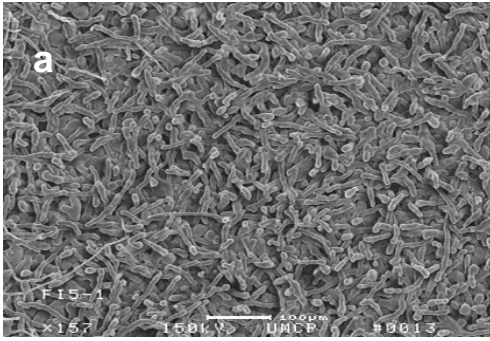
<i>Ichnotaxa = Bio-species</i>	Control	Organic Matter	Organic Matter + Fertilizer	Fertilizer
<b>Cyanobacteria</b>				
<i>Scolecia filosa = Plectonema terebrans</i>	0.2 (0.7)	0.1 (0.4)	1.3 (2.6)	2.1 (2.8)
<i>Fascichnus dactylus = Hyella caespitosa</i>	0.2 (0.5)	0.6 (0.7)	1.0 (2.5)	1.0 (2.4)
<i>Fascichnus frutex = Hyella gigas</i>	0.06 (0.2)	–	1.0 (2.5)	0.02 (0.07)
<i>Fascichnus parvus = Hyella pyxis</i>	–	0.1 (0.1)	–	–
<i>Eurygonum nodosum = Mastigocoleus testarum</i>	0.67 (1.2)	0.1 (0.2)	1.0 (1.2)	1.84 (2.57)
<i>Planabola isp. = Cyanosaccus piriformis</i>	–	0.03 (0.1)	0.1 (0.3)	–
<b>Total cyanobacteria</b>	<b>1.2 (1.2)</b>	<b>0.9 (0.7)</b>	<b>4.5 (4.4)</b>	<b>4.9 (2.7)</b>
<b>Green algae</b>				
<i>Fascichnus grandis = Acetabularia rhizoid</i>	0.03 (0.1)	–	0.09 (0.2)	0.01 (0.04)
<i>Ichnoreticulina elegans = Ostreobium quekettii</i>	0.61 (1.9)	0.02 (0.07)	0.72 (2.0)	–
<i>Rhopalia catenata = Phaeophila sp.</i>	13.3 (2.4)	11.7 (2.2)	45.8 (23.9)	50.9 (27.3)
<b>Total green algae</b>	<b>13.9 (3.4)</b>	<b>11.7 (2.2)</b>	<b>46.1 (23.7)</b>	<b>50.9 (27.3)</b>
<b>Fungi</b>				
<i>Saccomorpha sphaerula = Lithophythium gangliiforme</i>	1.7 (1.8)	5.1 (3.4)	1.4 (1.9)	1.35 (2.8)
<i>Saccomorpha clava = Dodgella priscus</i>	0.5 (1.2)	2.3 (3.5)	0.4 (0.6)	–



**Table 3.1** Continued

<i>Ichnotaxa = Bio-species</i>	Control	Organic Matter	Organic Matter + Fertilizer	Fertilizer
<i>Polyactina araneola = Conchyliastrum enderi</i>	–	0.02 (0.04)	–	–
<i>Orthogonum fusiferum = Ostracoblabe implexa</i>	0.7 (2.5)	1.9 (1.1)	1.5 (3.6)	0.8 (2.1)
<i>Orthogonum lineare = Unknown heterotroph</i>	–	0.4 (0.1)	–	–
<b>Bacteria*</b>				
<b>Coccolidal Form = Unknown producer</b>	–	–	0.005 (0.01)	–
<b>Total heterotrophs</b>	2.9 (1.6)	9.7 (3.7)	3.6 (3.8)	2.3 (3.7)
<b>Total</b>	18 (4)	21.2 (3.4)	53.8 (22.6)	58.1 (25.6)

Figure 3.1 Scanning electron micrographs of microboring casts in experimental substrata made from *Strombus gigas* shell exposed at 2m depth, Glovers Reef, Belize for 49 days. (a) Typical density of boring trace *Rhopalia catenata* produced by the green algae *Phaeophila* sp. in the fertilized treatment; (b) *Rhopalia catenata* (thick borings) and boring trace *Orthogonum fusiferum* produced by the fungus *Ostracoblabe implexa* (thin filaments) in the fertilized + organic matter treatment; (c) *Rhopalia catenata* and the boring trace *Saccomorpha spherula* produced by the fungus *Lithopythium gangliiforme* in the organic matter treatment; (d) density of boring trace *Rhopalia catenata* in the control treatment; (e) boring trace *Orthogonum lineare* produced by an unidentified fungus (f) boring trace *Saccomorpha clava* produced by the fungus *Dodgella priscus*; (g) *Orthogonum fusiferum* tunnel penetrating *Rhopalia catenata*'s tunnel; notice the various stages of degradation of the algae boring tunnel; (h) Detail of (g).



Both green algae and cyanobacteria were stimulated by the addition of inorganic nutrients and unaffected by organic matter (Table 3.2, Figure 3.2). These taxa were four times more abundant in treatments with added inorganic nutrients than the control and organic-matter-alone treatments (Tables 3.1 and 3.2). There was no statistical difference in green algae or cyanobacteria cover in the control and organic matter treatments.

Heterotrophs were positively affected by organic matter and negatively affected by inorganic nutrients, with no interaction between the two factors (Table 3.2). The addition of organic matter alone increased the heterotrophs' percent cover three times in relation to other treatments. The heterotrophs' percent cover in the organic matter + fertilizer treatment was not significantly different from cover in the control and fertilizer treatments.

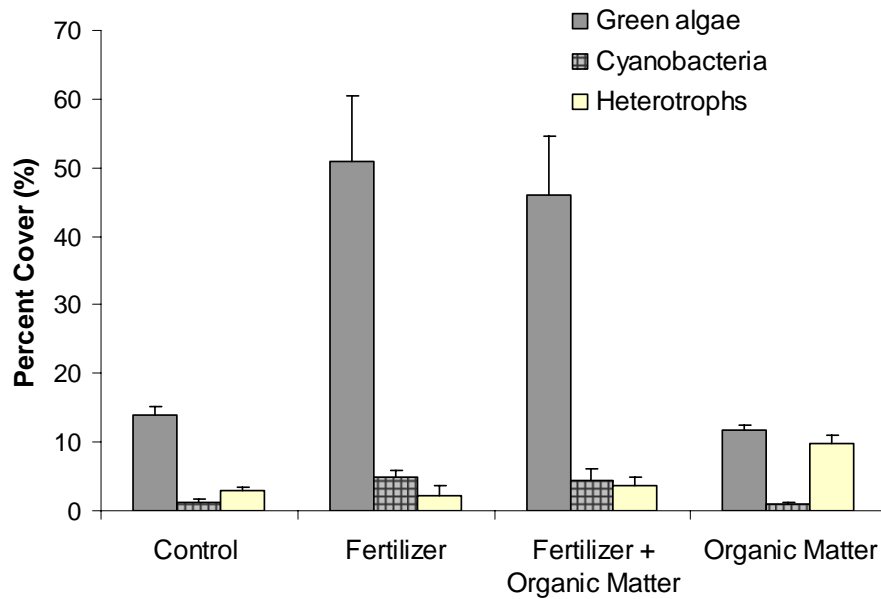
Total cover by all microbial endoliths was three times higher in treatments with added inorganic fertilizers than in other treatments (Tables 3.1 and 3.2). Total cover was not significantly different in the treatments with added fertilizers and the control and organic matter treatments (Table 3.2).

An estimation of the variance components for percent cover by green algae, cyanobacteria, and heterotrophs indicated that 84 to 98 % of the total variance was due to differences among shells within treatments and 2 to 15% to differences among sub-samples within shells (Table 3.2).

**Table 3.2** Two-way nested ANOVA on the effects of inorganic fertilizer and organic matter additions on microborer's logit transformed mean substratum cover (%) and bioerosion rates ( $\text{gCaCO}_3\cdot\text{m}^2\cdot\text{y}^{-1}$ ) by all microborers. Results of Tukey test for post-hoc means comparisons are included. OM = Organic matter treatment; F = fertilizer treatment; C = control treatment; OM + F = Organic matter and fertilizer treatment.

	Effect	DF	Variance Component	F-value	P-value	Tukey test			
<b>Green algae cover</b>									
Fertilizer	Fixed	1		47.68	<0.0001		<b>OM</b>	<b>F</b>	<b>OM + F</b>
Organic Matter	Fixed	1		0.44	0.5145	<b>C</b>	NS	***	***
Fertilizer × Organic matter	Fixed	1		2.03	0.7938	<b>OM</b>		***	***
Shell (treatment)	Random	22	0.3208			<b>F</b>			NS
Residual	Random	32	0.01167						
<b>Cyanobacteria cover</b>									
Fertilizer	Fixed	1		23.64	<0.0001		<b>OM</b>	<b>F</b>	<b>OM + F</b>
Organic Matter	Fixed	1		0.47	0.4982	<b>C</b>	NS	**	**
Fertilizer × Organic matter	Fixed	1		0.01	0.9082	<b>OM</b>		***	***
Shell (treatment)	Random	9	0.4388			<b>F</b>			NS
Residual (sub-samples)	Random	37	0.0086						
<b>Heterotroph cover</b>									
						<b>Tukey test</b>			
Fertilizer	Fixed	1		5.91	0.0220		<b>OM</b>	<b>F</b>	<b>OM + F</b>
Organic Matter	Fixed	1		10.6	0.0030	<b>C</b>	**	NS	NS
Fertilizer × Organic matter	Fixed	1		2.26	0.1686	<b>OM</b>		***	**
Shell (treatment)	Random	2	0.121			<b>F</b>			NS
Residual (sub-samples)	Random	32	0.023						
<b>Total cover</b>									
						<b>Tukey test</b>			
Fertilizer	Fixed	1		37.2	< 0.0001		<b>OM</b>	<b>F</b>	<b>OM + F</b>
Organic Matter	Fixed	1		0.01	0.9221	<b>C</b>	NS	***	***
Fertilizer × Organic matter	Fixed	1		0.71	0.4082	<b>OM</b>		***	***
Shell (treatment)	Random	17	0.552			<b>F</b>			NS
<b>Bioerosion rates</b>									
Fertilizer	Fixed	1		133.9	< 0.0001		<b>OM</b>	<b>F</b>	<b>OM + F</b>
Organic Matter	Fixed	1		0.31	0.5806	<b>C</b>	NS	***	***
Fertilizer × Organic matter	Fixed	1		0.46	0.5028	<b>OM</b>		***	***
Shell (treatment)	Random	15	0.038			<b>F</b>			NS
Residual (sub-samples)	Random	31	0.023						

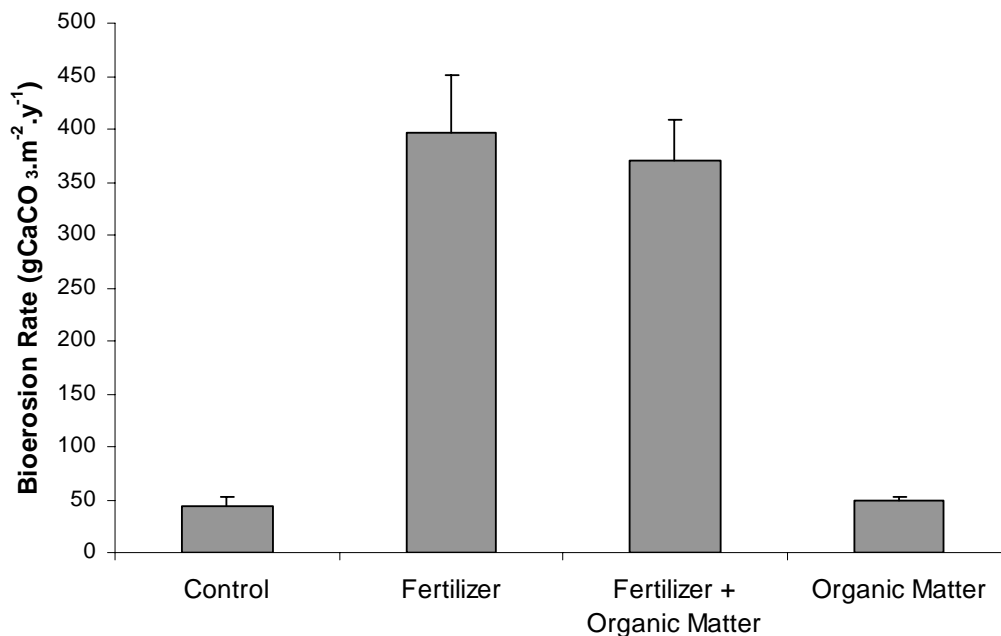
Note: For random effects, the variance components are reported, while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least of squares degrees of freedom \*\*\* $p < 0.0001$ , \*\* $p < 0.01$ , \*  $p < 0.05$



**Figure 3.2** Percent cover (mean  $\pm$  sem) of microbial endolithic taxa (green algae, cyanobacteria, heterotrophs) in different treatments in *Strombus gigas* shells exposed for a period of 49 days.

### Bioerosion rates

Bioerosion rates were significantly affected by the addition of inorganic nutrients but not by the addition of organic matter, with no interaction between the two factors (Table 3.2, Figure 3.3). Bioerosion rates were eight to nine times greater in the treatment with inorganic nutrients than in the control or organic-matter-alone treatments. There was no significant difference in bioerosion rates between the control and organic matter treatment. The difference in shells among treatments accounted for 62% of the total variance, and 38% of the total variance was due to differences in sub-samples within shells (Table 3.2).



**Figure 3.3** Bioerosion rates (mean  $\pm$  sem) by all microbial endoliths in different treatments in *Strombus gigas* shells exposed for a period of 49 days.

## Discussion

### Effectiveness of treatments

Measurements of nutrient levels in different treatments revealed that addition of inorganic nutrients increased nitrogen and phosphorus above levels considered normal for coral reefs (Kleypas et al. 1999; McClanahan et al. 2005). Wood dust was used as a source of particulate organic matter and as a form of simulating decaying plant matter. Wood generally has a C:N ratio of 150 to 1300 and a C:P ratio of 13,000 to 130,000 (Mellilo et al. 1984) and is therefore a suitable source of increased particulate carbon while containing undetectable levels of inorganic nutrients that would not confound the

experimental design. Inorganic nutrient concentrations measured in seawater from the cages confirmed that there was no elevation of inorganic nutrients in the organic matter treatment. I expected wood dust to increase suspended solids, and therefore water turbidity, in the organic matter treatments. However, suspended solids increased the most in treatments with added inorganic fertilizers (McClanahan et al. 2005), suggesting that inorganic fertilizers were a stronger factor than organic matter for this measure of water quality.

I used herbivore-exclusion cages (closed-top cages) to minimize the possible large effects of big grazers on this experiment that were previously studied and found to reduce microflora and bioerosion rates (Carreiro-Silva et al. 2005). Small grazers were able to enter the cages during the experiment and, therefore, herbivory was not entirely excluded. Treatments with added organic matter had a significantly lower number of damselfishes and parrotfishes entering the cages than other treatments (McClanahan et al. 2005), which may have influenced the results in these treatments.

### *Microbial endolithic community composition*

#### Organic matter effects

In agreement with my predictions, the addition of organic matter increased the abundance of heterotrophs (in particular, fungi), but only when organic matter was added alone, not when combined with inorganic nutrients. Lower fungi cover in the fertilizer + organic matter treatment suggests that green algae (particularly *Phaeophila* sp.), may have more efficient growth or nutrient uptake mechanisms and colonize available

substratum faster than heterotrophs. *Phaeophila* sp. is an early boring colonist with a short generation time and individuals or colonies have a rapid turnover (Kiene et al. 1995). Wissak et al. (2005) have also suggested decreased competition with heterotrophs as a possible explanation for the relatively higher abundance of endolithic fungi at deeper depths (>15 m) in the Swedish Kosterfjord.

However, because there was a lower number of grazing fish in the organic matter treatments, the observed effects in these treatments may be a result of the combined effect of the higher organic matter combined with lower herbivory. Nevertheless, comparisons with the results of my previous microbioerosion experiment (Carreiro-Silva et al. 2005), where herbivory was one of the factors tested, suggests that the observed effects are more likely due to higher organic matter than the lower herbivory inside cages. For example, the percent cover of fungi was not different for grazed and ungrazed treatments and was always below 4%. In the present experiment, I recorded highest fungal cover (10%) when organic matter was added alone, suggesting that organic matter was responsible for the increase in fungi cover.

Studies on the development of microboring communities through time (Kiene et al. 1995; Gektidis 1999) have shown that bacteria and fungi are generally slow to colonize newly exposed substratum. These authors found that, although heterotrophic endoliths were able to feed on the organic structures that exist in shells, they become abundant only after colonization by autotrophic borers. Endolithic fungi are able to feed on algae through specialized hyphae (haustoria) that penetrate algal cells (Golubic et al. 1975; Le Campion-Alsumard et al. 1995b; Priess et al. 2000). However, it is unknown if



this feeding takes place before or after the algae dies, therefore the nature of this relationship (i.e., saprophytic or parasitic) is unresolved.

I observed fungi invading algae filaments in several of my samples (Fig. 3.1g and h) and this was more common in the organic matter treatment, where fungi density was highest. This finding suggests that the addition of organic matter released fungi from carbon limitation and allowed them to increase more rapidly than in the normal succession, and that once they were established they started feeding on the limited algae that was available. I probably did not observe the same increase in endolithic fungi in the organic matter + fertilizer treatment because algae (particularly *Phaeophila* sp.) colonized the available substratum faster than fungi. It is possible that, if monitored through time, there would be a delayed response of fungi to organic matter in this treatment. It is also possible that, in some of the more densely colonized samples, deep borings of *Phaeophila* sp. may have obscured more shallow (close to the surface) borings by fungi, which may have led me to underestimate the real abundance of endolithic fungi in this treatment.

It should also be noted that heterotrophic organisms have been reported to grow at deeper depths in substratum containing neither organic substances or boring algae that they could feed on (Kiene et al. 1995; Vogel et al. 2000; Wissak et al. 2005). It is likely that other factors, apart from nutrient availability, play an important role in controlling the abundance of heterotrophic organisms; this needs further investigation.

Endolithic fungi have also been suggested to be potential pathogens of live corals (Le Campion-Alsumard et al. 1995b; Bentis et al. 2000). Several authors suggest that endolithic fungi are part of the microbial community living in the skeletons of healthy

corals. However, under environmental stress conditions that affect the calcification ability of corals (e.g., increased atmospheric CO<sub>2</sub>, elevated sea surface temperature or coastal eutrophication), fungi may become opportunistic pathogens, penetrating the coral skeleton and entering the coral tissue (Le Campion-Alsumard et al. 1995b; Bentis et al. 2000). Endolithic fungi are a suspected causative agent of a new source of coral mortality in Kenya (McClanahan et al. 2004), and their role as pathogens may be more common than acknowledged. Based on the results of the present study, I hypothesize that increases in particulate and dissolved organic matter in reef waters may stimulate the growth of endolithic fungi and increase the frequency of fungal attacks on coral tissue, playing an important role in the occurrence of fungi-related diseases in corals.

The addition of organic matter did not appear to negatively affect endolithic algae in the present study, as the substratum cover by endolithic algae was not significantly reduced in treatments with added organic matter (Table 3.2, Fig 3.2). Previous investigations on bioerosion rates along a cross-shelf transect on the northern Great Barrier Reef, Australia (Tribollet et al. 2002; Hutchings et al. 2005; Tribollet and Golubic 2005) recorded lower bioerosion rates by microborers in in-shore reef sites with elevated turbidity from suspended sediments, and suggested that these lower rates were a result of reduced light conditions and deposition of particles that inhibit the settlement and growth of autotrophic microbial endoliths. In the present experiment, turbidity was highest in treatments with added fertilizers, where substratum cover by algae was highest. Differences observed between these studies could be related to higher turbidity levels and higher particle settlement in the Great Barrier Reef study, where coral substrata were exposed for 1 to 4 years, in contrast to only 49 days in this study.

McClanahan et al. (2005) did observe some particle deposition on the experimental substrata in the organic matter treatments, which they suggested to be responsible for causing the smothering and reduced cover of coralline algae in these treatments. Therefore I cannot exclude the possibility that lower herbivory in organic matter treatments may have masked a negative effect of organic matter on algae. In other words, organic matter may have negatively affected algae, but if herbivores removed some of the algae, algal cover in organic matter treatments would not be lower than treatments without organic matter, resulting in non-significant differences among treatments.

#### Inorganic nutrients effects

The addition of inorganic nutrients increased surface cover of green algae four times above control levels. This result is similar to the results of my previous experiment, where I manipulated inorganic nutrients and herbivory (Carreiro-Silva et al. 2005). The cyanobacteria *Plectonema terebrans* was, however, considerably less abundant in the present experiment ( $2.1 \pm 2.8 \%$ ) than in the previous study ( $31.7 \pm 13.4 \%$ ). *Plectonema terebrans* is a low-light specialist (Kiene et al. 1995; Gektidis, 1999) and would benefit from reduced light levels. Consequently, the difference between my studies is probably due to changes in light or variable recruitment in this alga species. Kiene et al. (1995) reported high recruitment variability in endolithic cyanobacteria. Nonetheless, both of my studies were undertaken in the same reef area during the summer, so it may be that during the first experiment shells were overturned at times, which reduced light conditions for

endoliths growing in the inside part of the shell. Differences in recruitment and growth in different light conditions would help to uncover the reported high variation.

The lower abundance of *P. terebrans* in the fertilizer treatment resulted in lower total percent cover in this study compared to the findings of Carreiro-Silva et al. (2005) ( $58.1 \pm 25.6$  % as compared with  $85.2 \pm 11$  %). Although percent cover by different microborer taxa (green algae, cyanobacteria, and heterotrophs) changed significantly in different treatments (Table 3.2), the number of species recorded in each treatment varied less (Table 3.1). The exception was the lower number of fungi species in the fertilizer treatment as compared with other treatments. While organic matter increased the abundance of heterotrophs as a group, because of the small size of fungi, the green algae *Phaeophila* sp. was the dominant taxon in all treatments. Dominance of substratum cover by this algae is a characteristic of early boring communities (or a juvenile biocoenosis: Gektidis 1999; Vogel et al. 2000). At this stage of colonization, typically 30 to 90% of the bored surface is occupied by *Phaeophila* sp., which agrees with my findings. I did not observe any significant increase in the abundance of low-light specialists such as the green algae *Ostreobium quekettii* and the cyanobacterium *Plectonema terebrans* in treatments with organic matter (Table 3.1). Therefore, the expected light reduction associated with particle deposition in treatments with added organic matter was not strong enough to produce changes in the taxonomic composition of autotrophic microbial endoliths. It is likely that longer experiments are needed to fully describe changes in the succession of the boring community.

Microbial endoliths in this study were identified according to morphological descriptions. Although such approaches have traditionally been the major method of

identification, recent investigations comparing different methods for the identification of endolithic cyanobacteria (e.g., electron microscopy, cultivation, and molecular genetic techniques) suggest that morphological descriptions tend to underestimate the diversity of the microbial endolithic community compared to molecular genetic techniques (Chacón et al. 2006). Therefore, it is possible that I may have underestimated the diversity of microbial endoliths, in particular of filamentous fungi and cyanobacteria that are more difficult to identify morphologically (Golubic et al. 2005; Chacón et al. 2006). Studies that include molecular genetic techniques in the characterization of microbial endolithic organisms will help to better understand their diversity, ecology, distribution, and phylogenetic relationships, and are therefore a priority area of research (Golubic et al. 2005).

### **Bioerosion rates**

Inorganic nutrients increased bioerosion rates by a factor of eight to nine but bioerosion rates were unaffected by the addition of organic matter. The addition of organic matter changed the relative abundance of heterotrophs, but did not influence total bioerosion rates. Rates in the fertilized with inorganic nutrients treatments were the same order of magnitude as the rates I obtained in low herbivory and fertilized treatment in my previous experiment ( $370 \pm 39 \text{ g m}^{-2} \text{ y}^{-1}$  in the organic matter + fertilizer treatment and  $396 \pm 55 \text{ g m}^{-2} \text{ y}^{-1}$  in the fertilizer treatment compared to  $452 \pm 26 \text{ g m}^{-2} \text{ y}^{-1}$  in Carreiro-Silva et al. 2005). The response of microborers to the experimental addition of nutrients (N and P) has only been attempted as part of a fertilization study in the Great Barrier Reef (the ENCORE experiment - Kiene 1997; Koop et al. 2001). This ENCORE

experiment did not record any significant effects of nutrient additions on bioerosion rates by microbial endoliths in their experimental *Tridacna* shells. The maximum-recorded bioerosion rates of 20 to 30 g m<sup>-2</sup> y<sup>-1</sup> were lower than the rates I obtained in my control treatment (44 ± 8 g m<sup>-2</sup> y<sup>-1</sup>). Differences in these bioerosion rates are most likely related to a poor fertilization effect combined with a lack of control for herbivory (see discussion in Carreiro-Silva et al. 2005).

The bioerosion rates I obtained in treatments with added inorganic fertilizers in this study are comparable to the rates of 570 g m<sup>-2</sup> y<sup>-1</sup> measured in coral blocks on reefs with low herbivory and the occasional elevation on nutrients at Moorea Island (Wolanski et al. 1993; Chazottes et al. 1995; Peyrot-Clausade et al. 1995). However, higher bioerosion rates have been measured in coral blocks exposed for 1 to 3 years in reef sites located on the outer barrier or oceanic reefs in the Great Barrier Reef that experience little or no anthropogenic influence (1001-1420 g m<sup>-2</sup> y<sup>-1</sup>; Tribollet et al. 2002; Tribollet and Golubic 2005). In contrast, Chazottes et al. (2002), working on Reunion Island, recorded low bioerosion rates between 57 and 67 g m<sup>-2</sup> y<sup>-1</sup> in coral substrata exposed in reef areas experiencing N and P input and low grazing; these rates are comparable to the rates I obtained in the control treatments. Differences observed between these bioerosion rates could be related to the use of different substratum (shells versus coral), depth, length of exposure (Kiene et al. 1995; Vogel et al. 2000), location of the experiment and method used for the quantification of bioerosion rates.

Although organic matter enrichment reduced the abundance of small grazing damselfish and parrotfish, this is unlikely to have affected the conclusions of my experiment. Even if increased herbivory in the fertilized treatment as compared to the

organic matter + fertilizer treatment resulted in the underestimation of bioerosion rates in the first treatment, the effect of fertilizers in increasing bioerosion is still very clear.

## **Conclusions**

Results of this study and my previous study in the same location (Carreiro-Silva et al. 2005) suggest that the addition of inorganic fertilizers increases densities of microboring organisms, in particular green algae and cyanobacteria, and increases bioerosion rates by a factor of eight to ten in relation to control rates. These findings demonstrate a clear direct effect of increased nutrients, associated with coastal eutrophication, in increasing bioerosion of carbonate environments. Microborers are an important bioerosion agent of dead coral skeletons. The activity of these microborers together with bioerosion caused by macroborers (mainly worms, bivalves, and sponges) and grazers (herbivorous fishes and sea-urchins) can contribute to a decreased calcium carbonate budget of reefs and to a weakening of the coral reef framework. Bioerosion is particularly important in areas that suffered high coral mortality due to bleaching and diseases, because of increased amount of dead coral substratum available for bioerosion by boring and scraping organisms.

Investigations on the effects of inorganic fertilizers on algae abundance as part of this study (reported in McClanahan et al. 2005) and other previous studies in Belize (McClanahan et al. 2002, 2003), did not find a significant increase in abundance of frondose with added fertilizers. This suggests that microborers may be more sensitive to nutrient enrichment or may require less time of exposure to elevated nutrients to produce

a positive response. Therefore, although the role of increased nutrients in promoting the observed shifts from coral- to algae-dominated reefs remains controversial (Miller et al. 1999; Szmant 2002; Diaz-Pulido and McCook 2003; McClanahan et al. 2002, 2003, 2005), increased nutrients increase the degree of erosion of the coral reef framework by promoting the abundance of microborers, leading to the degradation of coral reef health.

My results suggest that increased organic matter in reef waters promotes the abundance of endolithic fungi. Based on these results, I hypothesize that elevated particulate and dissolved carbon from sewage and organic wastes in reef waters may be an important factor that could potentially influence the occurrence of fungi-related diseases in corals. Given the recent concern over increasing reports of disease outbreaks in corals, there is a clear need to investigate the potential role of endolithic fungi as pathogenic agents. At present, there is limited knowledge on the ecology, distribution and phylogenetics of fungi and other microborers. Studies that include molecular genetic techniques on the characterization of microborer communities and how they function are a priority area of research.

\* Information from Dr S. Golubic and Dr G. Radtke (personal communication) that I received after the dissertation defense date suggest that what I considered to be coccoid bacteria traces may be early stages of cyanobacteria in the order Pleurocapsales.



## Chapter 4

### **Effects of phosphorus and nitrogen on microbial endolithic communities and their bioerosion rates**

#### **Abstract**

I used herbivore-exclusion cages in Glovers Atoll, Belize to test the relative importance of nitrogen and phosphorus to microbial endolithic communities (algae, bacteria, and fungi) and their bioerosion rates of *Strombus gigas* shells in a 56-day fertilization experiment. I used a mixture-experiment design, where treatments were different proportions of nitrogen and phosphorus, while the total amount of fertilizer remained constant in all fertilized treatments. This approach allowed me to determine which nutrient was more limiting to microbial endoliths, and whether changes in their responses were proportional to nutrient concentrations in the treatments. I used the simplest case of a mixture-experiment design using only two components or factors in a four-treatment arrangement: (1) one control treatment; (2) treatment with pure nitrogen; (3) treatment with pure phosphorus, and (4) treatment with two equal parts of phosphorus and nitrogen fertilizers (0.5 P and 0.5 N). By the end of the experiment, green algae was highest compared with cyanobacteria and fungi in treatments with added nitrogen. Green algae cover did not increase proportionally with increasing nitrogen concentration in treatments. Two alternative explanations are proposed for this response (1) either green algae were co-limited by phosphorus and nitrogen; or (2) green algae cover was close to

its maximum at half of the amount of nitrogen fertilizer used. In contrast, cyanobacteria cover increased with increasing phosphorus concentration, suggesting that cyanobacteria were P-limited. Fungi were not significantly affected by nutrient addition. Bioerosion rates in treatments with added nitrogen were two times greater than bioerosion rates in the phosphorus alone treatment, and 15 times greater than the control treatment. I conclude that increased nutrient concentrations on coral reefs may increase microbial endolithic densities and bioerosion rates, and their variations in nutrient ratios can modify endolithic community composition.

**Keywords** algae, bioerosion, coral reefs, cyanobacteria, fungi, microbial endoliths, mixture-experiment design, nitrogen, phosphorus

## **Introduction**

Over the last few decades increased algal proliferation has affected coral reefs worldwide (Gardner 2003; Bellwood et al. 2004). Nutrient enrichment has been evoked as a major cause of the increased algal abundance (Lapointe 1992, 1997; Littler et al. 1991; Lapointe et al. 2004), although herbivory has also been demonstrated as an important controlling factor (Miller et al. 2001; Tacker et al. 2001; Williams and Pollunin 2001), with controversy remaining over the relative importance of each of these factors (Hughes et al. 1999; McCook 1999; McClanahan et al. 2002, 2003, 2005; Szmant 2002; Aronson et al. 2003). This increase in algal abundance is often accompanied by decreases

in coral cover, leading to phase shifts from coral to algal domination of coral reefs (Gardner et al. 2003; Bellwood et al. 2004). Thus, the identification and control of nutrients that enhance algal productivity has become a priority area for coral reef research and management (Kramer 2003). Productivity of non-calcareous algae may also benefit from anthropogenic increases in atmospheric carbon dioxide (Gao et al. 1993), and may have important synergistic effects when combined with increased nutrients, increasing shifts from coral-dominated to non-calcareous algae-dominated reefs with reduced framework accretion rates (Szmant 2002; Hallock 2005; Tribollet et al. 2006).

Investigations on the relative importance of nitrogen versus phosphorus limitation of coral reef algal communities have produced variable results (Lapointe et al. 1987, 1992; Fong 1993; Larned 1998; Kuffner and Paul 2001). Based on comparisons of atomic concentrations of inorganic nitrogen and inorganic phosphorus (N:P ratios) in seawater and in algae tissues, some studies have suggested that phosphorus is often the more limiting nutrient in carbonate environments where carbonate sediments can adsorb phosphorus (Lapointe et al. 1987, 1992; Littler et al. 1991). However, results of nitrogen- and phosphorus-enrichment bioassays using tropical macroalgae indicate that nitrogen limitation is also common (e.g., Fong et al. 1993; Delgado and Lapointe 1994; Larned 1998) and may be species-specific or habitat-specific.

Less is known about the relative importance of nitrogen and phosphorus to microphytobenthic turfs, although microcosm studies on benthic or mat-forming cyanobacteria suggest that cyanobacterial mats may be phosphorus limited, while diatoms are mainly limited by nitrogen (Fong et al. 1993; Kuffner and Paul 2001). One

important component of phototrophic microbial communities are microalgae and cyanobacteria that bore and live inside substrates, or endolithic algal communities.

Endolithic algae and cyanobacteria are ubiquitous inhabitants of tropical environments (Golubic and Schneider 2003; Tribollet et al. 2006), colonizing a multitude of carbonate substrates, including skeletons of live and dead corals (Le Campion-Alsumard et al. 1995b), coralline algae (Tribollet and Payri 2001), mollusk shells (Radtke 1993; Mao Che et al. 1996; Carreiro-Silva et al. 2005), limestone rocks (Schneider and Turunski 1983) and loose carbonate sediment grains (Tudhope and Risk 1985).

Microbial endoliths play important ecological and geological roles in reef environments. Recent experimental work by Tribollet et al (2006) has demonstrated that endolithic phototrophs (cyanobacteria and algae) are one of the major primary producers in coral reef ecosystems, with rates of net photosynthesis of as much as  $2\text{g C m}^{-2}\text{ day}^{-1}$ . In addition, studies on endolithic algae living within live coral skeletons suggest that endolithic algae may provide an alternative source of energy to bleached corals, enabling them to survive until the recruitment of new zooxanthellae (Fine and Loya 2002).

Geologically, microbial endoliths are an important agent of bioerosion of carbonate substrates (e.g., Chazottes et al. 2002; Tribollet et al. 2002, 2005; Carreiro-Silva et al. 2005) representing a significant destructive force in a reef's calcium carbonate budget. In addition to the erosion caused by their boring activity, microbial endoliths reinforce bioerosion of carbonate substrates by facilitating recruitment by macroborers (worms, sponges and mollusks) and by making it attractive for grazers as a source of food (Chazottes et al. 1995; Pari et al. 1998).

Results from my previous fertilization and herbivory experiments in Belize showed that bioerosion rates by microbial endoliths were enhanced eight to ten times by fertilization with inorganic nitrogen and phosphorus (Carreiro-Silva et al. 2005; Chapter 3) but reduced by half with the inclusion of herbivores (Carreiro-Silva et al. 2005). These studies have, however, tested the effects of elevated nutrient additions by adding inorganic nitrogen and phosphorus together. Therefore, the present study was specifically conceived to distinguish between the individual effects of phosphorus and nitrogen on microbial endolithic community composition and bioerosion rates.

Field studies of algal responses to nutrient additions have typically used factorial experimental designs testing the effect of nutrient concentrations on algae (Smith et al. 2001; Thacker et al. 2001; Fong et al. 2003; McClanahan et al. 2003). However, while nutrient concentrations may affect algal growth, the effect may also depend on nutrient concentration relative to other nutrients. Because changing the concentration of one nutrient while holding the concentration of other nutrients constant also changes the nutrient ratio, the two factors are often confounded (Cornell 2002).

Mixture-experiment design (Cornell 2002) is an alternative approach to factorial experiments that may be useful in determining the effects of nutrient proportions on algal recruitment and growth. Until recently, mixture-experiments were used almost exclusively to optimize mixture composition in engineering, pharmaceuticals and the food industry, with only one published example of its applicability to plant-nutrient interactions in ecological research (Bush and Phelan 1999). In the simplest mixture experiments, the response is assumed to depend only on the relative proportions of the ingredients or components in the mixture and not on the amount of the mixture. The

quantities of components must add up to a common total. In contrast, in a factorial design, the response varies depending on the amount of each factor. Thus, the factorial experiment measures the response in relation to the amount of each factor, while the mixture experiment investigate the changes in the response of interest that are affected by changing ingredient proportions within each mixture (Cornell 2002).

In this study, I used mixture methodology to measure the response of microbial endoliths in *Strombus gigas* shells to different proportions of nitrogen and phosphorus. My aim was to determine whether microbial endolithic organisms were limited by a single nutrient or co-limited by N and P, and whether changes in microbial endoliths' responses were proportional to the concentrations of nutrients in the mixture. I hypothesized that variations in nutrient ratios would induce taxonomic shifts in microbial endolithic community structure. My results support this hypothesis.

## **Materials and Methods**

### Site description

This study was conducted at Glovers Reef, Belize from June to August 2004. The reef is a coral-rimmed atoll 32-km long and 12-km wide, located approximately 45 km off mainland Belize (see map in McClanahan and Muthiga 1998). Experimental substrates were placed at 2-m depths on the windward side of a patch reef in the Conservation Zone of the atoll's lagoon, where resource extraction is prohibited. The reef

is remote and experiences no significant local organic pollution. The waters in this area are calm with a small ( $<0.5$  m) tidal range and slow current speeds ( $<1\text{ m s}^{-1}$ ). No waves or other physical disturbances such as hurricanes were experienced during the study period.

## Experimental design

To test for the effects of nitrogen and phosphorus on the microbial endoliths' community composition and bioerosion rates, I used a 2-component mixture-experiment design using herbivore-exclusion cages and fertilizers over a 56-day period.

In a mixture experiment, the independent factors are proportions of different components of a mixture, and their sum in a mixture must equal to 1. Therefore, the levels of one factor are not independent on the levels of the other factor. The measured response is assumed to depend only on the relative proportions of the ingredients and not on the total amount of the mixture. If  $x_1, x_2, \dots$ , are the variables representing proportions of  $q$  ingredients in the mixture, the values of  $x_i$  are constrained such that their sum in the mixture must equal to 1,

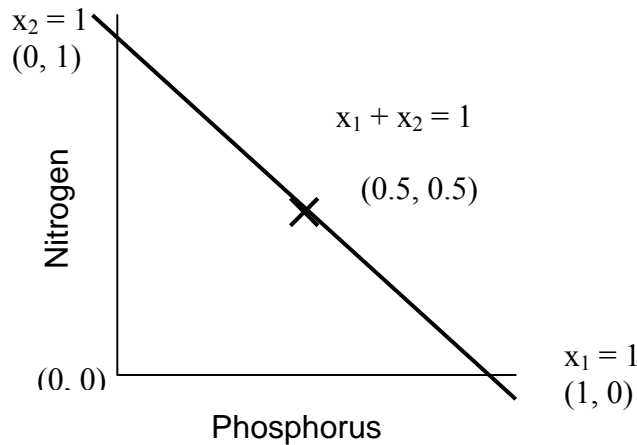
$$\sum_{i=1}^q x_i = x_1 + x_2 + \dots + x_q = 1.$$

If the value of  $x = 1$ , then the other component is absent from the mixture and the product is a pure or single-component mixture.

I used the simplest case of a mixture-experiment design using only 2 components or factors. The factors studied were inorganic nitrogen and phosphorus in a 4-treatment

structure: (1) treatment 1 was a control treatment without fertilizer addition, therefore the treatment consisted of environmental background conditions; (2) treatment 2 consisted of the addition of 1.5 kg of nitrogen (N) fertilizer; (3) treatment 3 consisted of the addition of 1.5 kg of phosphorus (P) fertilizer; and (4) treatment 4 consisted of 0.75 kg of N and 0.75 kg of P.

Figure 4.1 shows the experimental region or factor space for the Nitrogen-Phosphorus mixture experiment design. For two components, the factor space is a straight line. The allowable values for nitrogen ( $x_1$ ) and phosphorus ( $x_2$ ) are coordinate values along the line  $x_1 + x_2 = 1$ . The coordinates (0,1), (1,0) and (0.5, 0.5) are called mixture points and the coordinate system for mixture proportions is called a simplex coordinate system. In my experiment I used a control treatment (0, 0), one pure treatment of nitrogen (1, 0), one pure treatment of phosphorus (0, 1), and a mixture treatment with equal amounts of nitrogen and phosphorus (0.5, 0.5).



**Figure 4.1** Factor space showing the mixture points (i.e., treatments) used in the Nitrogen-Phosphorus mixture experiment. The treatments used were a control treatment (0, 0), one pure treatment of nitrogen (1, 0), one pure treatment of phosphorus (0, 1), and a mixture treatment with equal amounts of nitrogen and phosphorus (0.5, 0.5).



The experiment used 16 cages (50 x 50 x 20 cm) constructed with PVC frames and 3-cm mesh plastic caging material. Cages were tied to cement masonry blocks that kept them solidly on the reef bottom. The cage mesh size allowed for good water flow and light penetration and conditions in the cage were expected to resemble natural conditions. Nevertheless, results of actual bioerosion rates should be interpreted with caution due to possible caging effects.

Experimental substrates were made of *Strombus gigas* mollusk shells. Shells were used instead of coral blocks because their less porous structure produces better casts of boring organisms. This improves identification of boring traces, as well as measurements of surface cover and depth of penetration used for bioerosion rates estimates. In addition, blocks made of live coral often contain pre-existing traces of boring algae and fungi, which may compromise estimates of microborer surface cover and bioerosion rates due to treatment effects. By using the interior parts of shells in this study, pre-existing microborings were avoided. There are differences in substrate density between coral skeletons and mollusk shells, so bioerosion rate estimates for shells may not correspond to rates measured for corals. However, the objective of the study was to investigate how nitrogen and phosphorus interact and affect microbioerosion of carbonate substrates by comparing species composition and bioerosion rates between treatments, and not to determine absolute bioerosion rates that could be extrapolated to reefs in general.

Two pieces (~12 x 6 cm) of *Strombus gigas* shell fragments were placed in each cage, making a total of eight replicate shell samples exposed to each of the four treatments. I used the interior of the shell as the experimental substratum to avoid pre-

existing microborings. Samples of unsoaked shell fragment were collected and examined under a scanning electron microscope to determine if there was any evidence for borings in the fragments prior to their experimental soaking; there were none. Shell fragments were fixed to cages by drilling a hole in each of the conch shell pieces and attaching them to the bottom of the cages with black plastic cable ties such that the shell interiors faced upward. Cages were placed >1 m apart in a line aligned 90° to the dominant current direction such that neighboring cages would not slow the currents experienced by the cages and fertilizer would not influence the non-fertilized treatments. Every other day, cages were cleaned with wire brushes to remove algae and other settling organisms so as to reduce caging artifacts such as decreased light and obstruction of local water flow associated with increased algal growth on the mesh of cages.

Cages excluded large herbivorous fishes and large predators but allowed small fishes such as damselfishes (*Stegastes* spp.), wrasses, and small parrotfish (*Sparisoma aurofrenatum* and *Scarus iserti*) to enter and forage (McClanahan et al. 2003). Additionally, the chosen experiment site was uninhabited by sea-urchins, ensuring that results were not affected by sea-urchin foraging. The number of damselfish, parrotfish, and wrasses that occupied each cage was counted over a 3-min period on days 11, 25, 39, and 53 of the experiment. Following the count, one fish was arbitrarily selected from each cage and the number of bites it took in a 1 min period was recorded. This resulted in 64 observations of herbivory, 32 replicates per treatment distributed evenly across the four treatments.

I found there were no significant differences in average bite rates for damselfish and parrotfish between treatments or over time (MANOVA, all  $F < 2.00$ )., Therefore, I

averaged the number of bites per minute over the 16 cages and all four sampling periods (64 samples in total), multiplied by the respective number of fish in each cage, and then summed the values to derive a herbivory rate for each cage. The average herbivory rates in the four treatments for the four sampling periods were then compared.

#### Nutrient enrichment and sampling

Fertilization of the experimental substrates was achieved by placing 1.5 kg each of solid high phosphorus and high nitrogen slow release fertilizer in the high phosphate and high nitrate cages, respectively. The mixed fertilizer treatment contained 0.75 kg of high nitrogen fertilizer and 0.75 kg of high phosphate fertilizer. The high phosphate was a rock fertilizer (46% phosphate by weight). The nitrogen fertilizer was an Osmocote fertilizer (11.5 % nitrogen as nitrate, 11.5 % as ammonium, 0.5% sodium and 3.3% calcium by weight). Cages were fertilized with the above quantities on the 1<sup>st</sup> and 28<sup>th</sup> day of the experiment by placing fertilizer bags underneath cages, as described in Carreiro-Silva et al. (2005). There was fertilizer remaining in the fertilizer bags at the time of re-fertilization, suggesting that the fertilizer was still diffusing out when the fertilizer was replenished.

Water samples were collected from each cage on days 4 and 32 of the experiment, such that 32 water samples were taken balanced between the four treatments. Samples were collected 1-cm above the coral plates in acid-washed 1 L bottles and analyzed for nitrite (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), ammonium (NH<sub>4</sub>) and soluble phosphates (PO<sub>4</sub>) on the same day with a Hach DR/2500 spectrophotometer using the cadmium-reduction method for

nitrate and ascorbic acid method for phosphorus (Parsons *et al.* 1984). Due to the high variability and uncertainty of ammonium measurements, only concentrations of nitrogen as nitrate and nitrite were used to determine nitrogen concentration. Variability in the ammonium results were attributed to problems with reduction packets used for the ammonium samples.

### Sample preparation

Immediately after collection from the cages, shell fragments were fixed in 4% formaldehyde in sea water solution. I used two approaches to document the composition and densities of microbial endoliths in each treatment: 1) casts of the boring traces in the experimental samples were observed under scanning electron microscopy (SEM) and provided a documentation of the microbial endoliths' community composition and substrate percent cover, allowing the quantification of rates of bioerosion by the endoliths, and 2) observation of microbial endoliths under light microscopy for detailed identification and confirmation of organisms that produce the traces seen in the scanning electron microscopy casts.

For the cast's preparation, I cut and trimmed two 1 cm<sup>3</sup> cubes from the middle portion of each shell fragment using a diamond-blade rock saw. Organic remains in samples were dissolved with sodium hypochlorite for a period of 24 hours, then were rinsed with distilled water and dried overnight at 50 °C. Dried samples were impregnated under vacuum with epoxy resin (araldite) as described by Golubic *et al.* (1970). Embedded shell pieces were sawed along their longitudinal axes and placed in a solution

of hydrochloric acid (5% HCl) to eliminate the shell carbonate matrix and expose the boring trace casts. These casts and casts from unexposed shells were investigated by scanning electron microscopy. I analyzed a total of eight shell samples per treatment, and two 1 cm<sup>3</sup> sub-samples per shell.

For investigation by light microscopy, the soft epilithic overgrowth of shell pieces was removed under a dissecting microscope and diluted HCl was used to dissolve the remaining calcareous incrustation (coralline algae) and substratum. The emerging microbial endoliths were mounted on microscope slides and examined under a Zeiss Universal microscope (400x power).

#### Identification of microbial endoliths and their boring traces

Because the identification of microbial endolithic organisms and quantification of bioerosion rates were based on the morphology of the boring traces, I apply a dual nomenclature to microbial endoliths and refer to them using their ichnotaxonomy as a morphological classification of the traces, and the biological nomenclature for classifying the endolithic organisms that produced those traces. For example the boring trace *Eurygonum nodosum* is produced by the cyanobacterium *Mastigocoleus testarum*. Identification of the microbial organisms and their boring cast followed descriptions in Le Campion-Alsumard (1979), Le Campion-Alsumard and Golubic (1985), Radtke (1993), Radtke and Golubic (2005), and Wissak et al. (2005). I adopted the proposed changes (Radtke and Golubic 2005) of the name for cyanobacterial traces *Hyella* and

*Solentia* from *Fasciculus* Radtke 1991 to *Fascichnus*, and for traces of the green algae *Ostreobium* from *Reticulina* to *Ichnoreticulina*.

## Bioerosion rates

Microboring traces can be classified into several basic types based on their morphology, density of colonization, and depth of penetration in the experimental substrata. Boring intensities on experimental samples were determined by comparing the SEM images to a key of different densities (measured as percentage surface area covered) and depth of penetration by borings as described by Vogel et al. (2000). Scanning electron micrographs with several examples of boring intensities were prepared and the surface area and cross-sectional area of boring traces were carefully measured using a computer image analysis program (ImageJ, available at the National Institute of Health website). By comparing these key images to the small areas viewed with the SEM on each sample's upper surface, the areas could be rapidly classified as to their type and density of boring without having to measure these variables for every area observed. The depth of boring was measured in each sample by observing vertical sections through selected parts of the boring cast. Although it is not an absolute measure of bioerosion, the results obtained from this procedure provide an adequate method for comparing relative bioerosion rates between samples and treatments (Vogel et al. 2000).

Abundance of different microborers and the rate of microboring were measured by classifying 20 1-mm<sup>2</sup> areas of the 1 cm<sup>2</sup> sample's upper surface using the keys above.

The type of boring, density of the boring traces, and depth of boring penetration were recorded for each sample. The 20 1-mm<sup>2</sup> areas were selected systematically in each sample's upper surface following a predetermined pattern that was repeated in every sample as follows: I selected four 1-mm<sup>2</sup> areas, one at each corner of the sample, four 1-mm<sup>2</sup> areas at the center, and four 1-mm<sup>2</sup> areas on transects between the mid-points of every two sides of the sample. This systematic sampling, as opposed to random sampling, minimizes the possibility of over-sampling areas of unusual high or low densities, thus increasing the sensitivity of the sampling and improving estimates of mean erosion rates. The type of boring and density of the boring traces were classified under 300-1000x magnification. The volume of calcium carbonate removed by the microborers in each sample was estimated by multiplying the estimates of the area covered by the boring traces by their depth of penetration. This figure was then multiplied by the density of the substratum (2.65 g cm<sup>-3</sup>) to estimate the rates of calcium carbonate loss by microborers and expressed in g m<sup>-2</sup>. Bioerosion rates over the 56-day experiment were converted to g m<sup>-2</sup> year<sup>-1</sup> to present them in the form most commonly reported in previous studies

## Data Analysis

I used a mixed model one-way nested analysis of variance (ANOVA) to test for the effects of nitrogen and phosphorus mixture treatments on bioerosion rates and to determine the variation in bioerosion rates among shells within a treatment and among cages within a treatment (Mixed procedure, SAS Institute, 2004). The mixture treatments were fixed effects whereas both cages within treatments and shells within cages and

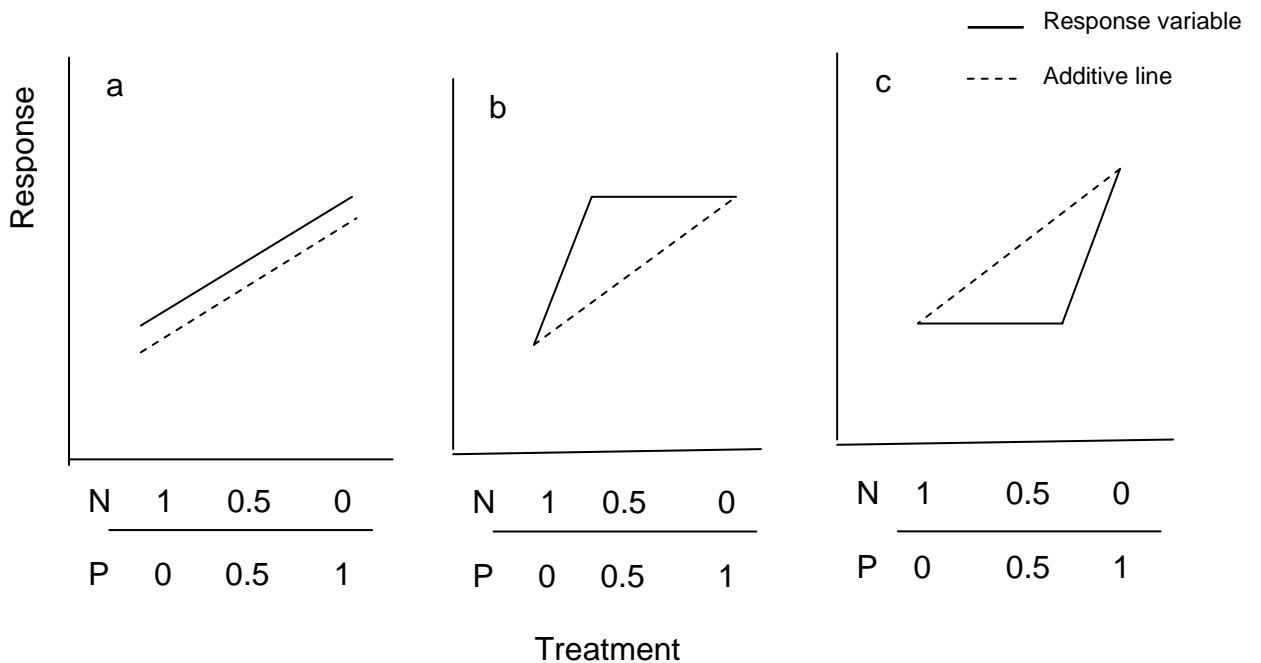
treatments were random effects. Fixed effects in the model were tested using the approximate F-tests of this procedure and the random effect was tested using the variance component approach (Littell et al. 2006). Residual variance component was interpreted as variability among sub-samples within each shell (the basal unit of replication). The percent variation explained by the nested factor relative to the total variation was estimated by dividing the variance component of the nested factor by the total variance (cages within treatments variance + shells within cages and treatments variance + residual variance). The analysis was performed on the log-transformed data to correct for lack of homogeneity of variance.

Treatment effects on percent substratum cover by different microboring taxa (green algae, cyanobacteria and heterotrophs) were analyzed using Proc GLIMMIX in SAS, which fits a generalized linear mixed model to the data (SAS Inst. 2004; Littell et al. 2006). Predicted values of percent substratum cover were logit-transformed to linearize data, and models were fit to the data using residual pseudo-likelihood. This generalized linear mixed model procedure assumed a pseudo-binomial error distribution because the data were recorded on a scale from 0 to 1, and a logit-link function (SAS Inst. 2004). Fixed and random effects in the model were the same as described above.

Objectives of the study were examined by planned comparisons. Accordingly, I tested whether there was a significant effect of the N, P and N + P treatments on mean percent substrate cover and bioerosion rates of the microbial endoliths by comparing the mean response in the control treatment against all other treatments. I tested differences in mean percent substrate cover and bioerosion rates in P and N treatments by comparing the mean response in both treatments. Finally, I tested whether the change in the



microbial endoliths' cover and bioerosion rates was proportional to changes in the proportions of nitrogen and phosphorus, by comparing the simple average value of the response in the N and P treatments and the response measured for the N + P treatment. When the effects of both components in a mixture are additive, the change in the mean response is proportional to changes in treatments and the response variable is best represented by a straight line joining the response of pure N treatment and the response of pure P treatment (Figure 4.2a). If the response variable for the mixture is higher or lower than the simple average response of the pure mixtures (above or below the additive line), then there is an interaction between nitrogen and phosphorus (Figure 4.2b, c), and the mean response is not proportional to changes in treatments.



**Figure 4.2** Example of hypothetical responses to mixture treatments: (a) the effect of both components in a mixture are additive; (b) positive interaction; and (c) negative interaction between the effects of both components in a mixture. N= Nitrogen, P= Phosphorus

A mixture response above the additive line indicates a synergistic effect of the two components in the mixture on the response variable (Fig 4.2b, Cornell 2002). By contrast, a mixture response below the additive line indicates an antagonistic effect of the two components in the mixture on the response variable (Fig 4.2c). However, it should be noted that the point of synergism or antagonism is not necessarily the one producing maximum response.

Data for nutrient concentrations and herbivory rates were analyzed using repeated measures ANOVA mixed model (SAS Inst. 2004), with time, treatments and their interaction as fixed factors and cages nested within treatments as random factors. I report the analysis for the best fit covariance structure by comparing the model fitting statistics from runs fitting different structures. Fisher's least significant differences (LSD) test (Sokal and Rohlf, 1995) was used to perform post-hoc means comparisons for significant effects.

## **Results**

### Experimental effects

Fertilization by both high phosphorous and high nitrogen fertilizer resulted in elevated levels of these two nutrients with no interaction or time effects (Table 4.1, Figure 4.3). Nitrate + nitrite concentrations in the nitrogen treatment were not significantly different from concentrations in the control and phosphorus treatments for

Time 1, but were significantly higher for Time 2 (LSD test  $p < 0.05$ ). Treatment with both nitrogen and phosphorus resulted in nitrate + nitrite concentrations approximately two times greater than control levels (Figure 4.3). Phosphate concentrations in the pure phosphorus treatment were two to three times higher than control levels (LSD test  $p < 0.001$ ), and were nearly two times greater in the mixture treatment when compared with the control treatment (LSD test  $p < 0.01$ ).

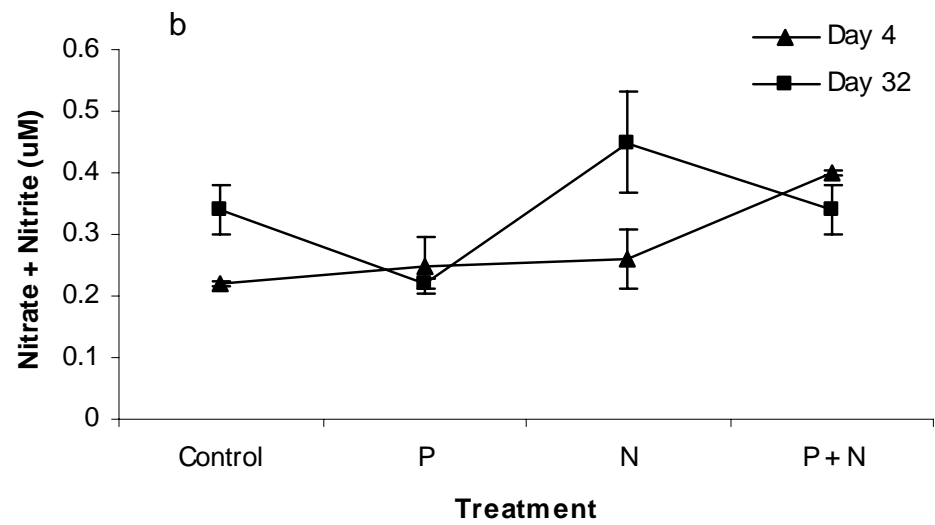
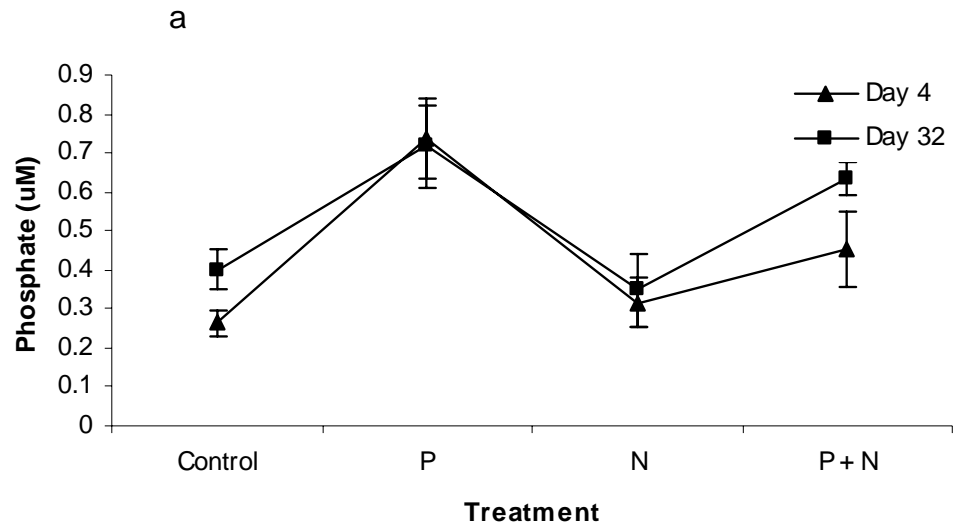
Total bite rates per cage per minute for fish that were able to enter the cages ranged between 90 to 165 bites per min per cage (Table 4.2a). There were no differences in the bite rates among treatments (Table 4.2b).

An estimation of the variance components for fish herbivory rates indicated that 8% of the total variance was due to differences among cages within treatments whereas 92% was due to unexplained variability within treatments.

**Table 4.1** Summary of repeated measures ANOVA statistics of nitrate +nitrite (NO<sub>3</sub>+ NO<sub>2</sub>) and phosphate (PO<sub>4</sub>) concentrations from the two sampling periods. ns = not significant, n = 4 for each treatment and time period.

<b>Nitrate + Nitrite (NO<sub>3</sub> + NO<sub>2</sub>)</b>									
	Effect	df	Variance Component	F	p		Fisher's LSD		
Treatment	Fixed	3		6.39	0.0078		Control	Nitrogen	Phosphate
Time	Fixed	1		2.87	0.1160	Nitrogen	0.0580		
Treatment x Time	Fixed	3		1.30	0.3193	Phosphate	NS	NS	
Residual (Time 1)	Random	12	0.00002			Mix	0.0024	NS	0.0038
Residual (Time 2)	Random	12	0.00002						
Residual Covariance Time 1 and 2	Random	0	0.00003						
<b>Phosphate (PO<sub>4</sub>)</b>									
Treatment	Fixed	3		11.83	0.0007	Nitrogen	NS		
Time	Fixed	1		1.96	0.1865	Phosphate	0.0003	0.0003	
Treatment x Time	Fixed	3		0.73	0.5551	Mix	0.0196	0.0196	0.0364
Residual (Time 1)	Random	12	0.002						
Residual (Time 2)	Random	12	0.0002						
Residual (covariance Time 1 and 2)	Random	0	0.003						

Note: For random effects, the variance components are reported, while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least of squares degrees of freedom



**Figure 4.3** Concentrations ( $\mu\text{M}$ ) of (a) phosphates ( $\text{PO}_4$ ) and (b) nitrogen as the sum of nitrate ( $\text{NO}_3$ ) and nitrite ( $\text{NO}_2$ ) concentrations (mean  $\pm$  SEM) for each treatment.

**Table 4.2** (a) Herbivory rates (mean bites.cage<sup>-1</sup>.min<sup>-1</sup> ± SEM ) for four sampling periods and (b) summary repeated measures ANOVA statistics on the effects of time and treatment on herbivory rates. SEM = Standard error of the mean; N=4 for each treatment and time period.

(a)

	Herbivory Rate (bites.cage <sup>-1</sup> .min <sup>-1</sup> )									
	Day 11		Day 25		Day 39		Day 53		Total	
Treatment	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	110.7	41.1	97.2	65.0	197.3	146.6	134.6	43.3	134.9	39.5
P	200.6	87.8	100.5	45.2	156.7	54.9	202.6	73.6	165.1	32.0
N	77.1	38.1	107.4	62.8	46.2	34.7	141.2	79.9	93.0	27.1
P + N	66.9	41.8	121.1	60.3	153.2	77.0	97.2	51.1	109.6	27.6

(b)

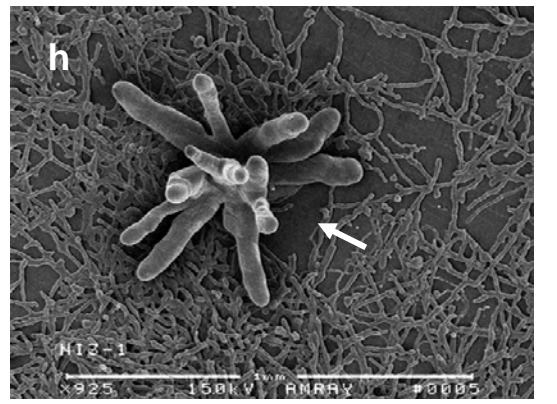
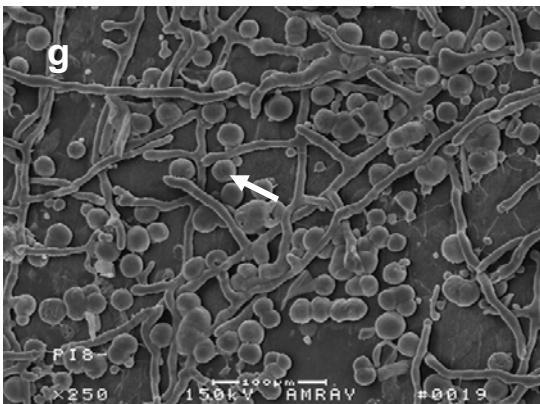
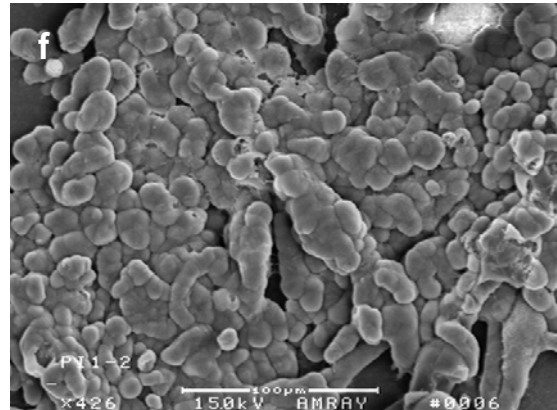
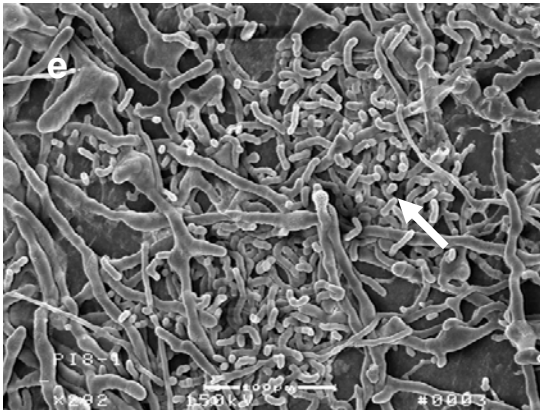
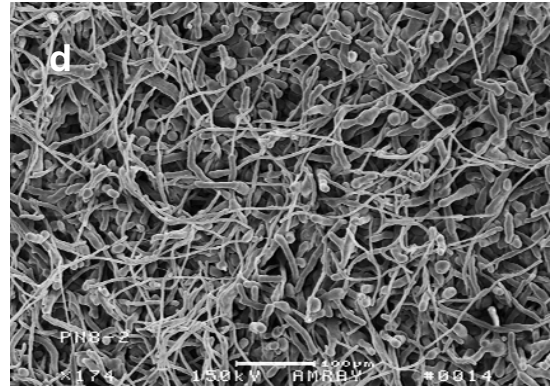
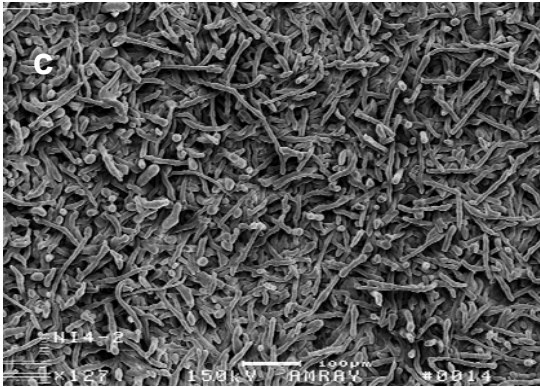
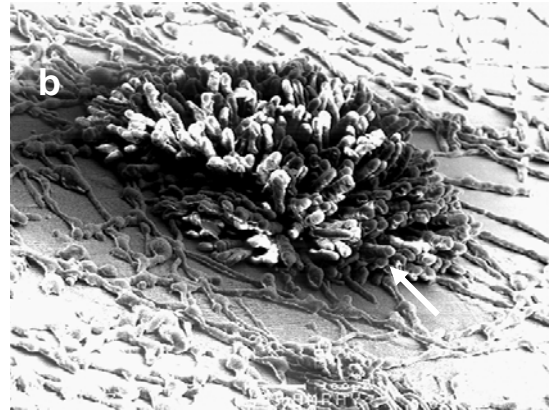
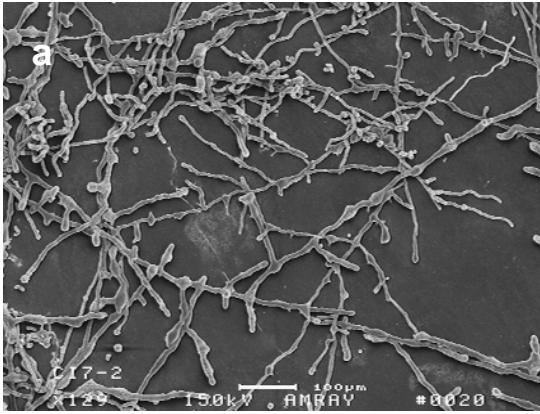
Factor	Herbivory rate				
	Effect	df	Variance Component	F	p
Treatment	Fixed	3		0.67	ns
Time	Fixed	3		0.31	ns
Time x Treatment	Random	9		0.47	ns
Cage (Treatment)	Random	1	1631		
Residual		36	16966		

Note: For random effects, the variance components are reported, while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least of squares degrees of freedom

## Microbial endolithic community composition

I identified a total of 15 different microborer traces in *Strombus gigas* shells corresponding to seven species of cyanobacteria, three species of green algae, and five species of fungi (Table 4.3). Boring traces by the green algae *Phaeophila* sp. were the dominant traces in all treatments (Table 4.3, Figure 4.4). The second most abundant trace corresponded to the cyanobacterium *Hyella balani* in the pure phosphorus treatment ( $8.6 \pm 9.4\%$ , mean  $\pm$  SD). Traces produced by the fungus *Saccomorpha spherula* were more abundant in the phosphorus treatment ( $2.7\% \pm 2.9\%$ , mean  $\pm$  SD). Substrate cover by other microborer traces was very variable and generally less than 2%.

**Figure 4.4** Scanning electron micrographs of microboring casts in experimental substrata made from *Strombus gigas* shell exposed at 2 m depth, Glovers Reef, Belize for 56 days. (a) Typical density of boring trace *Rhopalia catenata* produced by the green algae *Phaeophila* sp. in the control treatment; (b) *Rhopalia catenata* (thick borings) in the background and boring trace *Fascichnus frutex* produced by the cyanobacterium *Hyella gigas* (center colony) in the phosphorus treatment; (c) density of boring trace *Rhopalia catenata* in the nitrogen treatment; (d) *Rhopalia catenata* and the boring trace *Solecia filosa* (thin filaments) produced by the cyanobacterium *Plectonema terebrans* in the N + P treatment; (e) *Rhopalia catenata* and the boring trace *Fascichnus dactylus* produced by the cyanobacterium *Hyella caespitosa* (f) boring trace *Fascichnus acinosus* produced by the cyanobacterium *Hyella balani* (g) Boring trace *Planabola* isp. (spherical chambers) produced by the cyanobacterium *Cyanosaccus piriformis*; (h) boring trace *Fascichnus grandis* produced by the rhizoid of the green algae *Acetabularia*.





**Table 4.3** Percent cover of microboring traces (*ichnotaxa*) and their producers (*bio-species*) in experimental substrata made from *Strombus gigas* shell and exposed to different treatments for 56 days. Values are Mean (Standard deviation).

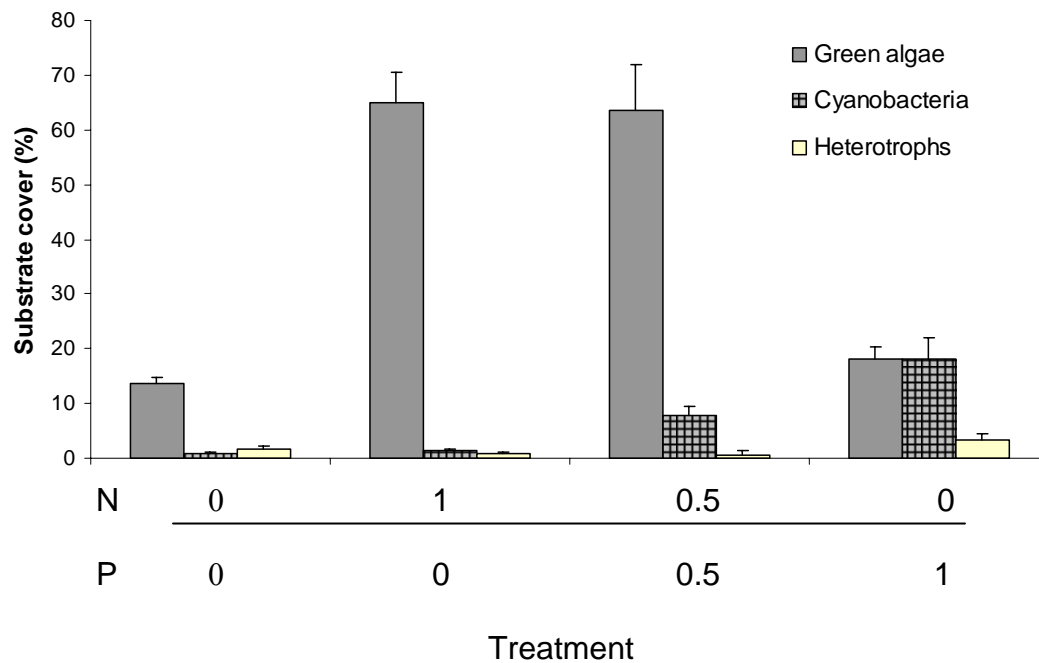
<i>Ichnotaxa = Bio-species</i>	Control	P	N	P + N
<b>Cyanobacteria</b>				
<i>Scolecia filosa = Plectonema terebrans</i>	0.03 (0.08)	1.45 (2.1)	0.87 (1.0)	5.1 (4.5)
<i>Fascichnus dactylus = Hyella caespitosa</i>	0.42 (0.31)	1.08 (1.54)	0.4 (0.7)	1.1 (1.8)
<i>Fascichnus frutex = Hyella gigas</i>	0.08 (0.24)	2.23 (2.1)	0.05 (0.15)	0.1 (0.4)
<i>Fascichnus parvus = Hyella pyxis</i>	–	0.33 (1.2)	–	–
<i>Fascichnus acinosus = Hyella balani</i>	–	9.7 (8.9)	0.14 (0.45)	0.3 (0.8)
<i>Eurygonum nodosum = Masticoleus testarum</i>	0.29 (0.53)	4.93 (6.0)		0.8 (1.2)
<i>Planabola isp. = Cyanosaccus piriformis</i>	0.1 (0.15)	0.63 (1.8)		
<b>Total cyanobacteria</b>	<b>0.93 (0.24)</b>	<b>19.5 (10.0)</b>	<b>1.42 (1.1)</b>	<b>7.5 (4.0)</b>
<b>Green algae</b>				
<i>Fascichnus grandis = Acetabularia rizhoid</i>	–	–	2.02 (1.22)	1.03 (1.9)
<i>Ichnoreticulina elegans = Ostreobium quekettii</i>	0.58 (1.17)	0.38 (0.95)		
<i>Rhopalia catenata = Phaeophila sp.</i>	13.7 (2.79)	17.7 (8.7)	63.1 (13.2)	62.6 (17.9)
<b>Total green algae</b>	<b>14.3 (2.9)</b>	<b>18.2 (5.2)</b>	<b>65 (13.7)</b>	<b>63.6 (20.1)</b>
<b>Fungi</b>				
<i>Saccomorpha sphaerula = Lithophythium gangliiforme</i>	0.3 (0.4)	2.48 (2.77)	0.2 (0.3)	0.14 (0.23)
<i>Saccomorpha clava = Dodgella priscus</i>	0.84 (0.93)	–	–	0.036 (0.12)
<i>Polyactina araneola = Conchyliastrum enderi</i>	0.02 (0.05)	–	–	0.0005 (0.0002)
<i>Orthogonum fusiferum = Ostracoblabe implexa</i>	0.31 (0.45)	0.4 (0.68)	0.71 (0.93)	0.44 (0.72)
<i>Orthogonum lineare = Unknow heterothroph</i>	0.25 (0.55)	–	–	–
<b>Total heterotrophs</b>	<b>1.6 (1.1)</b>	<b>3.1 (3.0)</b>	<b>0.84 (0.94)</b>	<b>0.58 (0.7)</b>
<b>Total</b>	<b>16.0 (3.0)</b>	<b>40.5 (15.9)</b>	<b>67.1 (14.2)</b>	<b>70.2 (20.1)</b>

The addition of fertilizers significantly affected substrate cover by green algae and cyanobacteria, but did not affect fungi cover (Table 4.4). Green algae cover increased by a factor of four with nitrogen addition (in both N and N + P treatments) as compared with the control and P treatments (planned comparisons  $p < 0.0001$ , Table 4.4, Figure 4.5).

**Table 4.4** One-way nested ANOVA (mixed model) on the effects of nitrogen and phosphorus mixture treatments on microbial endoliths logit-transformed substrate cover (%) and log-transformed bioerosion rates ( $\text{gCaCO}_3/\text{m}^2$ ) by all microborers.

	Effect	D. F.	Variance Component	F-value	P- value
<b>Green algae cover</b>					
	Treatment	3		39.40	<0.0001
	Cage ( treatment)	1	0.02367		
	Shell (cage*treatment)	1	0.03505		
	Residual	23	0.02612		
<b>Cyanobacteria cover</b>					
	Treatment	3		17.60	0.0004
	Cage ( treatment)	2	0.2572		
	Shell (cage*treatment)	2	0.1560		
	Residual	15	0.005955		
<b>Heterotrophs cover</b>					
	Treatment	3		5.03	0.0257
	Cage ( treatment)		0.5797		
	Shell (cage*treatment)		0.01454		
	Residual		0.01764		
<b>Bioerosion rates</b>					
	Treatment			57.31	<0.0001
	Cage ( treatment)		0 (0)		
	Shell (cage*treatment)		0.02149		
	Residual		0.01579		

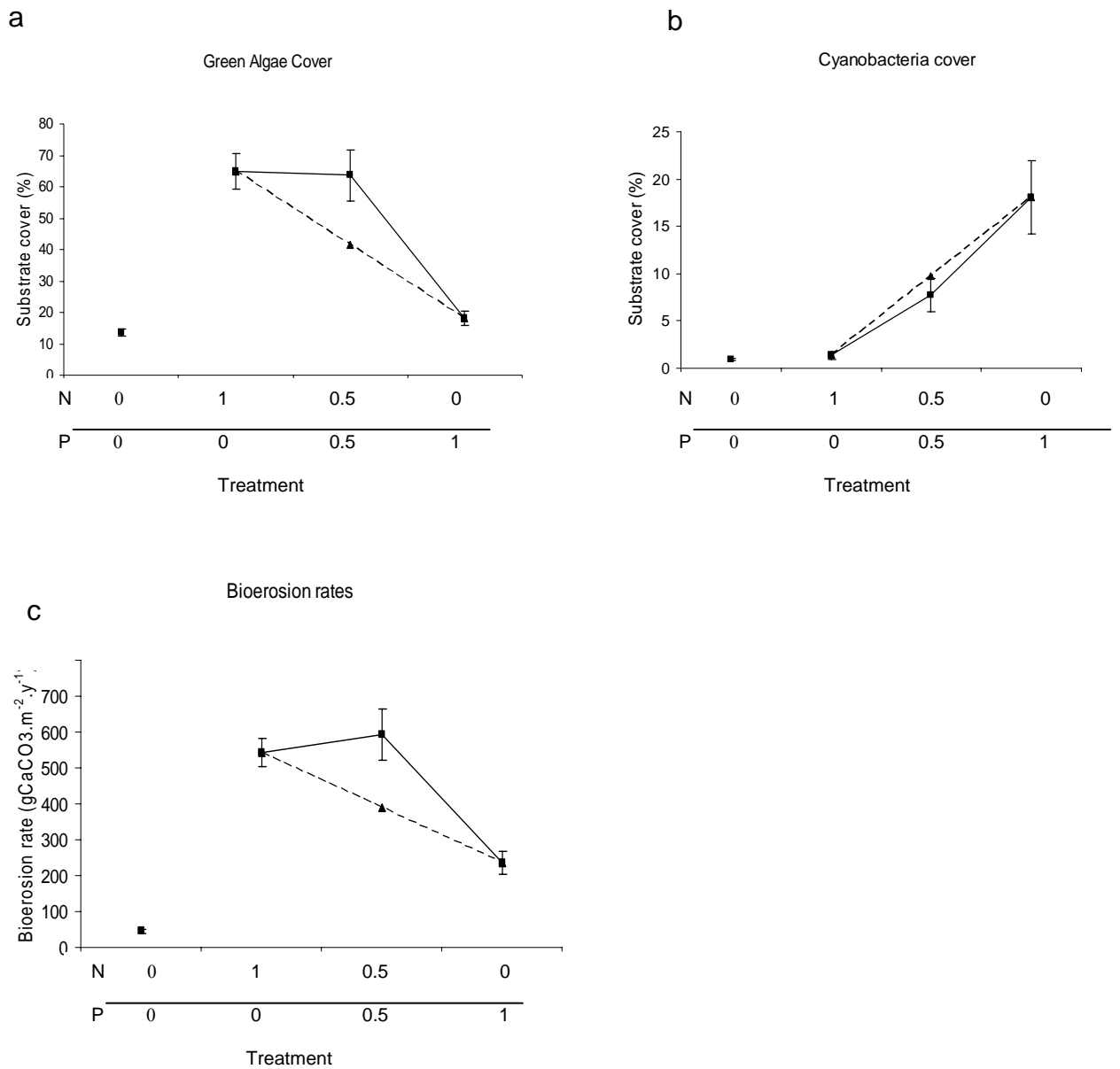
Note: For random effects the variance components and the ratio between the nested factor variance component and residual variance are reported, while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least of squares degrees of freedom



**Figure 4.5** Substrate percent cover (mean  $\pm$  sem) by microborer's taxa (green algae, cyanobacteria, fungi) in different treatments in *Strombus gigas* shells exposed to various proportions of nitrogen and phosphorus for a period of 56 days. N=8 for each treatment.

The lowest green algae cover was recorded in the control treatment and was not significantly different from cover in the phosphorus treatment (planned comparison  $p > 0.05$ ). Green algae cover in the mixture treatment was significantly different from the simple average of algae cover in the pure treatments (planned comparison  $p < 0.01$ ), indicating that a change in fertilizer proportions was not proportional to a change in algae cover (interaction of N and P, Figure 4.6a).

The addition of phosphorus alone increased cyanobacteria's percent cover 20 times in relation to control levels, while the addition of phosphorus and nitrogen increased cyanobacteria cover five to seven times in relation to cover in the nitrogen alone and control treatments respectively (planned comparisons  $p < 0.001$ , Table 4.4,



**Figure 4.6** Diagram showing substrate cover (mean  $\pm$  sem) by different microbial endolithic taxa (green algae, cyanobacteria and fungi) and bioerosion rates by all microbial endoliths, for different proportions of phosphorus and nitrogen. The dotted lines indicate the expected cover if the change in cover were proportional to an increase in either N or P. The asterisk indicates a significant difference from the expected simple average of microborers' cover in the pure treatments.

Figure 4.5). Cyanobacteria cover was lowest and not statistically different in the control and nitrogen treatments (planned comparison  $p > 0.05$ ). I did not find a significant difference between cyanobacteria cover in the mixture treatment and the simple average of their cover in the pure treatments (planned comparison  $p > 0.1$ , Figure 4.6b). Thus, cyanobacteria cover increased proportionally with increasing phosphorus in the mixture

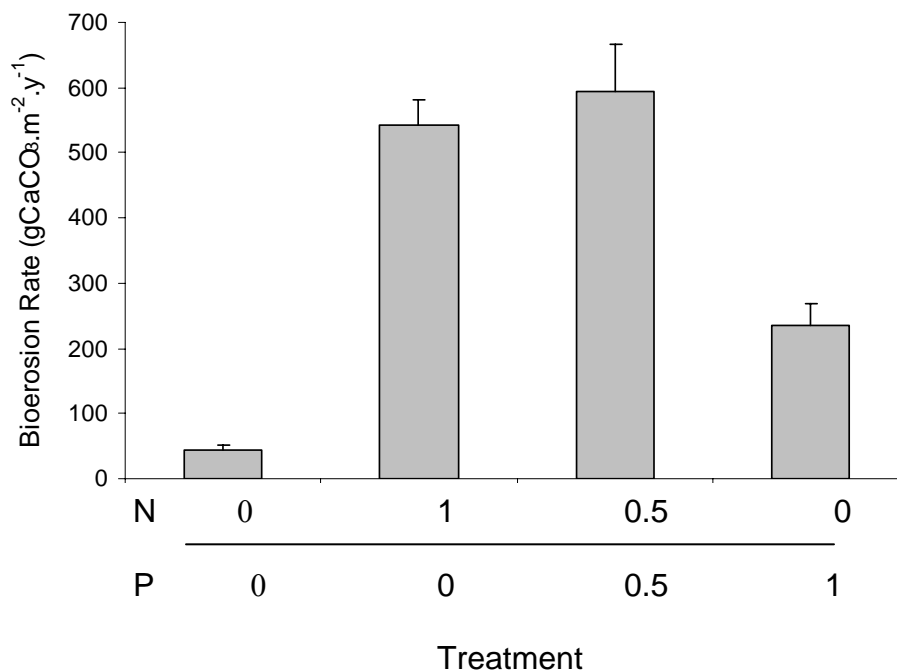
An estimation of the variance components for green algae indicated that 28% of the total variance was due to differences among cages within treatments, 41% was due to differences among shells within treatments, and 31% due to differences among sub-samples within shells (Table 4.4). Variance components for cyanobacteria and heterotroph cover indicated that most of the total variance was due to differences among cages within treatments (61% for cyanobacteria and 95% for heterotrophs). Differences in cyanobacteria cover among shells within treatments explained 37% of the total variance whereas differences among sub-samples within shells explained only 2% of the total variance. Differences on heterotroph cover among shells within treatments and among sub-samples within shells explained only 5% of the total variance.

#### Bioerosion rates

Nitrogen and phosphorus addition either alone or in combination increased bioerosion rate above control levels (Table 4.4). Bioerosion rates were 15 times greater in treatments with added nitrogen ( $544 \pm 39 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$  and  $593 \pm 72 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$  in the N and N+P treatments, respectively) than in the control treatment ( $40 \pm 7 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) and four times greater in the pure phosphorus treatment ( $235 \pm 33 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) than in the control treatment (planned comparisons  $p < 0.0001$ , Tables

4.3 and 4.4, Figure 4.7). Rates were two times greater in the nitrogen treatment than in the phosphorus treatment (planned comparison  $p < 0.001$ ). I found a significant difference in bioerosion rates in the N + P treatment and the simple average of bioerosion rates in treatment with pure N and P (planned comparison  $p < 0.01$ ), indicating that a change in fertilizer proportions was not proportional to a change in bioerosion rates (interaction of N and P, Figure 4.6d).

Variance component estimates indicated that bioerosion rate estimates among cages within treatments were consistent and did not contribute much to the total variance (Table 4.4). Differences in bioerosion rates among shells within treatments explained 58% of the total variance, and differences among sub-samples within shells explained 42% of the total variance.



**Figure 4.7** Bioerosion rates (mean  $\pm$  sem) by all microborer in different treatments in *Strombus gigas* shells exposed for a period of 56 days. N=8 for each treatment.

## Discussion

### Effectiveness of treatments

Measurements of nutrient levels in different treatments showed that the addition on nitrogen and phosphorus doubled concentrations of these nutrients in fertilized treatments in relation to control levels. Nutrient levels in fertilized treatments were above levels considered normal for coral reefs (Kleypas et al. 1999). I used a mixture experiment, where treatments were different proportions of nitrogen and phosphorus while the total amount of fertilizer was maintained constant in all fertilized treatments. I used two different proportions of nitrogen and phosphorus, with the N+P treatment composed of two equal parts of phosphorus and nitrogen fertilizers (0.5 P and 0.5 N), and the nitrogen and phosphorus treatments were composed of only P or N fertilizer. Therefore the pure treatments (N and P treatments) had twice the amount of fertilizer added to the mixture treatment (N+P treatment). Accordingly, I expected that the concentration of nitrate + nitrite and phosphate in the pure treatments would be double that of their concentration in the mixture treatment. However, while this was true for the phosphorus treatment, nitrate + nitrite concentrations in the pure nitrogen treatment were not significantly different from nitrate + nitrite levels in the mixture treatment. It should be noticed, however, that half of the nitrogen added to the nitrogen fertilized treatments was in the form of ammonia, which due to technical difficulties was unable to be measured. Therefore, the total available nitrogen in nitrogen fertilized treatments was most likely two times above measured concentrations. Ammonia is often the preferred

inorganic nitrogen source by algae (Graham and Wilcox 2000), and is likely to have the highest contribution to the measured response of algae in nitrogen treatments.

I used herbivore exclusion cages to minimize the possibly large effects of big grazers on this experiment that were previously studied and found to reduce micorborers' colonization and bioerosion rates (Carreiro-Silva et al. 2005). Small grazers were able to enter the cages but their herbivory rates did not differ among treatments, eliminating any indirect effects of unequal herbivory in my results.

#### Microbial endolithic community composition

Nutrient addition increased microbial endoliths' colonization of shell substrates above control levels suggesting that they were nutrient-limited. Green algae cover increased by a factor of four with nitrogen addition (in both N and N + P treatments) as compared with the control and P treatments (Table 4.4, Figure 4.5). In contrast, the addition of phosphorus alone promoted the relative increase of cyanobacteria with respect to green algae, while fungi did not significantly respond to nutrient addition (Table 4.4). Thus, the relative availability of N and P in conjunction with the differential response of green algae and cyanobacteria to nutrient availability was responsible for the new community structure found in different treatments.

The proportional increase of cyanobacteria cover with increasing P concentration suggests that cyanobacteria were P-limited (Figure 4.6b). In contrast, green algae cover was lowest in the P treatment but did not increase proportionally to increasing nitrogen concentration in the mixture (Figure 4.6a). According to mixture design theory, a mixture response significantly above the additive line indicates a synergistic effect of the



two components in the mixture on the response variable. In this case, the percent cover of green algae above the additive line suggests that green algae were co-limited by nitrogen and phosphorus. An alternative explanation is that green algae are nitrogen-limited, and green algae growth was at saturation at half of the amount of nitrogen fertilizer used.

In order to improve our understanding and distinguish between these two alternative explanations, I would have to repeat this experiment including intermediate ratios of P and N between the mixture treatment and the pure N treatment. If I recorded a curvilinear relationship between increasing nitrogen in the mixture and green algae cover, then this would indicate that green algae were co-limited by N and P. In contrast if I recorded no change in green algae cover after the addition of equal parts of N and P, then this would indicate that green algae were N-limited and that their growth would have reached a saturation state.

The positive response of endolithic cyanobacteria to phosphorus additions recorded in this study is consistent with results from fertilization studies in both tropical and temperate environments. Fong et al. (1993) found increased biomass of cyanobacterial mats with phosphorus additions compared with other phototrophs in microcosm experiments representing shallow coastal lagoons in Southern California. Microcosm experiments by Kuffner and Paul (2001) also demonstrated phosphorus limitation of two benthic mat-forming cyanobacteria from Cocos Lagoon, Guam. In temperate environments, Pinkney et al. (1995), employing nutrient addition bioassays with intertidal cyanobacterial mat communities, found increased cyanobacteria growth in relation to diatoms with phosphorus additions. In addition, Camacho and de Wit (2003) reported that phosphorus additions favored the development of benthic cyanobacteria

mats with respect to diatoms on a benthic microbial mat from a hypersaline lake in NE Spain.

High N:P ratios often favor cyanobacteria in fresh and saltwater systems because of their ability to fix nitrogen (Sellner 1997). Therefore cyanobacterial abundance is often limited by the availability of other nutrients such as phosphorus and iron, both of which are required for the synthesis of nitrogenase (Paerl 1990).

The changes I observed in the relative cover of endolithic green algae and cyanobacteria in different treatments are in agreement with the resource ratio theory (Tilman 1982) that predicts changes on community structure as a consequence of changing resource supply ratios. Tilman's (1982) theory of resource competition has been demonstrated to provide a mechanistic explanation of how resource-supply ratios competitively regulate phytoplankton (Tilman 1982), zooplankton (Rothhaupt 1988) and microbial (Smith 1993) community structure. More recently, Camacho and Wit (2003) demonstrated the applicability of this theory to benthic microbial mat communities.

The resource-ratio theory is based on the assumption of a dynamic relationship between resources and consumers. In nature, populations compete for a host of potentially limiting resources, and this theory suggests that the long-term coexistence of competing species is observed only when the growth rate of each species is limited by a different nutrient. Thus, the theory predicts that a directional change in resource supply ratios for two or more species competing for those resources should result in a directional shift in their competitive dominance. In my experiment, directional changes caused by inorganic nitrogen and phosphorus additions were reflected in a directional shift in the competitive dominance of cyanobacteria and green algae. Cyanobacteria were most

abundant when phosphorus was added alone (low N:P ratio), but green algae were the dominant cover in treatments with added nitrogen (higher N:P ratio). When such ratios are low, nitrogen should be limiting the growth of most of the microbial endolithic species present. Cyanobacteria were dominant in this treatment probably because of their ability to fix atmospheric nitrogen. In contrast, under high N:P ratios phosphorus becomes limiting. The dominance of green algae in treatments with higher N:P ratios suggests that green algae were superior competitors for phosphorus than cyanobacteria.

Although fungi percent cover was slightly higher in the P-alone treatment than other treatments, this difference was not statistically significant, probably because cover was very low (less than 3%) in all treatments. Results from our previous study on the effects of organic matter and inorganic nutrients additions on microbial endolithic communities (Chapter 3) showed that the addition of N and P did not change fungi cover in relation to control levels, but fungi were stimulated by the addition of organic matter, suggesting that they were carbon-limited.

#### Species-specific responses to nutrients

The green algae *Phaeophila* sp. was the dominant species in all treatments. Percent cover by this species increased from 14% in the control treatment to 63% in the N+P and N alone treatments, indicating that it was nutrient -limited. *Phaeophila* sp. is a pioneer short-lived species that typically dominates early boring communities (Kiene et al. 1995; Gektidis 1999; Vogel et al., 2000; Carreiro-Silva et al. 2005). I observed the same response by *Phaeophila* sp. to the addition of N + P in our previous fertilization

experiments (Carreiro-Silva et al. 2005; Chapter 2). Boring traces of green macroalgae *Acetabularia* rhizoid were only present in treatments with added nitrogen, while traces of the green algae *Ostreobium queketti* were only recorded in the control and phosphorus-alone treatment. Nevertheless the percent cover by these species was generally lower than 2% and therefore their present in different treatments is most likely related to random recruitment patterns than to a specific response to the treatments.

Cyanobacteria belonging to the genus *Hyella* were particularly abundant in the P alone treatment (Table 4.3). The higher abundance of *Hyella* in this treatment is probably a direct response to low N:P ratios because *Hyella* species were generally uncommon in other treatments and in our previous experiments (Carreiro-Silva et al. 2005; Chapter 3).

The species *Hyella balani* was the most abundant cyanobacteria species in the P treatment. This is particularly interesting since this species has not been recorded in our previous studies (Carreiro-Silva et al. 2005; Chapter 3). Furthermore, this species was rarely observed in experimental studies in Lee Stocking Island, Bahamas (Radtke 1993; Vogel et al. 2000) and was completely absent from experiments at One Tree Island, Australia (Vogel et al. 2000). *Hyella balani* is typically more abundant in the intertidal zone (Le Campion-Alsumard 1979; Le Campion-Alsumard and Golubic 1985; Glaub 1999), but has been recorded up to 6 m depth in Eilat, Israel (Gektidis et al. 2007). Our results suggest that the addition of phosphorus may have released this species from P-limitation, increasing its recruitment and growth in subtidal areas where it rarely occurs.

The cyanobacteria *Plectonema terebrans* was, however, more abundant in the N+P treatment than the P-alone treatment ( $6 \pm 4\%$  compared to  $1.45 \pm 2.1\%$ , mean  $\pm$  stdev). Although higher abundance of *Plectonema terebrans* in the N+P as compared

with other treatments was also recorded in our two previous fertilization experiments, its specific abundance was variable. For example, its cover was only  $2.1 \pm 2.8$  % in experiment 2 but reached  $31.7 \pm 13.4$  % in experiment 1. These differences may be related to variable recruitment in this algal species, since both studies were undertaken in the same reef area and during the summer. High variability in endolithic cyanobacteria's recruitment has been reported by Kiene et al. (1995).

Our identification of boring cyanobacteria was based on the morphological identification of their cells and boring traces, which is the major method of identification of microbial endoliths. However, the distinction among different *Hyella* species is quite difficult (Radtke and Golubic 2005; Chácon et al. 2006), and it is possible that I may have underestimated the diversity of these microbial endoliths. Future studies using molecular genetic techniques will help uncover the true richness in cyanobacteria species composition.

#### Bioerosion rates

The addition of inorganic nutrients both individually or when added together significantly increased bioerosion rates in relation to control levels (Table 4.4, Figure 4.7). Bioerosion rates were 15 times higher in the mixture and N-alone treatments when compared to control levels. The addition of phosphorus alone increased bioerosion rates by a factor of six in comparison to control levels. The fast-growing early boring green algae *Phaeophila* sp was the main agent of erosion in treatments with added nitrogen, covering as much as 63% of the experimental substrate. In contrast, cyanobacteria were

the main agent of erosion in the pure phosphorus treatment. Here, although substrate cover by cyanobacteria was very similar to cover by green algae (Table 4.3, Figure 4.4 and 4.5), because cyanobacteria colonies (in particular the genus *Hyella*) grow perpendicular to the substrate, they are able to remove more calcium carbonate than green algae. For example, the species *Hyella gigas* penetrated as much as 200  $\mu\text{m}$  of the substrate, in comparison with a maximum of 50  $\mu\text{m}$  penetration by *Phaeophila* sp.

The magnitude of increase in bioerosion rates in treatments with added nitrogen is slightly higher than the bioerosion rates recorded in our previous studies. In Carreiro-Silva et al. (2005) and Chapter 3 I found an increase in bioerosion rates of eight to ten times in treatments with added inorganic nitrogen and phosphorus, when compared to control levels, while I found a 15– time increase in this study. These differences are related to higher depths of penetration by microborers in the present study. For example, traces of *Acetabularia* rhizoid (Figure 4.3) in nitrogen fertilized treatments sometimes reached 350  $\mu\text{m}$  deep.

Our studies in Belize represent the first direct experimental evidence of the effect of nutrients in increasing microborer bioerosion rates. Studies on the response of microbial endoliths to fertilizers additions as part of the ENCORE project (Kiene 1997; Koop et al. 2001) did not find any significant effects of fertilizers on microbioerosion. Kiene (1997) measured rates of 20 to 30  $\text{g m}^{-2} \text{y}^{-1}$  in *Tridacna* shells, which are comparable to the rates I obtained in the control treatment. A poor fertilization effect combined with a lack of control for herbivory are likely to be responsible for the different results (see discussion in Carreiro-Silva et al. 2005).

Comparisons between bioerosion rates obtained in this study and those in the literature are hampered by the use of different experimental substratum, depth, length of exposure (Kiene et al. 1995; Vogel et al. 2000), location of the experiment, and method used for the quantification of bioerosion rates. For example, bioerosion rates obtained in the treatments with added inorganic fertilizers in this study are comparable to rates of 570  $\text{g m}^{-2} \text{y}^{-1}$  measured in coral blocks after 2 months exposure on reefs with low herbivory and the occasional elevation on nutrients at Moorea Island (Wolanski et al. 1993; Chazottes et al. 1995; Peyrot-Clausade et al. 1995). However, higher bioerosion rates have been measured in coral blocks exposed for 1-3 years in reef sites located on the outer barrier or oceanic reefs in the Great Barrier Reef (Australia) experiencing very little or no anthropogenic influence (1001-1420  $\text{g m}^{-2} \text{y}^{-1}$ ; Tribollet et al. 2002; Tribollet and Golubic 2005). In contrast, Chazottes et al. (2002) working on Reunion Island recorded bioerosion rates between 57 and 67  $\text{g m}^{-2}$  in coral substrata exposed in reef areas experiencing N and P input and low grazing, which are comparable to the rates I obtained in the control treatment ( $40 \pm 8 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ). Other factors such as temperature, salinity, wave energy condition and degree of grazing by herbivorous fishes and sea-urchins may also influence the degree of bioerosion (Kiene 1997; Vogel et al. 2000; Carreiro-Silva et al. 2005; Tribollet and Golubic 2005). Therefore, future studies that specifically control for variations on these factors are needed.

## Conclusions

- Results from this and previous experiments (Carreiro-Silva et al. 2005; Chapter 3), consistently show a significant effect of nutrients in stimulating microbial endoliths' substrate colonization and bioerosion rates. High water column N:P ratios appears to cause the greatest increase for substrate cover and bioerosion rates, mainly by stimulating the fast-growing pioneer green algae *Phaeophila* sp. Microbioerosion rates in treatment with added nitrogen were eight to 15 times greater than control levels in all three experiments.
- Changes in nutrient ratios changed microbial endolithic community structure, with the addition of nitrogen alone or in combination with phosphorus stimulating green algae and the addition of phosphorus alone stimulating cyanobacteria. Additionally, results from our previous experiment (Chapter 3) showed that fungi were stimulated by organic matter addition.
- Although the role of increased nutrients in promoting the observed shifts from coral- to algae-dominated reefs remains controversial (Miller et al. 1999; Szmant 2002; McClanahan et al. 2002, 2003, 2005; Diaz-Pulido and McCook 2003), results of our microbioerosion studies show a clear direct effect of increased inorganic nutrients in increasing microbioerosion of carbonate environments. This suggests that microborers may be more sensitive to nutrient enrichment or may require less time of exposure to elevated nutrients to produce a positive response.



## Chapter 5

### **The importance of nutrients and herbivory on the microbioerosion of experimental carbonate substratum in Eastern Africa reefs**

#### **Abstract**

Microbial endolithic communities (algae, bacteria, and fungi) and their bioerosion rates were studied at three to six months intervals using herbivore-exclusion cages and experimental substratum made of *Lambis chiragra* mollusk shells on nine reefs along the East African coast with different levels of nutrients and number of grazing fish and sea urchins. The aim was to determine the relative importance of nutrients and herbivory in controlling patterns and rates of microbioerosion based on previous experimental studies in Glovers Reef Belize. Hypotheses tested were that (1) rates of microbioerosion would be correlated with spatial variations in nutrient availability; (2) grazing by herbivorous fish and sea urchins would reduce measurable microbioerosion rates; (3) sea urchins would be more influential to microbioerosion rates because their grazing is more intense and localized than herbivorous fish; (4) herbivory would arrest community succession of microborers at an early stage; (5) high concentrations of nitrogen would preferentially stimulate green algae, whereas high phosphorus would stimulate cyanobacteria, and carbon would stimulate heterotrophs. Microbial endolithic community composition changed with time, from a community dominated by the green algae *Phaeophila* sp. and the cyanobacterium *Masticoleus testarum* to a community with increasing abundance of the cyanobacterium *Plectonema terebrans* and the green algae *Ostreobium quekettii*. This

change was more noticeable in ungrazed than grazed substratum, suggesting that herbivores, by removing the superficial layers of the substratum during grazing, prevent microborer full ecological succession. No clear relationship was found between nutrient levels and percent cover of bioeroding taxa. Both nutrified reefs and “pristine” reefs experienced high colonization by microbial endoliths and bioerosion rates. Instead, total percent cover and bioerosion rates of microborers were negatively correlated with current speed and cover of encrusting coralline algae. These results, together with the observations of lower bioerosion rates in substratum covered by coralline algae, suggest that coralline algae may inhibit colonization of microborers by reducing light availability reaching endoliths and prevent the expected effects of high water column nutrient concentrations on microborers.

**Keywords:** algae, cyanobacteria, ecological succession, endoliths, eutrophication, fisheries closures, fungi, Indian Ocean, water flow

## **Introduction**

There is a growing interest in the role of microbial endoliths, or microborers (bacteria, fungi, and algae), as agents of bioerosion of calcium carbonate substratum in coral reefs. While early studies have seldom measured microbioerosion rates as part of bioerosion estimates of reefs (Kiene 1988; Sammarco and Risk 1990; Kiene and Hutchings 1994; Risk et al. 1995; Reaka-Kudla et al. 1996), more recent studies have recognized that microborers play an important role in the calcium carbonate budget of

reefs (Chazottes et al. 1995; Peyrot-Clausade et al. 1995, 1999; Chazottes et al. 2002; Tribollet et al. 2002; Carreiro-Silva et al. 2005; Tribollet and Golubic 2005). Microborers are ubiquitous inhabitants of carbonate substrates in coral reefs, participating in the erosive morphogenesis of coastal limestones (Schneider and Turunski 1983), in the breakdown of coral skeletal and other substrates (Chazottes et al. 1995; Mao Che et al. 1996; Vogel et al. 2000; Chazottes et al. 2002; Tribollet et al. 2002; Carreiro-Silva et al. 2005; Tribollet and Golubic 2005), and in the production and modification of sediment grains (Schneider and Turunski 1983; Tudhope and Risk 1985).

Previous research has focused on the identification of microbial endolithic organisms and their traces (Radtke 1993; Kiene et al. 1995; Vogel et al. 2000; Radtke and Golubic 2005), on the degree of colonization of different types of substrate (e.g., coral skeleton, shells, limestone: Golubic et al. 1975; Perkins and Tsentas 1976; Kobluk and Risk 1977; Kiene et al. 1995; Le Campion-Alsumard et al. 1995b; Vogel et al. 2000). There have been studies of their usefulness as paleo-environmental indicators (Glaub 1999; Vogel et al. 2000), the distribution and colonization levels of microbial endoliths across reefs (Chazottes et al. 1995, 2002; Tribollet et al. 2002; Tribollet and Golubic 2005), and on the response of microborers to the experimental addition of nutrients (Kiene 1997; Carreiro-Silva et al. 2005). These studies have provided an initial insight into the potential controls of substrate colonization levels and species community assemblages.

Factors that may affect the distribution and colonization levels of microborers include type of substrate (Kiene et al. 1995; Vogel et al. 2000), light availability (Kiene et al. 1995; Vogel et al. 2000; Gektidis 2007), grazing by herbivores (Chazottes et al. 2002;

Tribollet et al. 2002; Carreiro-Silva et al. 2005; Tribollet and Golubic 2005), and nutrient concentrations (Chazottes et al. 2002; Carreiro-Silva et al. 2005).

Grazing by herbivorous fishes and sea urchins and variations in nutrient concentrations are particularly important controlling factors of microbioerosion in shallow reef areas (< 20 m depth). The significance of these factors has been demonstrated in experiments in the offshore reefs of Belize (Carreiro-Silva et al. 2005). These studies showed that fertilization with inorganic nitrogen and phosphorus enhanced colonization by microbial endoliths and their bioerosion rates by a factor of 8 to 15, but that the inclusion of herbivores reduced observed bioerosion rates by half. Herbivorous fish influence bioerosion rates by feeding on endolithic algae, thus decreasing measurable bioerosion rates, and potentially masking nutrient effects on microbioerosion (Carreiro-Silva et al. 2005).

Field studies on the relationship between nutrients, herbivory levels, and microbioerosion rates have given variable results (Zubia and Peyrot-Clausade 2001; Chazottes et al. 2002; Tribollet et al. 2002). Two studies (Zubia and Peyrot-Clausade 2001; Chazottes et al. 2002) found higher bioerosion rates by microborers in reefs subjected to eutrophication compared to more oligotrophic reefs. Chazottes et al. (2002) recorded high microbioerosion rates in association with low grazing while Zubia and Peyrot-Clausade (2001) found higher rates in heavily grazed sites. Studies by Tribollet et al. (2002) and Tribollet and Golubic (2005) in the Great Barrier Reef found the lowest microbioerosion rates to occur in inshore waters subjected to high terrigenous inputs and suggested that low rates resulted from low light levels that restricted colonization of microborers in the presence of high nutrients. A contributing factor to the lack of

agreement among these studies is that reefs often experience different environmental conditions and a combination of human and natural disturbances, which are often difficult to tease apart.

To better understand the factors that influence microbioerosion rates in coral reefs, I conducted a study on the spatial variations of bioerosion rates in nine East African reefs. These reefs differed in nutrient levels, numbers of sea urchin and fish, and benthic community structure based on factors such as fishing intensity and nearness to shore and human population densities. In Eastern Africa, increasing urbanization of coastal areas and increased intensive agricultural activities in catchments has led to increases in the rate of land runoff (Obura et al. 2000; Fleitman et al. 2007). This runoff includes sediment, nutrients from fertilizers, and sewage that are discharged into coastal waters after heavy rains. For example, there is evidence that Malindi Marine National Park (MNP) has been receiving increasing sediment and nutrients from the Sabaki River associated with an increase in land use that has promoted soil loss for the past 50 years (Dunne 1979; Fleitmann et al. 2007). A second park, Watamu MNP, lacks the sediment problem but receives terrestrial run-off from a local creek and groundwater, and many reef sections are dominated by fleshy algae, even in the presence of large herbivorous fishes (McClanahan et al. 1999). In addition, a bleaching episode in 1998 has increased the number of dead coral colonies (McClanahan et al. 2001), opening substratum for borers and grazers, with the potential for an increase in the amount of bioerosion occurring on affected reefs.

The aim of this study was to determine the relative importance of nutrients and herbivory in controlling patterns and rates of microbioerosion, and to examine how these

interact with other physical-chemical and ecological variables in reefs. Hypotheses tested were that (1) rates of microbioerosion would be positively correlated with water column nutrient concentrations; (2) grazing by herbivorous fish and sea urchins would arrest succession; (3) sea urchins would reduce microbioerosion rates more than herbivorous fish because their grazing is more intense and localized; (4) herbivory would reduce microborerosion rates; (5) high concentrations of nitrogen would preferentially stimulate green algae, whereas high phosphorus would stimulate cyanobacteria, and carbon would stimulate heterotrophs.

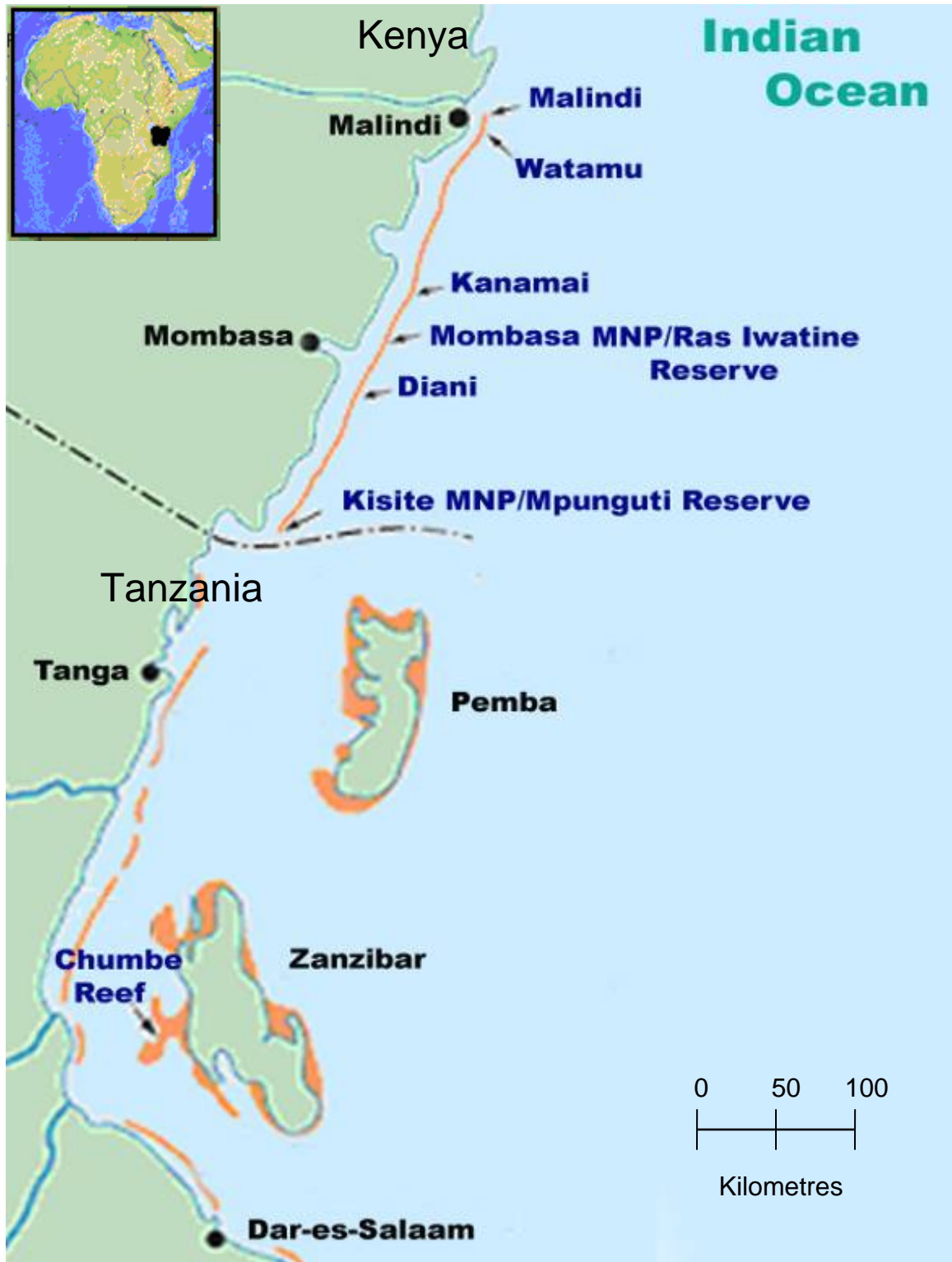
## **Materials and Methods**

### Oceanographic conditions and study sites

Physical, chemical, and biological oceanography processes in East African coastal waters are controlled by the behavior of the Inter-Tropical Convergence Zone that creates two distinct seasons - the northeast and southeast monsoons (McClanahan 1988).

Meteorological parameters in the southeast monsoon (March to October) are characterized by high cloud cover, rainfall, river discharge, terrestrial runoff, and wind energy, whereas solar insolation and temperatures are low. Oceanographic parameters are characterized by cool water, a deep thermocline, high water-column mixing and wave energy, and rapid currents. These meteorological and oceanographic parameters are reversed during the northeast monsoon (October to March).

Study sites included five reefs in marine protected areas or fisheries closures and four unprotected reefs in Eastern Africa (Fig. 5.1) that are exposed to various levels of pollution and with varying numbers of herbivorous fish and sea-urchins. Protected fisheries closures locations included (i) three fringing reef parks — the Malindi, Watamu and Mombasa Marine National Parks (MNP) — that exclude all forms of fishing as well as coral and shell collection, that are characterized by high numbers of grazing fish, but that are close to urban areas and are therefore subjected to rural and urban waste and runoff; (ii) one fringing reef park — Chumbe Reef Sanctuary in Northern Tanzania — characterized by high numbers of grazing fish and that does not experience any notable sources of pollution; and (iii) one offshore patch reef park — Kisite Marine National Park. This reef is remote and experiences no notable form of land-based pollution and is characterized by high numbers of grazing fish. The Malindi and Watamu MNP have been protected from fishing and shelling since 1968, and Kisite MNP has been protected since 1978. Mombasa MNP was declared a marine protected area in 1989, but fishing was eliminated in 1991. The Chumbe Reef Sanctuary was established in 1994 and is part of a privately owned conservation area that includes the whole island.



**Figure 5.1** Study sites along Kenya's and Tanzania's coast. Sites included four Marine National Parks (Malindi, Watamu, Mombasa, Kisite, and Chumbe Island), two unprotected reefs (Kanamai and Diani) and two marine reserves (Ras Iwatine and Mpunguti). Coral reefs are shown in orange.



Unprotected locations open to most forms of fishing included Diani, Kanamai and Ras Iwatine fringing reefs, which experience heavy fishing and are dominated by high numbers of sea urchins. These reefs are located close to urban areas and are subjected to rural and urban waste and run-off. An offshore patch reef — Mpunguti Marine Reserve that experiences moderate fishing but is remote and experiences no form of pollution — was also included. The Kanamai and Diani reefs have no protective legislation and experience coral collection in addition to heavy fishing. Diani is one of the most developed tourist resort-hotel areas in Kenya, in addition to supporting fishing communities that extend back more than 200 years (Obura et al. 2000). It is also the most degraded shallow reef on the Kenyan coast, due to extraction of fish and other organisms. Ras Iwatine and Mapunguti have been gazetted as marine reserves since 1978 and 1972 respectively, but protective management is almost non-existent. Each reef harbors lagoonal areas and although each lagoon differs in its location and relationship to environmental factors, study sites in the reefs are similar as far as being protected from strong waves, having a substrate of calcium carbonate, and being shallow at low tides (<1.5 m). Reefs are distributed along approximately 390 km of the coastline.

Malindi's coral reef lagoon is a depression in a patch reef (North Reef) approximately 1 km from the shore, and forms the northern end of Kenya's fringing reef. It is influenced by a freshwater discharge from the Sabaki River that lies 15 km north of the Park. Watamu's reef lagoon consists of coral growing on a reef edge approximately 2 km north of the Mida Creek discharge (saltwater lagoon) and about 150 m from shore. The Watamu reef flat is lower (< 0.3 above datum) than other sites, is rarely exposed except on extreme low tides, and experiences greater wave and current activity. Mombasa

MNP is located 1 km from the shore and is a long stretch of lagoonal fringing reef with a tidal channel formed by a depression on the reef. Kisite MNP, at the southernmost part of Kenya's coast, is located 8 km seawards of Wasini Island. The reef at this location forms a shallow shelf 10 to 20 m deep, with adjacent small patch reefs. Mapunguti marine reserve is located 1.7 km north of Kisite MNP and has the same reef conformation as the park. Chumbe Reef Sanctuary is a fringing reef located on the eastern shore of Chumbe Island, 14 km southwest of Zanzibar Town, Tanzania (Horrill et al. 2000). The two unprotected reefs (Kanamai and Diani) are also lagoonal fringing reefs 200 and 300 m from shore, respectively. Ras Iwatine is a hard-substrate dominated lagoon separated from Kenyatta Beach by a deep channel and is located 200 m from shore.

In general, major environmental differences between the protected and unprotected reefs are that protected reefs have some unique physical feature (i.e., patch reef, drop off reef, and surge channel), and are generally slightly deeper (mean depth of sampled areas in lagoons during low spring tides = 1 m). The unprotected reef lagoons included in this study are perhaps more typical of most of Kenya's fringing reef lagoons and are somewhat shallower (mean low water depth = 0.47 m). Reef lagoons are composed of coral sand and seagrass with discontinuous patches of hard coral (Hamilton and Brakel 1984; McClanahan and Mutere 1994) that are inhabited by the greatest diversity of fish and invertebrates (McClanahan 1994).

## Experimental design

The experimental design was completely randomized, with one controlled source of variation — exposure to herbivory. On each reef, I exposed carbonate substrates made of shells of the gastropod *Lambis chiragra* to two levels of herbivory by attaching shell pieces to the inside of cages that excluded larger herbivorous fishes and sea-urchins, and by attaching shell pieces to the outside of the cages to allow them to be exposed to herbivory. The experiment was conducted between August 2002 and April 2003. One cage per reef site was collected in November 2003 after 3 months exposure and the other cage was collected in April 2003 after 6 months exposure, to allow for changes in microendolithic community composition and bioerosion rates over time of exposure.

Experimental substrates were made of *Lambis chiragra* mollusk shells instead of coral blocks because their less porous structure produces better casts of boring organisms. This improves identification of boring traces, as well as measurements of surface cover and depth of penetration used for bioerosion rates estimates. In addition, blocks made of live coral often contain pre-existing traces of boring algae and fungi, which may compromise estimates of microborer surface cover and bioerosion rates due to treatment effects. By using the interior parts of shells in this study, pre-existing microborings were avoided. There are differences in substrate density between coral skeletons and mollusk shells, so bioerosion rate estimates for shells may not correspond to rates measured for corals. However, the objective of the study was to investigate the effects of herbivory and water quality in bioerosion rates by comparing species composition and bioerosion

rates among reefs and over time, and not to determine absolute bioerosion rates that could be extrapolated to reefs in general.

Experiments used triangular cages (30-cm sides and 15-cm height) constructed with 3 cm meshed plastic caging material, with two cages on each of two reef sites (4 cages per reef). Six pieces (~4 x 6 cm) of shell fragments were randomly assigned to each cage; three pieces were tied to the outside and three to the inside of the cages, making a total of six replicate shell samples exposed to each of the two levels of herbivory. I used shell interior parts as experimental substrate to avoid pre-existing microborings. Samples were fixed to cages by drilling a hole in each of the conch shell pieces and attaching them to the cages with plastic cable ties.

The microendolithic species in each treatment and their rates of bioerosion were investigated by scanning electron microscopy and light microscopy, as described in Chapters 2, 3 and 4.

#### Field measurements

Measurements of benthic cover, and sea-urchin and fish biomass were obtained from data collected by the Coral Reef Conservation Project (CRCP, Wildlife Conservation Society). The project has been monitoring benthic cover, sea-urchin abundance, and fish populations in Kenyan coral reefs since 1987. Here I report data collected between 2002 and 2005. Methods used in benthic cover and herbivore biomass measurements are briefly described below:

### *Benthic substrate cover*

Sessile benthic communities were studied by the line-intercept method using 12 to 18 haphazardly placed 10-m line transects per site. Cover of benthic macrobiota under the line > 3 cm in length was classified into nine categories (hard coral, soft coral, algal turf, coralline algae, calcareous algae (*Halimeda*), fleshy algae, seagrass, sand, and sponge) and their lengths were measured to the nearest centimeter (McClanahan and Shafir 1990). An estimate of topographic complexity of the reef was determined by pressing the 10-m line along the contour of the reef, then measuring the straight line distance that the line traveled and dividing this by 10 m (McClanahan and Shafir 1990).

### *Sea-urchin biomass*

Sea-urchins were identified to species and counted in nine to 12 haphazardly placed 10 m<sup>2</sup> circular plots per site. The wet weight of each species was estimated from length-weight correlations for individual species (McClanahan and Shafir 1990). Total sea urchin wet weight was estimated by summing the wet weights of each species.

### *Fish biomass*

Biomass of fish belonging to the families Scaridae and Acanthuridae was estimated using two 5 m × 100 m belt transects per site (McClanahan 1994; McClanahan and Kaunda-Arara 1996). Only fish belonging to these two families were considered because they are the primary consumers of endolithic algae (Bruggeman et al. 1996; Glynn 1997). Wet-weight estimates were made, estimating fish length and placing it into 10 cm size intervals. No individuals < 3 cm in length were recorded. Wet weights per

family were estimated from length-weight correlations established from measurements of common species in each family (McClanahan and Kaunda-Arara 1996).

## Water quality

### *Sample collection*

Nitrogen availability (as nitrate, nitrite and ammonia concentrations), phosphorus availability (as ortho-phosphate concentration), suspended particulate matter (SPM), particulate organic matter (POM), chlorophyll *a*, temperature and water flow were monitored by the Coral Reef Conservation Project at 16 reef lagoon locations (2 sites in 8 reefs) in Kenya (see study sites, Fig. 5.1). All sites were 0.5 to 2 m deep, depending on tidal height. Each site was surveyed 10 times, approximately 12 weeks apart between September 2002 and August 2005. Environmental data for Chumbe Island Sanctuary were obtained from Horrill et al. (2000).

Every 12 weeks, water samples were collected close to the substratum using Nalgene bottles previously washed with a solution of 0.1 N HCl. One to three replicate water samples were collected using 1-liter Nalgene bottles for nitrate, nitrite, ammonia, and phosphate analysis. In addition, five replicate water samples were collected using 3-liter dark-colored jars for chlorophyll *a*, total suspended matter, and particulate organic matter measurements.

Water temperature was measured with a water temperature logger (Hobo Temp; Onset Corporation Ltd.), which recorded hourly measurements in each reef station. Current speed was estimated using clod cards deployed at each of the study sites, following descriptions in McClanahan et al. (2005).

### *Analytical procedures*

Water samples used for nitrate, nitrite, ammonia, and phosphate measurements were placed inside an icebox immediately after collection. Nutrients concentrations were measured no more than 4 hours after collection with a Hach DR/2500 spectrophotometer using the cadmium reduction method for nitrogen and the ascorbic acid method for phosphorus (Parsons et al. 1984) at the Coral Reef Conservation Project headquarters.

Chlorophyll *a*, suspended particulate matter, and particulate organic matter concentrations were analyzed at the local government marine research center, the Kenya Marine and Fisheries Research Institute. Suspended particulate matter (SPM) was measured by filtering 3 liters of seawater onto pre-weighed Nucleopore filters (0.45  $\mu\text{m}$  pore size) that were subsequently oven-dried and weighed. Whatman GF/F filters (0.45  $\mu\text{m}$  nominal pore size), previously treated in a muffle furnace (450  $^{\circ}\text{C}$ , 24h), were used for particulate organic matter. Duplicate 2-liter seawater samples were filtered on 0.45  $\mu\text{m}$  pore size GF/F filters and kept frozen pending chlorophyll-*a* determination.

Suspended particulate matter was assessed by a gravimetric method using a Mettler M3 balance (accuracy  $\pm 1 \mu\text{g}$ ) after desiccation (70 $^{\circ}\text{C}$ , 24h). After removal of carbonates by HCl vapor in a desiccator (Parsons et al. 1984), particulate organic matter was analyzed by combustion using an Elemental Analyzer. Chlorophyll *a* was extracted by soaking filters in 90% acetone overnight in the dark at 4 $^{\circ}\text{C}$  and measured with a Shimadzu 1201 spectrofluorometer according to Parsons et al. (1984).

## Data analysis

To compare the degree of relatedness of study sites, I performed cluster analysis on the site averages for physical-chemical variables and ecological variables measured in each study site, using Ward's method (JMP software, Sall and Lehman 1996). Physical-chemical variables included nutrient concentrations, SPM, POM, chlorophyll *a*, temperature, and current speed. Ecological variables included substrate cover data (live and dead coral, sponges, algae turfs, coralline algae and macrophytes) and sea-urchin and herbivorous fish biomass. The Ward method estimates the contribution of the variables to each cluster by computing the squared distance between each cluster's (class) center of gravity and the overall center of gravity (here the origin)(Sall and Lehman 1996).

I used a mixed model two-way nested analysis of variance (ANOVA) to test for differences in inorganic nutrient concentrations, chlorophyll *a*, SPM, POM, temperature and water flow among reefs, and seasons (northeast monsoon and southeast monsoon) (Mixed procedure, SAS Institute, 2004). Reefs and seasons were fixed effects whereas both sites within reefs were random effects. I considered the northeast monsoon as the months between October and February and the southeast monsoon as the months between March and September. The analysis on phosphate, chlorophyll *a*, POM concentrations, and water flow was performed on the log-transformed data to correct for lack of homogeneity of variance.

I used a mixed model three-way nested analysis of variance (ANOVA) to test for differences in bioerosion rates among reefs, grazing levels (inside/outside cages) and time (3 and 6 months exposure), and to examine the variation in bioerosion rates among sites within a reef (Mixed procedure, SAS Institute, 2004). I treated reefs, grazing levels, and



time as fixed effects, and sites within reefs, variations in grazing levels, and time of exposure within sites and reefs as random effects. Fixed effects in the model were tested using the approximate F-tests of this procedure, and the random effect was tested using the variance component approach (Littell et al. 2006). The analysis was performed on the log-transformed data to correct for lack of homogeneity of variance.

Predicted values of percent substrate cover by different microboring taxa (green algae, cyanobacteria and heterotrophs) were logit-transformed to linearize data and were analyzed using the Proc GLIMMIX in SAS, which fits a generalized linear mixed model to the data (SAS Inst. 2004; Littell et al. 2006). Models were fit to the data using residual pseudo-likelihood, which fits mixed linear models using residual maximum likelihood. This generalized linear mixed model procedure assumed a pseudo-binomial error distribution because the data were recorded on a scale from 0 to 1, and a logit-link function (SAS Inst. 2004). Fixed effects in the model were reefs, grazing levels, and time, whereas random factors were sites within reefs, and variations in grazing levels and time of exposure within sites and reefs.

Because there were no data for the first 3 months exposure in Diani, running the full model with reef×time interactions (reef×time and reef×grazing×time) made main effects sources of variation invalid. Therefore, the analysis was run using the full model (including all interactions) but without Diani reef, to determine if reef×time interactions were non-significant. After verifying that time interactions (reef×time and reef×grazing×time) were non-significant, the analysis excluding these time interactions but including Diani reef was rerun. The model used for all analyses was: response = reef grazing reef×grazing time grazing×time.

The percent variation explained by the nested factor relative to the total variation was estimated by dividing the variance component of the nested factor by the total variance (sites within reefs variance + residual variance). Nested factors with percent variation less than 4% were removed from the random statement to increase test power for fixed effects. I used Fisher's Least Significant Difference Test (LSD, Sokal and Rohlf 1995) to perform post-hoc means comparisons for significant effects.

Pearson correlation coefficients were used to determine the relationship between the physical-chemical variables (Sokal and Rohlf 1995), densities of herbivorous fishes and sea-urchins and microborers' abundance and bioerosion rates. Microborer percent cover and bioerosion rates measurements after 3 months exposure were correlated with physical-chemical variables during the southeast monsoon, whereas measurements made after 6 months exposure were correlated with physical-chemical variables during the northeast monsoon.

## **Results**

### Characterization of study sites

#### *Physical-chemical variables*

Mean nitrate + nitrite concentrations varied between 0.21 and 0.42  $\mu\text{M}$ , with lowest concentrations recorded in Diani during the northeast monsoon and highest concentrations recorded in Kisite MNP during the southeast monsoon. Concentrations were significantly greater in Kisite MNP than in Diani, Malindi Mpunguti and Ras

Iwatine during the southeast monsoon (Appendix 1, Table 5.1, Fig. 5.2a, LSD  $p < 0.05$ ). Concentrations were also greater in Mombasa MNP than in Malindi MNP and Mpunguti reserve during the same season (LSD  $p < 0.05$ ). No significant differences in nitrate+nitrite concentrations were recorded between seasons except for Kisite and Mombasa (Table 5.1, LSD  $p < 0.01$ ).

Mean ammonia concentrations were lowest at Watamu MNP ( $1.75 \mu\text{M}$ ) during the southeast monsoon and highest at Ras Iwatine ( $3.79 \mu\text{M}$ ) during the northeast monsoon, and were significantly higher in Malindi ( $3.59 \mu\text{M}$ ) during the southeast monsoon than in all other reefs (Fig. 5.2b, Table 5.1, LSD,  $p < 0.01$ ). Concentrations were generally higher during the northeast monsoon, but these differences were only significant for Kanamai, Mombasa and Ras Iwatine reefs (LSD,  $p < 0.001$ ).

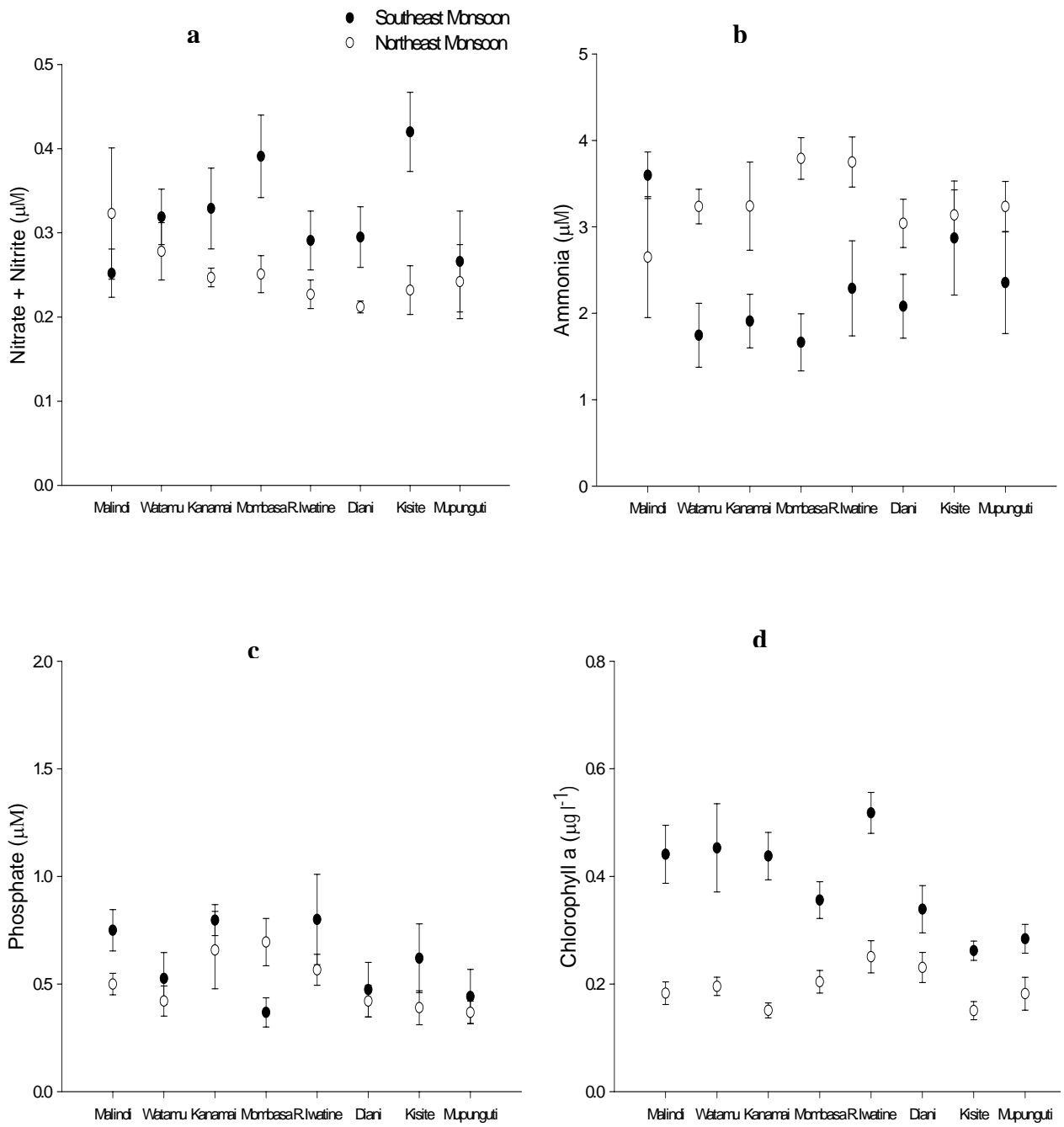
Phosphate concentrations were significantly different among reefs, but not different among seasons (Fig. 5.2c, Table 5.1). Concentrations were significantly higher in Malindi, Ras Iwatine, and Kanamai ( $0.5\text{-}0.8 \mu\text{M}$ ) than in Watamu, Diani, Mpunguti and Kisite ( $0.4\text{-}0.5 \mu\text{M}$ ) for both seasons (LSD,  $p < 0.05$ ). No significant differences among any other reefs were found.

Chlorophyll *a* showed a strong seasonal variability for all reefs studied, with minimum values during the northeast monsoon and maximum values during the southeast monsoon (Fig. 5.2d). Significantly higher concentrations were recorded during the southeast monsoon in Ras Iwatine reserve ( $0.5 \mu\text{g l}^{-1}$ ) when compared with Diani, Kanamai, Mpunguti and Kisite (LSD,  $p < 0.05$ ). Lowest chlorophyll *a* concentrations were recorded in Kisite ( $0.2\text{-}0.3 \mu\text{g l}^{-1}$ , LSD,  $p < 0.05$ ). There were no differences in chlorophyll *a* concentrations among reefs during the northeast monsoon.

**Table 5.1** Results of 2-way ANOVA on inorganic nutrients, chlorophyll a, total particulate matter and particulate organic matter, among reefs and seasons (southeast monsoon and northeast monsoon).

Variable	DF	Variance component	F-value	P-value
<b>Nitrate+Nitrite</b>				
Reefs	7		1.16	0.3324
Seasons	1		13.22	0.0005
Reefs × Seasons	7		1.97	<b>0.0698</b>
Site (reef)		0.0009		
Residual		0.008		
<b>Ammonia</b>				
Reefs	7		0.75	0.6320
Seasons	1		20.88	<0.0001
Reefs × Seasons	7		3.03	<b>0.0071</b>
<b>Phosphate</b>				
Reefs	7		2.10	<b>0.0534</b>
Seasons	1		1.60	0.2103
Reefs × Seasons	7		1.29	0.2666
<b>Chlorophyll a</b>				
Reefs	7		2.00	<b>0.0542</b>
Seasons	1		121.66	< <b>0.0001</b>
Reefs × Seasons	7		1.48	0.1712
<b>Total Particulate Matter</b>				
Reefs	7		1.20	0.2998
Seasons	1		60.85	< <b>0.0001</b>
Reefs × Seasons	7		1.43	0.1916
<b>Particulate Organic Matter</b>				
Reefs	7		1.87	0.0737
Seasons	1		37.30	<0.0001
Reefs × Seasons	7		4.54	< <b>0.0001</b>
<b>Temperature</b>				
Reefs	7		2.35	<b>0.0239</b>
Seasons	1		2.86	0.0922
Reefs × Seasons	7		0.81	0.5285
<b>Current Speed</b>				
Reefs	7		2.61	0.0149
Seasons	1		0.03	0.8532
Reefs × Seasons	6		2.30	<b>0.0381</b>
Season*site(reef)		0.7340		
Residual		5.4729		

Note: For random effects the variance components are reported while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least squares degrees of freedom. Random effects with covariance ratios less than 4% were removed from the random statement to increase test power for fixed effects. Significant effects are in bold



**Figure 5.2** Inorganic nitrate+nitrite (a), ammonia (b), and phosphate (c) concentrations, chlorophyll *a* (d), particulate organic matter (e), total particulate matter (f), temperature (g) and current speed (h) in different reefs and seasons (southeast monsoon and northeast monsoon).

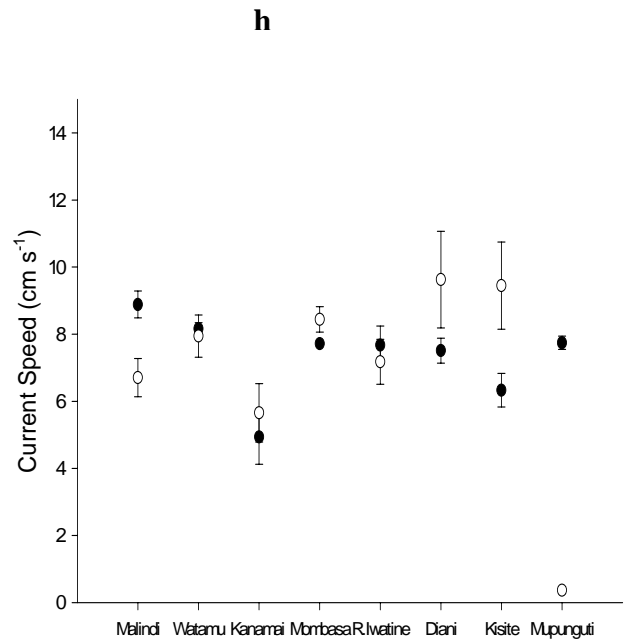
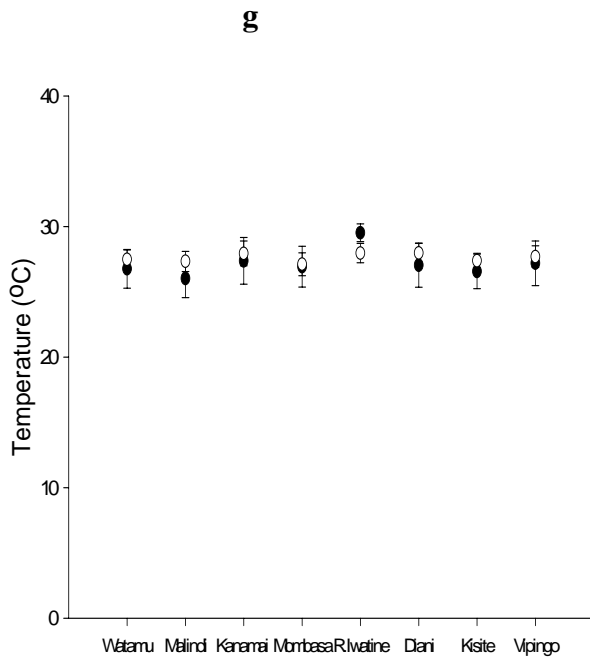
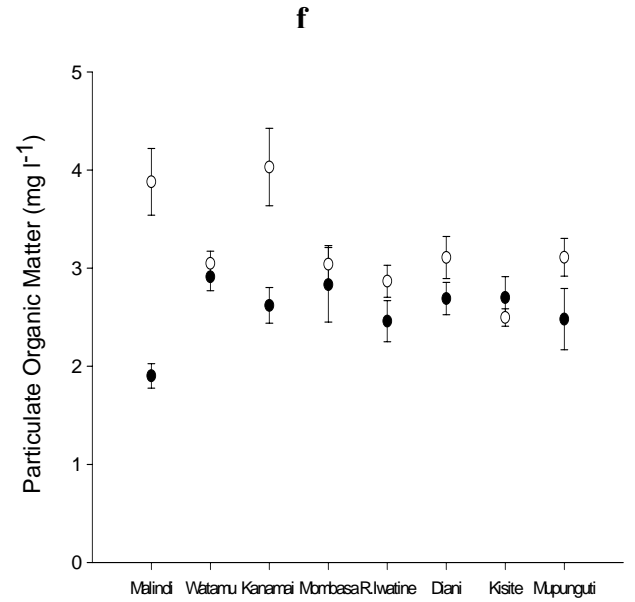
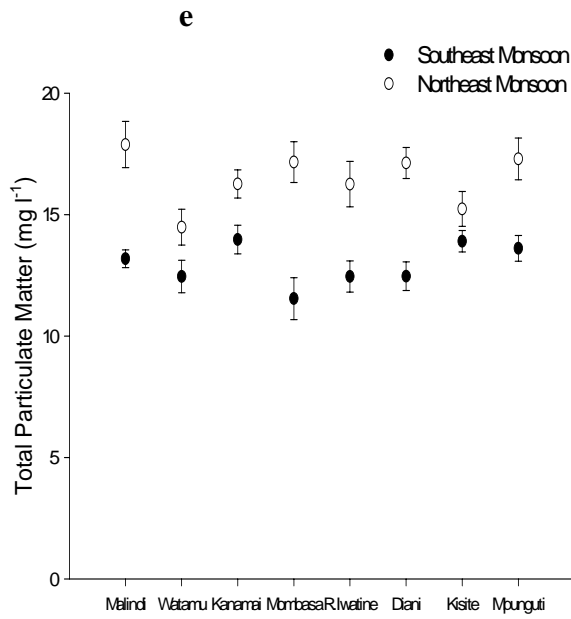


Figure 5.2 Continued

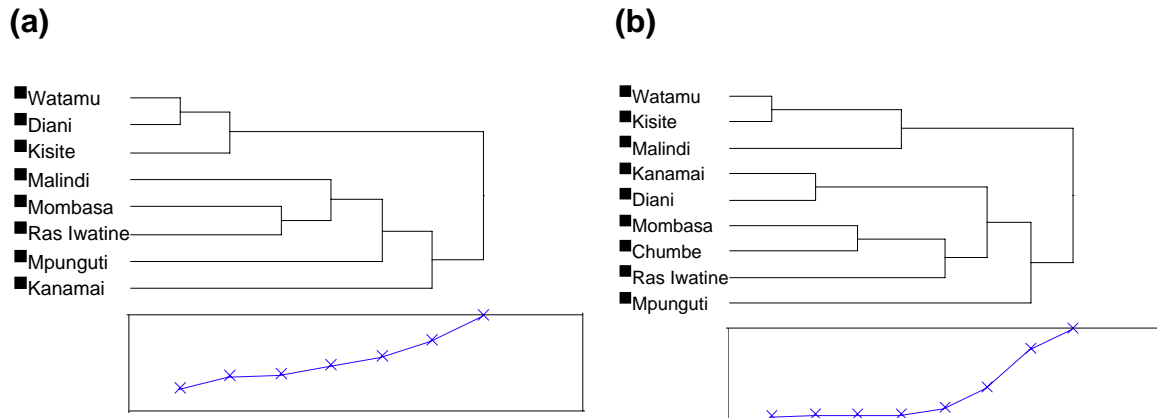
Mean total particulate matter concentrations ranged between 12.5 and 17.3 mg l<sup>-1</sup>, with significantly higher concentrations during the northeast monsoon when compared with the southeast monsoon (Table 5.1, Fig. 5.2e, p<0.001). However, concentrations did not differ among reefs (Table 5.1).

Although mean particulate organic matter concentrations tended to be higher during the northeast monsoon, differences were only significant for Malindi, Kanamai, Mpunguti and Ras Iwatine (Table 5.1, Fig. 5.2f, LSD p<0.01). Highest concentrations were recorded in Malindi and Kanamai during the northeast monsoon (4 mg l<sup>-1</sup>) and in Watamu during the southeast monsoon (3 mg l<sup>-1</sup>, Table 5.1, LSD p<0.05).

Mean seawater temperature was significantly different among reefs, but not different between seasons (Table 5.1, Fig. 5.2g). Highest seawater temperature was recorded in Ras Iwatine (28-30 C°) when compared to all other reefs, except for Kanamai (LSD p<0.05).

There were no significant differences on current speed measured in different reefs with the exception of current speed being significantly lower in Kanamai (5-6 m/s) than other reefs in both seasons (Table 5.1 Fig. 5.2h, LSD, p<0.05).

Cluster analysis of physical-chemical variables revealed two major clusters of study sites (Fig. 5.3a), with several subclusters. Within the first major cluster, Watamu and Diani presented the lowest values of most chemical variables, followed by Kisite, which presented low values of most variables with the exception of high nitrate + nitrite.



**Figure 5.3** Cluster analysis of the study sites for (a) physical-chemical and (b) ecological variables.

The second major cluster was composed of reefs that presented intermediate to high nutrient levels. Within this group Mombasa and Ras Iwatine were most similar, presenting similar values of most variable except for phosphate and chlorophyll *a*. Malindi was distinguished from other reefs by higher levels of ammonia and particulate organic matter, while Mpunguti presented low to intermediate levels of most chemical variables. Kanamai reef was distinguished from other reefs by presenting high values of most chemical variables and highest maximum water temperature and lowest current velocity during the northeast monsoon.

*Ecological variables*

Hard coral cover and algal turf cover were the dominant benthic cover types in all reefs, followed by coralline algae, calcareous algae, fleshy algae, soft coral, and sponge cover (Table 5.2). Cluster analysis of ecological variables revealed two major clusters of study sites (Fig. 5.3b). These clusters were, however, quite different from the clusters



based on physical-chemical variables. The first cluster was composed of three marine national parks (Watamu, Kisite and Malindi) characterized by high number of herbivorous fishes, high substrate complexity (rugosity), and low number of sea-urchins. Within this cluster, Malindi MNP is distinguished from other reefs by presenting higher cover of coralline algae, lower cover of turf algae and lower herbivorous fish biomass. Several small sub-clusters form the second major cluster group. Within this group Kanamai and Diani present the greatest similarities. Both reefs experience heavy fishing pressure and are characterized by low biomass of herbivorous fishes and high biomass of sea-urchins. Mombasa MNP and Chumbe Sanctuary are both protected areas that present high coral cover and high fish biomass, but have intermediate biomass of sea-urchins. Ras Iwatine reserve has lower cover of live coral and lower fish biomass, but has similar benthic cover of other taxa. Mpunguti reserve was distinguished from other reefs by having higher cover of soft coral and higher biomass of sea-urchins than other reefs.

**Table 5.2** Percentage cover (mean  $\pm$  standard deviation) of reef substrata by benthic organisms, and biomass of sea-urchins and herbivorous fishes in studied sites. The proportion of sand was removed from data and only cover on hard substrata (100%) was considered. Reefs studied include four Marine National Parks (Malindi, Watamu, Mombasa, Kisite, and Chumbe Island), two unprotected reefs (Kanamai and Diani) and two marine reserves (Ras Iwatine and Mpunguti). N= 72 per reef for substrate cover and sea-urchin biomass; n= 12 for fish biomass.

Reef	Coverage of reef substrata (%)								Biomass of sea-urchins	Biomass of herbivorous fishes
	Living hard corals	Algal turfs	Calcareous Algae	Fleshy Algae	Coralline Algae	Soft Coral	Sponge	Rugosity		
<b>Malindi</b>	20.5 $\pm$ 3.6	18.0 $\pm$ 8.7	22.3 $\pm$ 7.4	0.7 $\pm$ 0.6	34.2 $\pm$ 5.7	3.9 $\pm$ 2.1	0.5 $\pm$ 0.5	1.3 $\pm$ 0.03	7.7 $\pm$ 9.4	428.2 $\pm$ 121.4
<b>Watamu</b>	12.3 $\pm$ 1.7	36.1 $\pm$ 5.8	22.1 $\pm$ 1.6	5.2 $\pm$ 0.5	24.3 $\pm$ 6.5	0	0.02 $\pm$ 0.02	1.3 $\pm$ 0.01	7.3 $\pm$ 6.4	615.7 $\pm$ 191.8
<b>Kanamai</b>	29.3 $\pm$ 2.9	60.9 $\pm$ 5.0	1.1 $\pm$ 1.3	1.5 $\pm$ 1.4	1.1 $\pm$ 1.0	5.5 $\pm$ 4.0	0.6 $\pm$ 0.4	1.2 $\pm$ 0.05	4002.9 $\pm$ 525.9	5.3 $\pm$ 3.6
<b>Ras Iwatine</b>	7.0 $\pm$ 0.9	50.9 $\pm$ 0.8	1.0 $\pm$ 0.1	25.8 $\pm$ 0.03	10.4 $\pm$ 1.9	2.5 $\pm$ 1.9	2.4 $\pm$ 0.2	1.2 $\pm$ 0.01	2953.0 $\pm$ 514.6	2.4 $\pm$ 1.8
<b>Mombasa</b>	30.9 $\pm$ 9.8	46.4 $\pm$ 9.2	0.6 $\pm$ 0.1	2.9 $\pm$ 1.2	14.4 $\pm$ 5.8	3.7 $\pm$ 2.8	1.2 $\pm$ 0.8	1.3 $\pm$ 0.04	2005.4 $\pm$ 905.9	370.6 $\pm$ 81.9
<b>Diani</b>	31.2 $\pm$ 13.8	47.6 $\pm$ 9.4	1.3 $\pm$ 0.8	10.6 $\pm$ 5.5	6.8 $\pm$ 5.1	2.0 $\pm$ 1.3	0.8 $\pm$ 0.6	1.2 $\pm$ 0.08	4294.2 $\pm$ 2349.9	4.0 $\pm$ 3.5
<b>Mpunguti</b>	20.9 $\pm$ 4.3	49.9 $\pm$ 15.9	0.01 $\pm$ 0.03	0.6 $\pm$ 0.5	10.0 $\pm$ 2.1	16.7 $\pm$ 12.4	1.9 $\pm$ 0.7	n.d.	7780.4 $\pm$ 1163.0	75.1 $\pm$ 13.9
<b>Kisite</b>	43.9 $\pm$ 11.5	37.4 $\pm$ 6.7	0.1 $\pm$ 0.1	3.7 $\pm$ 1.3	11.1 $\pm$ 1.7	3.5 $\pm$ 2.6	0.4 $\pm$ 0.3	1.2 $\pm$ 0.01	9.6 $\pm$ 6.9	599.6 $\pm$ 237.9
<b>Chumbe</b>	65.7 $\pm$ 1.0	26.8 $\pm$ 2.4	0	0.3 $\pm$ 0.4	7.2 $\pm$ 1.1	0.04 $\pm$ 0.06	0	1.2 $\pm$ 0.01	2335.6 $\pm$ 948.2	326.3 $\pm$ 135.9

## Microbial endolithic community composition

Experimental substrates contained 16 different boring traces (Appendix 2). These comprised seven ichnotaxa attributed to the activity of cyanobacteria, four to chlorophytes, four of fungal origin and one bacterium\*. During the first three months of substrate exposure, traces by the green alga *Phaeophila* sp., the cyanobacteria *Masticoleus testarum* and *Plectonema terebrans* were the most abundant traces in ungrazed substrates in all reefs studied. *Plectonema terebrans* was particularly abundant in Kanamai and Malindi (30 and 44% respectively), whereas other reefs were dominated by *Phaeophila* sp. and *Masticoleus testarum* (Appendix 2a, Fig. 5.4a). Boring traces by the fungus *Ostracoblabe implexa* (Fig.5.4b) were recorded in all reefs studied but were abundant only in Watamu, Kanamai, Ras Iwatine and Kisite. Substrate cover by other boring traces was generally less than 5%.

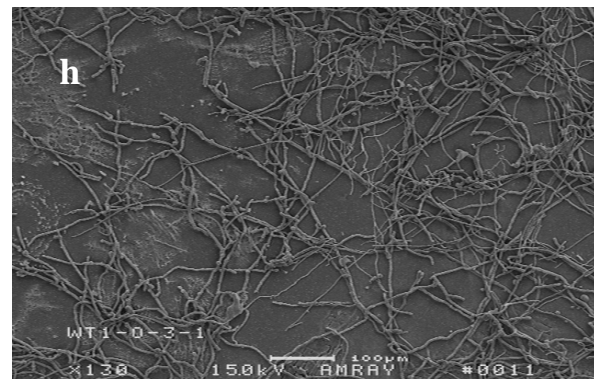
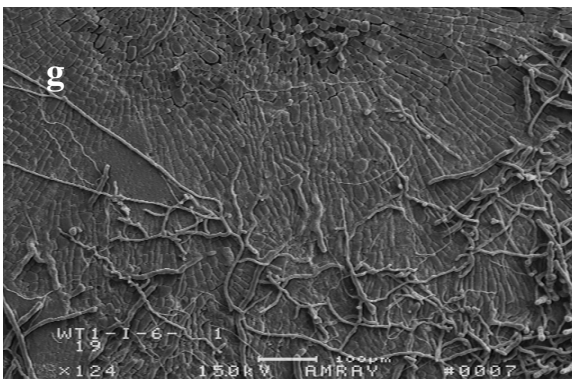
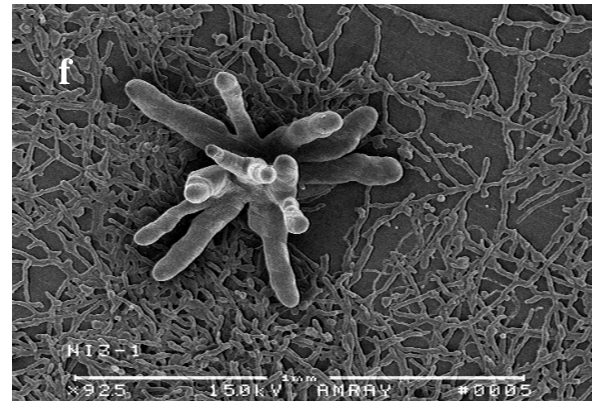
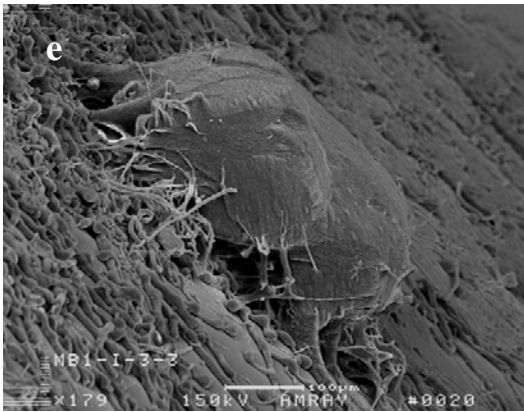
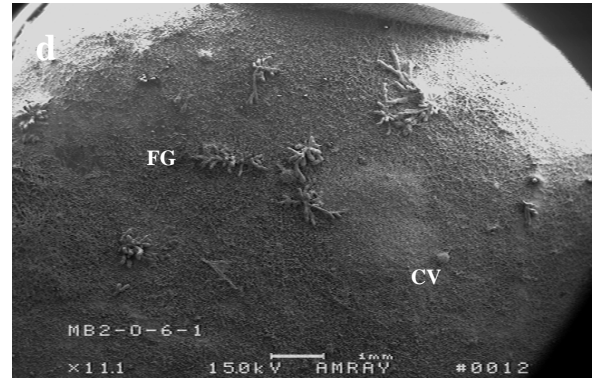
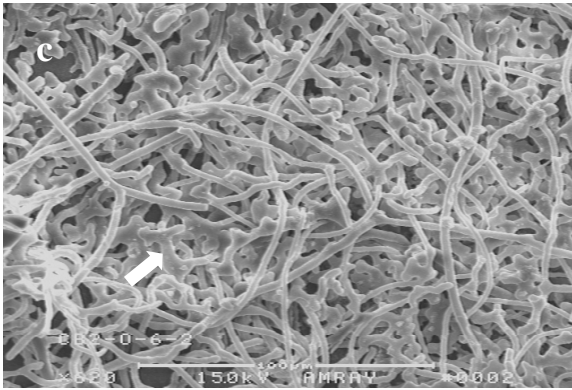
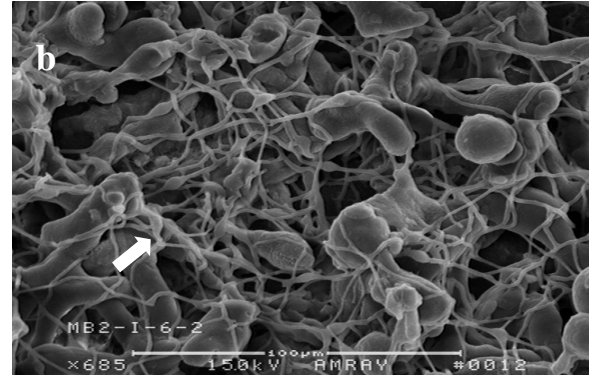
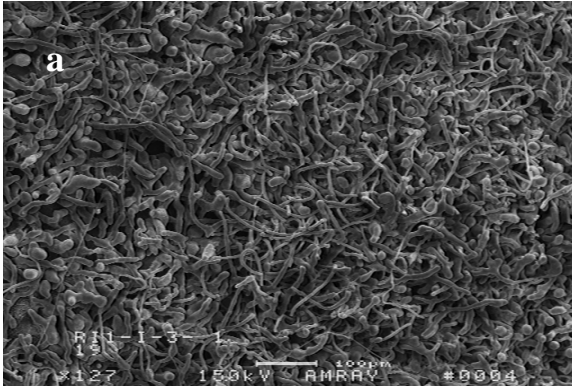
Microborer composition changed with time, but these changes were more noticeable in substrates inside cages than in substrates exposed to grazers. For example, boring traces by *Plectonema terebrans* increased in all reefs after 6 months exposure, with the exception of Mombasa, and became the dominant trace in ungrazed substrates in several reefs (e.g., Diani, Kisite and Chumbe Sanctuary, Fig. 5.4c).

Other changes included increased cover of boring traces by the late colonizer green algae *Ostreobium queketti*, (Fig 5.4c) traces of rhizoids of the green macroalgae *Acetabularia*, and traces of green macroalgae codium-stage (Fig. 5.4 d,e,f). There were, however, no changes in the number of species recorded with time or exposure to grazers.

Analysis of variance of the percent cover by different taxa (cyanobacteria, green algae and heterotrophs) revealed no significant differences among reefs (Table 5.3). This lack of statistical significance was related to the high variability in substrate cover in sites within reefs. An estimation of the variance components for percent cover by different taxa indicated that 15 to 46% of the total variance was due to differences in sites within reefs.

I did however record a significant effect of grazing on cyanobacteria (Table 5.3). This taxon was more abundant in substrates inside cages than substrates exposed to grazers in Malindi and Mpunguti.

**Figure 5.4** Scanning electron micrographs of microboring casts in experimental substrata made from *Strombus gigas* shell exposed on different reefs for a period of 3 and 6 months. (a) Typical assemblage of boring traces after 3 months exposure. Thick borings are *Rhopalia catenata* produced by the green algae *Phaeophila* sp.; thinner borings are *Eurygonum nodosum* produced by the cyanobacterium *Masticoleus testarum*. (b) High density of *Orthogonum fusiferum* produced by the fungus *Ostracoblabe implexa* in Kanamai; (c) Boring trace *Ichnoreticulina elegans* produced by the green algae *Ostreobium quekettii* (arrow), after 6 months exposure. Thinner long filaments are the boring trace *Eurygonum nodosum* produced by the cyanobacterium *Masticoleus testarum*; (d) High density of boring trace *Cavernula pedunculata* (CV) produced by green algae codium-stage and boring traces *Fascichnus grandis* (FG) produced by rhizoids of the green algae *Acetabularia* in Mombasa MNP after 6 months exposure (e) detail of *Cavernula pedunculata* (CV) produced by green algae codium-stage; (f) detail of *Fascichnus grandis* (FG) produced by rhizoids of the green algae *Acetabularia*. Notice the larger dimensions of these boring traces compared with others; (g) Trace of crustose coralline algae skeleton covering the substrate in Watamu MNP. Notice that there is little endolithic algae growing beneath it; (h) Typical assemblage in grazed substrates.



**Table 5.3** Three-way nested ANOVA on microborer's logit transformed mean substrata cover (%) and total bioerosion ( $\text{gCaCO}_3 \text{ m}^{-2}$ ) and bioerosion rates ( $\text{gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) for different reefs, time of exposure (3 months and 6 months) and grazing (exposed or unexposed to grazers).

Variable	Effect	DF	Variance Component	F-value	P-value
<b>Cyanobacteria</b>					
Reef	Fixed	8		1.89	0.1820
Grazing	Fixed	1		18.68	<b>0.0001</b>
Time	Fixed	8		6.53	<b>0.0160</b>
Reef × Grazing	Fixed	1		0.96	0.4856
Grazing × Time	Fixed	1		0.78	0.3843
Site (reef)	Random		0.06653		
Grazing × Time × Site(Reef)	Random		0.1770		
Residual	Random		0.1542		
<b>Green Algae</b>					
Reef	Fixed	8		1.36	0.3511
Grazing	Fixed	1		1.51	0.2562
Time	Fixed	8		6.11	<b>0.0210</b>
Reef × Grazing	Fixed	1		1.26	0.3296
Grazing × Time	Fixed	1		0.12	0.6824
Grazing × Site (Reef)	Random		0.03157		
Grazing × Time × Site (Reef)	Random		0.02108		
Residual	Random		0.08670		
<b>Heterotrophs</b>					
Reef	Fixed	8		2.28	0.1568
Grazing	Fixed	1		0.12	0.6806
Time	Fixed	8		0.39	0.5351
Reefs × Grazing	Fixed	1		1.22	0.3184
Grazing × Time	Fixed	1		0.17	0.6821
Site(Reef)	Random		0.1145		
Grazing × Time × Site(Reef)	Random		0.09148		
Residual	Random		0.04146		
<b>Total Bioerosion rate (<math>\text{gCaCO}_3 \text{ m}^{-2}</math>)</b>					
Reef	Fixed	8		3.90	<b>0.0032</b>
Grazing	Fixed	1		22.02	<b>&lt;0.0001</b>
Time	Fixed	8		92.65	<b>&lt;0.0001</b>
Reef × Grazing	Fixed	1		0.24	0.9807
Grazing × Time	Fixed	1		1.06	0.3098
Grazing × Time × Site(Reef)	Random		249.4		
Residual (Time 3 M)	Random		668.3		

Residual (Time 6 M)	Random	1212.2
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**Table 5.3** Continued

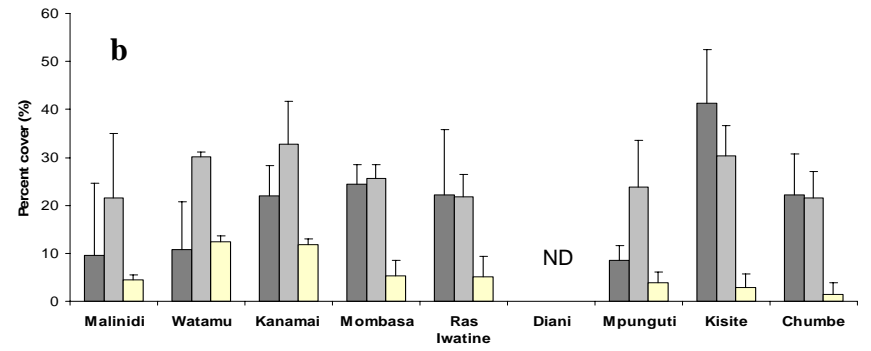
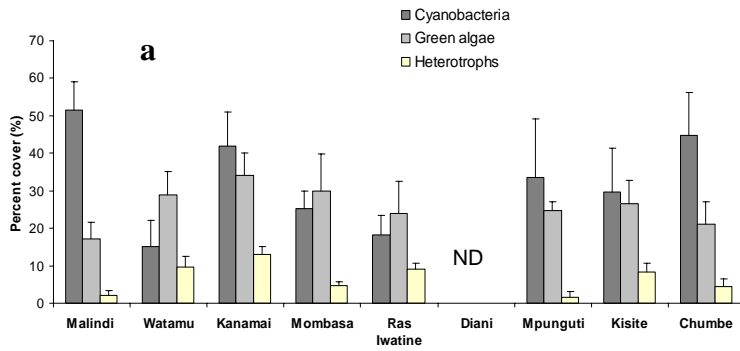
Variable	Effect	DF	Variance Component	F-value	P-value
<b>Yearly Bioerosion rate (gCaCO<sub>3</sub> m<sup>-2</sup> y<sup>-1</sup>)</b>					
Reef	Fixed	8		3.63	<b>0.0041</b>
Grazing	Fixed	1		18.09	<b>&lt;0.0001</b>
Time	Fixed	8		0.59	0.4460
Reef × Grazing	Fixed	1		0.18	0.9938
Grazing × Time	Fixed	1		0.36	0.5543
Grazing × Time × Site(Reef)	Random		2260.5		
Residual (Time 3 M)	Random		11122		
Residual (Time 6 M)	Random		4515.1		

Note: For random effects the variance components are reported, while for fixed effects the F-ratios and their probabilities are reported. DF are ordinary least squares degrees of freedom. Random effects with covariance ratios less than 4% were removed from the random statement to increase test power for fixed effects. Significant effects are in bold.

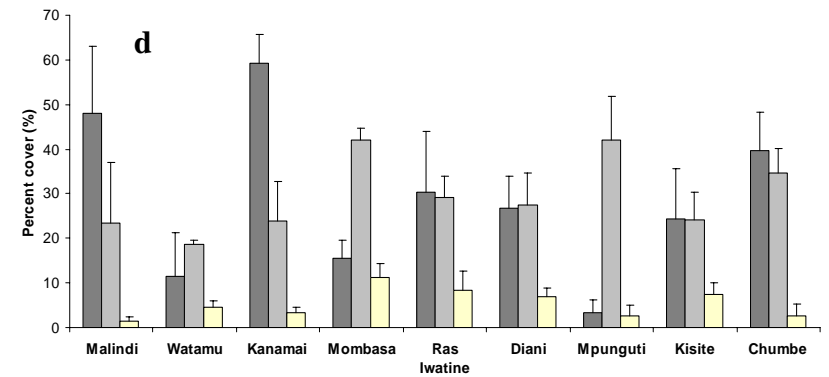
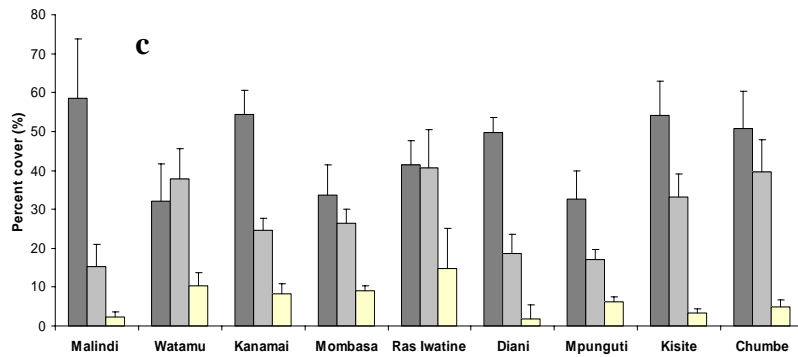
Although green algae cover was not different in grazed and ungrazed substrates (Table 5.3), the reduction in cyanobacteria cover in grazed substrates changed microborer community composition by increasing the relative dominance of green algae in relation to cyanobacteria, particularly during the first three months of exposure (Fig. 5.5).

Differences in substrate cover over time were significant for cyanobacteria and green algae, but not for heterotrophs (Table 5.3). Cyanobacteria increased with time in Watamu, Kanamai, Ras Iwatine and Kisite, mostly due to an increase in abundance of *Plectonema terebrans*, whereas green algae increased with time in Ras Iwatine, Kisite and Chumbe due to an increase in *Ostreobium queketti* boring traces (Appendix 2b, Fig 5.5).

### Three months' exposure



### Six months' exposure



**Figure 5.5** Substrate percent cover (mean  $\pm$  sem) by microborer's taxa (green algae, cyanobacteria, heterotrophs) in experimental substrata made from *Lambis chiragra* shells in different reefs (a) inside cages (ungrazed) and (b) outside cages (grazed) for a period of three months; and (c) inside cages (ungrazed) and (d) outside cages (grazed) for a period of six months. N=6 per reef.

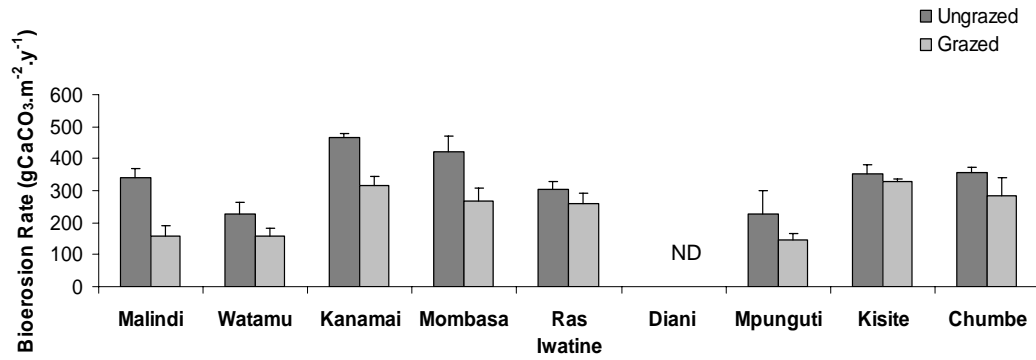


## Bioerosion rates

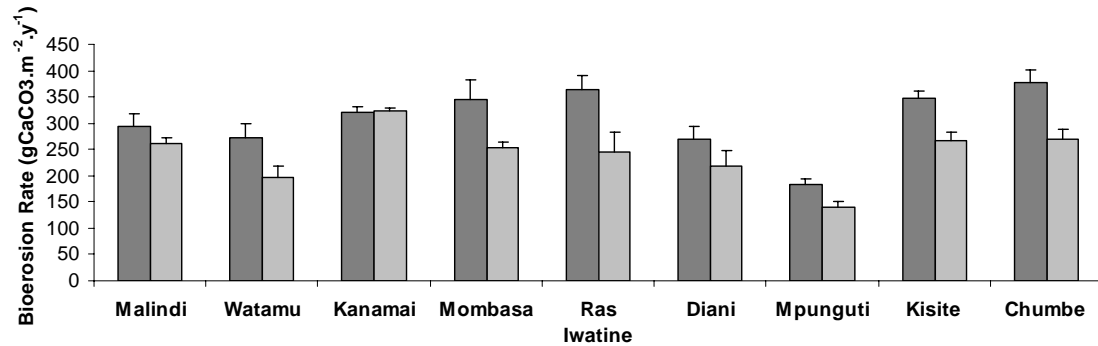
Results from a three-way nested ANOVA revealed that bioerosion rates varied significantly among reefs (Table 5.3), and across time and grazing levels (interaction factors  $p > 0.05$ ). Highest mean rates were observed in Kanamai, Mombasa MNP, Ras Iwatine Reserve, Kisite MNP and Chumbe Sanctuary ( $350\text{-}468 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ , Fig. 5.6). Lowest rates occurred in Mpunguti Reserve ( $139\text{-}226 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) and Watamu MNP ( $228\text{-}272 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ , Fig. 5.6, Table 5.3, LSD  $p < 0.05$ ). Bioerosion rates in Kanamai ( $321\text{-}421 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) were also significantly greater than in Diani ( $211\text{-}269 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) and Malindi MNP ( $156\text{-}342 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ). Although bioerosion rates in shells exposed to grazers were 15% to 54% lower than in grazed substrates for all reefs except Kanamai, grazing significantly decreased bioerosion rates only in Malindi MNP, Mombasa MNP, and Chumbe Sanctuary (Fig. 5.6, Table 5.3, LSD  $p < 0.05$ ).

While total bioerosion (absolute measure after 3 and 6 months:  $\text{gCaCO}_3 \text{ m}^{-2}$ ) significantly increased through time for all reefs except Mpunguti, bioerosion rates (bioerosion per unit time:  $\text{gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) did not (Table 5.3).

**a) Three months 'exposure**



**b. Six months' exposure**



**Figure 5.6** Bioerosion rates (mean ± sem) by all microborers in experimental substrata made from *Lambis chiragra* shells in different reefs exposed to different levels of grazing (ungrazed and grazed) and different times: (a) three months exposure; and (b) six months exposure. N=6 per reef.

Relationship between microborer community composition, microbioerosion rates and environmental and ecological variables

Percent cover by microbial endolithic taxa (green algae, cyanobacteria and heterotrophs) was not significantly correlated with any physical-chemical variables (Appendix 3) or with herbivorous fish and sea-urchin biomass (Appendix 4). Microborer total cover was, however, moderately negatively correlated with current speed after the first 3 months of the study ( $r=-0.77$ ,  $p=0.0418$ ), but not after 6 months (Appendix 3).

Bioerosion rates were also negatively correlated with current speed during the first 3 months of exposure ( $r= -0.77$ ,  $p=0.0449$ ), but not affected by any other physical or chemical variable throughout the rest of the study (Appendix 2). No correlation between herbivorous fish or sea-urchin biomass and bioerosion rates was found (Appendix 4).

## **Discussion**

### Site characteristics

Terrestrial run-off of nutrients and sediments to near-shore coral reef areas in Kenya has been a well recognized problem (McClanahan 2000). However, few chemical measurements are available. For example, nutrient measurements prior to this study were only made by Mwangi et al. (1999) in Mombasa.

Based on chemical variable analysis, we can recognize a gradient of study sites from reefs with low values of most chemical variables to reefs with high values. At one extreme are Kisite MNP, Watamu MNP and Diani with low levels of most of the measured variables. At this end, however, Kisite MNP presented average nitrate+nitrate concentrations above concentrations considered normal for coral reefs (Kleypas et al. 1999). Because this reef is an offshore platform reef far from any significant source of terrestrial run-off and upwelling areas, high nitrate+nitrate concentrations are most likely the result of nitrogen fixation within the lagoon.

Although Watamu MNP occasionally receives terrestrial run-off from a creek located close to the park (McClanahan and Obura 1997; McClanahan et al. 2001), nutrient concentrations in reef waters were within normal levels, probably because of low reef height and high wave and current activity in this reef, which promotes lagoon-ocean water exchange. High particulate organic matter during the southeast monsoon is probably a result of macrophyte vegetation biomass, which is high in this reef (Table 5.1, McClanahan et al. 2001). Nutrient concentration data for Chumbe Sanctuary (Horrill et al. 2000) showed minimum nitrogen and phosphorus concentrations (undetectable concentrations) in reef waters, suggesting that this reef is at the lower end of the gradient of unpolluted to polluted reefs.

Mpunguti and Ras Iwatine Reserves and Mombasa MNP had intermediate values of most variables. Mpunguti Reserve is an offshore platform reef far from any source of land-based pollution. Mombasa MNP is close to a large urban center, Mombasa town, and to numerous beach hotels, but the large tidal amplitude (4 m) and strong tidal currents allow good lagoon-ocean water exchange and the direct supply of low nutrient

oceanic waters that dilutes any land-based pollutants. Nutrient concentrations recorded in Mombasa MNP were similar to values recorded in 1995 by Mwangi et al. (1996), showing no deterioration in water quality during the past 10 years. Ras Iwatine reef lagoon, although located only 500 m from Mombasa MNP, is closer to shore (200 m) than Mombasa MNP (1 km). High concentrations of phosphorus and chlorophyll *a* at this location indicate that this reef may be exposed to terrestrial run-off. Although phosphorus concentrations here were two times above levels considered normal for coral reefs (Kleypas et al. 1999), chlorophyll *a* concentrations were still below the critical eutrophication threshold proposed for coral reefs ( $0.5 \mu\text{g l}^{-1}$ ; Bell 1992).

Malindi MNP and Kanamai consistently presented highest concentrations of most chemical variables. Malindi MNP has been receiving increasing sediment and nutrients from the Sabaki River for the past 50 years, associated with an increase in land uses that promote soil loss (Dunne, 1979; Fleitmann et al. 2007). High concentrations of phosphate and particulate organic matter in this reef during the northeast monsoon indicate the river origin of these nutrients. Short rains during October to December coincide with northeast monsoon winds that entrain inshore currents and river discharge towards coral reefs in Malindi MNP during the northeast season (McClanahan and Obura 1997).

Kanamai's reef lagoon is shallower than other reefs at low tide (0.47 m), has higher reef height above datum, and does not have a tidal channel that connects it to the open sea (McClanahan and Maina 2003). This isolation from the open sea together with its proximity to the shore (200 m from shore) causes retention of nutrients recycled in the lagoon and makes it more susceptible to terrestrial run-off. The high abundance of sea-urchins on this reef may also contribute to the high particulate organic matter recorded.

Sea-urchins typically release 67% to 81% of ingested algal biomass as fecal matter, playing a significant role in the production of particulate organic matter in nearshore benthic ecosystems (Mills et al. 2000; Mamelona and Pelletier 2005). Other distinctive characteristics of this reef are the highest maximum water temperature and lowest water current during the northeast monsoon, which are also related to reef morphology and isolation from the open sea.

Nutrient concentration data for Chumbe Sanctuary (Horrill et al. 2000) show minimum nitrogen and phosphorus concentrations (undetectable concentrations) in reef waters, suggesting that this reef is at the lower end of the gradient of unpolluted to polluted reefs.

Microbial endolithic community composition and bioerosion rates over space and time

Although significant differences in nutrient concentrations in different reefs were detected, there were no significant differences in microborer taxa (green algae, cyanobacteria and heterotrophs) among reefs. This lack of significant differences was probably related to high variability in substrate cover among samples within a site and sites within a reef, in combination with a small sample size (three samples per site, two sites per reef). Several experimental studies of bioerosion have demonstrated spatial and temporal variations in bioerosion rates by macroboring organisms (Hutchings 1986; Hutchings et al. 1992, 2005). Hutchings et al. (1992) suggest this variability may be related with factors operating at a very small scale that influence patterns of recruitment and settlement of macroborers larvae. This may also be the case for microborers' spores.

In addition, there was no significant correlation between nutrient concentrations in different reefs and cover by different boring taxa. These results contrast with results of controlled experiments in Belize (Carreiro-Silva et al. 2005; Chapters 3 and 4) where different microborer taxa were found to be stimulated by different nutrients: green algae were stimulated by high concentrations of nitrogen, cyanobacteria by high phosphorus, and fungi by carbon. For example, mean cyanobacteria cover after 3 months exposure was high in both reefs that experienced high phosphorus concentrations, (Malindi and Kanamai) as well as in “pristine” reefs such as Chumbe Sanctuary. Mean cyanobacteria cover in these reefs was two times greater than cyanobacteria cover measured in the high phosphorus treatment in experiments in Belize. Results after 6 months exposure are more difficult to interpret because the cyanobacterium *Plectonema terebrans* increased with time of exposure in cages.

Green algae cover was much lower than in experiments in Belize (Carreiro-Silva et al. 2005; Chapters 3 and 4) and did not vary greatly among reefs. Heterotrophs were more abundant in reefs with high particulate organic matter, such as Watamu and Kanamai, but they were very variable in other reefs.

Microborer community composition changed with time in most reefs, from a community dominated by the green algae *Phaeophila* sp. and the cyanobacterium *Masticoleus testarum* to a community with greater abundance of the cyanobacterium *Plectonema terebrans* and to a lesser extent the green algae *Ostreobium queketti*. Similar patterns of microbial endolith succession with time have been described by Kiene et al. (1995), Gektidis (1999), and Vogel et al. (2000). Both *Phaeophila* sp. and *Masticoleus testarum* are pioneer species, characteristic of early boring communities (or a juvenile

biocoenosis: Gektidis 1999; Vogel et al. 2000). This community is then slowly replaced after 3 months by low-light specialists such as the cyanobacterium *Plectonema terebrans* and the green algae *Ostreobium queketti*, which are able to grow under reduced light conditions caused by epilithic algal overgrowth of substrata, and in deeper parts of the substratum. *Ostreobium queketti* is generally slower to colonize substrates than *P. terebrans* and becomes abundant only after one year of exposure (Kiene et al. 1995). However, this species was particularly abundant in Malindi MNP, Kisite MNP and Chumbe Sanctuary after only 3 months exposure. The reason for the high abundance of this species in these particular reefs is difficult to interpret.

Changes over time were, however, less noticeable in grazed substrates. Several past studies suggest that grazing fish may influence species composition and succession of the macroborer community (Risk and Sammarco 1982; Sammarco et al. 1987; Kiene and Hutchings 1994; Risk et al. 1995). Grazing and associated removal of the surface substrata prevents the full ecological succession. Consequently, the newly exposed substratum is colonized by early boring colonists but not larger and slower colonizing macroborers. Results of the present study indicate that herbivory is also important in differentiating the microborer community, with ungrazed substrates having greater abundance of late colonizers *Plectonema terebrans* and *Ostreobium quekettii*. Similar results were observed in the Belize study (Carreiro-Silva et al. 2005), where herbivory in the presence of nutrients appeared to reduce *Plectonema terebrans* cover.

Le Bris et al. (1998) observed similar patterns in microborer colonization of experimental substrate exposed for 5 years in French Polynesia. These authors found that the cyanobacterium *Masticoleus testarum* was only present at sites where there was



significant external erosion by sea-urchins. In contrast, blocks in reef sites with little external erosion were dominated by the late colonizer *Ostreobium queketti*. In addition, Chazottes et al. (2002) observed higher colonization of *Masticoleus testarum* in association with high grazing and cover by epilithic algae turfs, while *Plectonema terebrans* and *Ostreobium queketti* were more commonly observed in association with crustose coralline algae and macroalgae in reefs with low grazing.

### Bioerosion rates

Contrary to expectations, there was no significant correlation between nutrient levels in reefs and bioerosion rates. Both nutrified reefs (e.g., Kanamai and Ras Iwatine) and “pristine” reefs (e.g., Kisite and Chumbe MNP) presented high bioerosion rates. These results contrast with results of controlled experiments in Belize, where I found an 8- to 15-fold increase in bioerosion rates in treatments with added inorganic nutrients as compared to control levels (Carreiro-Silva et al. 2005; Chapters 3 and 4). Nutrient concentrations in Kisite MNP and Chumbe Sanctuary were similar to or lower than control conditions in the experiments (except for nitrate+nitrite in Kisite during the southeast monsoon), while nutrient levels in Kanamai and Ras Iwatine were similar to the concentrations used in the fertilized treatments. Nevertheless, rates recorded in all these reefs were close to rates measured in the fertilized treatments in Belize (370-593 gCaCO<sub>3</sub> m<sup>-2</sup> y<sup>-1</sup>).

Conversely, bioerosion rates recorded in reefs presenting the lowest bioerosion rates, such as Mpunguti Reserve and Watamu MNP, were two to five times greater than

rates recorded in the control treatments of the experiments. However, it is possible that the longer time of exposure of the experimental substrates in this study (3 and 6 months) compared with experiments in Belize (49-56 days) may have contributed to the observed differences in bioerosion rates.

High bioerosion rates in “pristine” reef areas have been recorded in the Great Barrier Reef, Australia (Tribollet et al. 2002; Tribollet and Golubic 2005). These authors measured high bioerosion rates in coral blocks exposed for 1 to 3 years in reef sites located on the outer barrier or oceanic reefs in the Great Barrier Reef experiencing very little or no anthropogenic influence ( $1001\text{-}1420\text{ g CaCO}_3\text{ m}^{-2}\text{ y}^{-1}$ ). Contrasting results among these studies suggest that other factors, apart from nutrients, may be important controlling factors of microbioerosion.

In the present study, bioerosion rates recorded during the first 3 months of exposure were negatively correlated with current speed. This negative effect of current speed on microbioerosion rates is likely to be related with higher epilithic coralline algae cover of experimental substrates. The reefs that present highest current speed, such as Watamu, Malindi, and Ras Iwatine, were also the reefs where I observed greatest epilithic cover by crustose coralline algae on experimental shells (Fig. 5.4g). Furthermore, benthic crustose coralline algae cover was negatively correlated with bioerosion rates (Pearson  $r=-0.7427$ ,  $p=0.0348$ ,  $n=8$ ) but positively correlated with current speed on the same reefs (Pearson  $r=0.8006$ ,  $p=0.0306$ ,  $n=8$ ), meaning that bioerosion rates were lowest in reefs that presented highest current speed and benthic coralline algae cover. Hydrodynamic energy is an important control of coralline algae development, increasing their capacity to acquire nutrients (Bosence 1983). In turn, by reducing light, coralline algae may reduce

the colonization and abundance endolithic boring organisms. The effect of current speed is less apparent after 6 months exposure because low-light specialists, such as *Ostreobium queketti* and *Plectonema terebrans*, had time to colonize the substrates. For example, both Watamu and Ras Iwatine present lowest total percent cover after 3 months exposure, but total cover increased by 26 and 37% respectively in the following 6 months with the increase in *Plectonema terebrans* and *Ostreobium queketti* cover (Appendix 2). These patterns are not as obvious in Malindi MNP, where *Plectonema terebrans* cover was high after only 3 months exposure. The reasons for this higher abundance are unclear, as nutrient conditions were very similar in both Malindi and Ras Iwatine.

Bioerosion studies in French Polynesia found highest bioerosion rates after 1 year exposure in substrates covered by coralline algae and in areas with high levels of nitrate and ammonia (Chazottes et al., 2002). Microbial endolithic communities in these substrates were mainly composed of *Ostreobium queketti*, a late borer, and low-light specialist. It is possible therefore that, given enough time for late borers to develop, the effect of nutrients on microbioerosion rates would become more evident.

Differences in current speed, however, do not explain the high bioerosion rates obtained in “pristine” reefs. A potentially important factor influencing microbioerosion rates in tropical reefs may be the pool of nutrients that recycle and concentrate in reef sediments and framework crevices (Kiene 1997). Cryptic habitats and sand make up an important part of the volume of coral reefs and their surfaces provide a large interface for the exchange of nutrients with the water column (Tribble et al. 1988). Studies by Risk and Muller (1983) and Ferrer and Szmant (1988) on the nutrient environments within coral skeletons revealed that nitrogen and phosphorus concentrations were higher in

skeletal and pore waters than in the overlying water column. More recent research support these findings (Rasheed et al. 2002; van Duyl et al. 2006). Rasheed et al. (2002) studied nutrient regeneration in reef framework and coral sand in Gulf of Aqaba (Red Sea) and found that nutrients released from framework cavities were two times higher than those in surrounding waters, while nutrients released from reef sediments exceeded those of the water column by a factor of 15 to 80. In addition, van Duyl et al. (2006) investigated inorganic nutrients dynamics in coral reef cavities in Curacao, Netherlands Antilles, and showed that coral cavities are an important source of  $\text{NO}_x$  (nitrate+nitrite) and  $\text{PO}_4^{-3}$  to the water column.

High concentrations of nutrients in the reef framework and in coral sand may be responsible for the high bioerosion rates recorded in oligotrophic reefs included in our study (Kisite MNP and Chumbe Sanctuary). These reefs' high structural complexity provides numerous cavities and crevices for nutrient regeneration and accumulation, which would increase nutrient concentrations close to the reef bottom. For example, Kisite MNP was greatly affected by coral bleaching in 1998 that killed around 45% of the corals, increasing the substrate available for colonization by benthic cryptofauna and flora. It is possible that the high nitrate + nitrite concentrations recorded in this reef, are a result of remineralization within reef crevices. More research is needed to clarify the potential contribution of these sources of nutrients to microbial endoliths recruitment and growth.

While the total amount of calcium carbonate removed by microborers increased with time (3 to 6 months, Table 5.4), bioerosion rates (per unit time) did not. When a substrate has become densely occupied by endoliths, boring rates slow down because

continued boring is only possible up to the maximum light compensation depth (Golubic et al. 1975; Le Campion-Alsumard 1979). For example, in this study total bioerosion in ungrazed substrates increased the least in Kanamai, where total percent cover and bioerosion rates were highest after 3 months ( $89 \pm 7.4$  % total cover,  $468 \pm 12$  gCaCO<sub>3</sub> m<sup>-2</sup> y<sup>-1</sup>, Table 5.3, Figure 5.4). This means that microbial bioerosion when acting alone is light-limited and thus a self-stabilizing process that becomes progressive only in the presence of grazing (Chazottes et al. 1995; Golubic and Schneider 2003). With continuing grazing, the zone of microbial endoliths and the amount of carbonate they remove moves like a front through the substrate. Likewise, by introducing additional shading and protection from grazing, crustose coralline algae limit the progression of microbial boring and further destruction of the substrate.

Herbivory affected bioerosion rates but only in reefs within marine protected areas (Malindi MNP, Mombasa MNP, and Chumbe MS) where herbivorous fish were the dominant grazers. These results are contrary to my expectations. Sea-urchins are often referred to as more efficient grazers than fish (Bak 1990; Reaka-Kudla et al. 1996) because grazing by sea-urchins is generally more localized and intense than fish grazing. Therefore I expected that the measurable microboring activity (that is, the “residual” microbioerosion) should be lower in reefs where sea-urchins are the main grazers. Microbioerosion rates measured by Chazottes et al. (2002) in areas with high numbers of sea-urchins ( $40-70$  gCaCO<sub>3</sub> m<sup>-2</sup> y<sup>-1</sup>) were lower than rates reported in the present study and on Great Barrier Reef, where fish were the dominant grazers. The lesser influence of sea-urchins on microbioerosion observed in this study could, however, be related to the position of the experimental substrates in cages. Although I tried to position the shells

outside cages at the vertices of the cage so that they could touch the reef floor, it is possible that they were difficult for sea-urchins to access. In contrast, being swimmers, fish could more easily access the experimental substrates. Nevertheless, it is unclear why grazing was more effective in some MNP than in others. For example, both Watamu MNP and Kisite MNP have higher biomass of fish than Malindi MNP and Mombasa MNP, but there was no effect of herbivory on these reefs. There are differences in the types of herbivorous among these reefs and future work will need to investigate their possible influences.

#### Implications for paleoecological reconstructions

Traces of microboring organisms have a long geological history associated with reefs and as a result provide a valuable paleontological tool for reconstructing ancient reef environments (Golubic et al. 1975; Golubic and Schneider 2003). For example, microboring traces of specific species or trace communities (i.e., ichnoconozones) have been used as paleobathymetric indicators (Vogel et al. 1999; Glaub 1999).

In the present study and in a series of previous experiments in Belize, the focus was on the relationship between nutrient conditions in reefs and microborer community composition and their bioerosion rates. These studies were expected to provide indicator species or indicator communities of particular nutrient conditions in a reef that could ultimately be used as paleoecological indicators of nutrient conditions in the fossil record. However, although controlled experiments in Belize showed that different taxa responded differently to varied nutrients (i.e., green algae responded to nitrogen, cyanobacteria to

phosphorus, and fungi to carbon), changing the microborer community composition, these patterns were not detected in the present study. These contrasting results indicate that other physical-chemical and environmental variables in the reef (such as current speed and coralline algae cover in this study) may influence the development and composition of microbial endolithic communities, and therefore individual species or taxa may offer limited use as indicators of nutrient conditions in the fossil record.

Nevertheless, differences observed in microbial endolithic communities in grazed and ungrazed substrates could have some applicability to interpreting in the fossil record. Bioerosion by grazers is difficult to quantify in fossil substrates because the amount of carbonate removed by grazing cannot be measured (Chazottes et al. 1995). In addition to preserved grazing traces, the assessment of “residual” bioerosion by microbial endolithic communities may, therefore, constitute an indirect measure of grazing intensity (Chazottes et al. 1995). Consequently, my results suggest that microborer community composition, with respect to the presence of early bioeroding species such as *Phaeophila* sp and *Masticoleus testarum*, could be used as an indicator of intense grazing. In other words, substrates that had only early bioeroders could represent substrates that were more heavily grazed, while substrates with late colonizers such as *Ostreobium quekettii* could represent substrates that were not as heavily grazed.

## **Conclusions**

Contrary to the original hypotheses and previous experimental manipulations in Belize, significant relationship between nutrient concentrations and microbial endolithic community composition and bioerosion rates among reefs were observed. Instead,

bioeroders' total percent cover and bioerosion rates were negatively correlated with current speed and benthic crustose coralline algae in reefs. I suggest that crustose coralline algae growing on top of experimental substrates may have been partly responsible for the lack of a relationship between bioerosion rates and nutrient concentrations in reef waters. Coralline algae may have negatively influenced microborer colonization and bioerosion rates by reducing light conditions within substrates, even in reefs that experienced high nutrient concentrations. Simultaneous measurements of epilithic algae cover and endolithic algae within the substrate will help clarify the influence of coralline algae on microbioerosion rates. Another factor that contributed to this lack of relationship was the observation of near-maximum bioerosion rates in reefs considered "pristine". It is possible that high concentrations of nutrients in the reef framework and coral sand may have been responsible for the high rates observed, and this deserves further research. This study suggests that many physical, chemical and ecological processes in reefs interact in determining rates of bioerosion. Clearly more studies are needed to fully understand these interactions.

\* Information from Dr S. Golubic and Dr G. Radtke (personal communication) that I received after the dissertation defense date suggest that what I considered to be coccoid bacteria traces may be early stages of cyanobacteria in the order Pleurocapsales.



## Chapter 6

### **Are bioerosion rates good indicators of coral reef health?**

#### **Abstract**

Coral reefs worldwide are suffering unprecedented degradation. One important ecological consequence of such degradation is an increase in biological erosion, or bioerosion, of the coral framework by boring and grazing organisms. Therefore, it has become essential to understand the factors that control the processes and agents of bioerosion. Nutrient enrichment has often been evoked as a primary control of bioerosion. However, high bioerosion rates have been recorded in both eutrophic and “pristine” reefs. Critical examination of nutrient effects on bioerosion shows that reef macroborers most directly reflect increases in nutrient conditions. While there is experimental evidence that microborers are also influenced by nutrient enrichment, field studies have produced variable results because of interactions with epilithic algae cover and grazing pressure. Sea-urchins appear to be more directly controlled by over-harvesting of their predators. However, they may also be influenced by increased nutrients, particularly in reef areas that have also been affected by bleaching, because of increased algae growth on the newly available substrate as a source of food. High bioerosion rates by herbivorous fish appear to be normal in healthy reefs. Evidence suggests that increased abundance of macroborers and sea-urchins most directly reflect human pressures in coral reefs, such as nutrient enrichment and overfishing. Therefore, in reef monitoring programs, macroborer abundance may be a useful indicator of eutrophication, with sea-urchin numbers a

reliable indicator of over-fishing of their predators. Because high bioerosion rates can also be obtained in “pristine” reefs (mainly due to high abundances of herbivorous fishes), bioerosion rates should be used as indicators of reef health only when used together with rates of carbonate accretion to determine if the reef is accreting or eroding.

**Keywords:** bioerosion; calcium carbonate budget; nutrients; organic matter; overfishing; reef “health”

## **Introduction**

Coral reefs have been suffering unprecedented degradation from increasing fishing pressure, pollution, diseases, and coral bleaching. Recent reports indicate that 20% of the world’s coral reefs have been destroyed and show no immediate prospects of recovery, 24% are under imminent risk of collapse due to anthropogenic stressors, while another 26% are under a longer-term threat of collapse (Wilkinson 2004). For example, coral cover in many Caribbean reefs has declined by up to 80% (Wilkinson 2004).

One important consequence of coral reef degradation is a reduction in net accretion and growth. Reef framework growth is determined by the balance between calcium carbonate production, mainly due to calcification of corals and coralline algae, and calcium carbonate destruction, mainly due to bioerosion (Glynn 1997). The balance between production and destruction determines whether a reef will grow, remain steady, or be eroded (Kleypas et al. 2001). Factors that increase bioerosion rates or decrease calcification rates may rapidly tip the balance in favor of framework destruction.

Nutrient enrichment associated with increased agriculture activity and urbanization near coastal areas has been often evoked as a primary control of this balance (Hallock 1988; Risk et al. 1995; Edinger et al. 2000; Chazottes et al. 2002). Increased nutrients negatively affect coral calcification and recruitment and at the same time promote macroalgal and bioeroder proliferation, and may lead to conditions where reef erosion exceeds calcium carbonate accretion. The effect of nutrients on coral reefs is further exacerbated in reef areas affected by coral mortality that has resulted from bleaching and diseases because of an increase in available substrate for colonization by algae and bioeroders.

The relationship between nutrients and bioerosion has been documented in the fossil record (Hallock and Schlager 1986; Hallock 1988), in museum collections (Highsmith 1980), and in modern coral reefs (Rose and Risk 1985; Sammarco and Risk 1990; Risk et al. 1995; Edinger et al. 2000; Holmes et al. 2000; Ward-Paige et al. 2005; Carreiro-Silva et al. 2005). Hallock and Schlager (1986) and Hallock (1988) suggest that nutrient availability may have increased bioerosion rates in the past and may have been responsible for the demise or the drowning of reefs or carbonate platforms in the geological record. Highsmith (1980) found a strong relationship between the percentage of massive corals infested with boring bivalves and levels of phytoplankton productivity at several geographic locations. Studies on modern reefs have documented increased abundance of macroborers (worms, bivalves, and sponges) in response to enhanced nutrient availability in different geographic locations (e.g. Great Barrier Reef, Australia: Sammarco and Risk 1990; Risk et al. 1995; Tribollet et al. 2002; Tribollet and Golubic 2005; Osnorno et al. 2005; French Polynesia: Pari et al. 2002; Indonesia: Edinger et al.

2000; Holmes et al. 2000). More recently, Carreiro-Silva et al. (2005) showed that erosion by microborers was enhanced 10-fold by fertilizer application.

Based on this evidence, bioerosion rates have been suggested as a useful monitoring tool to determine the degree of nutrient enrichment in reefs (Holmes et al. 2000) and as a measure of coral reef “health” when used together with calcium accretion rates (Edinger et al. 2000). However, other studies have shown that sites regarded as pristine, as well as eutrophic sites, may exhibit high rates of bioerosion (Tribollet et al. 2002; Hutchings et al. 2005). In addition there is often considerable variation in bioerosion between sites within a reef system (Kiene and Hutchings 1994; Tribollet et al. 2002; Hutchings et al. 2004; Tribollet and Golubic 2005), which may make it difficult to distinguish natural fluctuations between rates and agents of bioerosion and fluctuations due to anthropogenic influences (Hutchings et al. 2005).

This chapter aims to (1) critically review and synthesize evidence for the effects of nutrients on bioerosion by different groups of bioeroders and geographic regions; (2) discuss the effect of bioerosion in the inorganic/organic carbon cycle of reefs; (3) identify the main factors controlling bioerosion rates, and (4) discuss the usefulness of bioerosion rates as indicators of nutrient enrichment and coral reef “health”.

This review is not intended to give detail on the bioeroders’ taxa and the mechanisms by which they destroy the substrate. Instead, it focuses on the physical, chemical, and ecological controls of the bioerosion process. For detail on the diversity of bioeroders and their modes of destruction of the substrate, the reader may consult Golubic et al. (1975), Hutchings (1986), and Glynn (1997).

## **Evidence of nutrient effects on bioerosion**

Agents of bioerosion, i.e., bioeroders, are a diverse group, including at least 12 phyla of animals as well as several groups of plants, protozoans, bacteria and fungi (Neumann 1966; Warne 1975, Golubic et al. 1975, Hutchings 1986, Hallock 1988). Bioeroders are generally divided into two groups: external scraping organisms (sea urchins and fishes) and internal bioeroders (Hutchings 1986). The latter group can be subdivided in two subgroups based on size: macroborers (primarily sponges, worms, crustaceans, molluscs, bryozoans, and cirripeds) where boring diameters on calcareous substrata are  $>100 \mu\text{m}$  and microbial endoliths or microborers (mainly bacteria, fungi, and algae) where boring diameters are  $<100 \mu\text{m}$ .

Bioeroders break down calcareous substrata in a variety of ways (Hutchings 1986). Herbivorous grazers scrape and erode substrata while feeding on associated algae. Most internal bioeroders are borers that erode calcareous substrata by digesting the organic matrix, secreting acid that dissolves calcium carbonate, or mechanical abrasion. The great majority of internal bioeroders colonize and erode dead skeletons, although a few species of microalgae, fungi, boring sponges, and bivalves invade live coral (Glynn 1997).

Below I review and synthesize the evidence for nutrient effects on bioerosion by different groups of bioeroders (e.g., microborers, macroborers, and grazing fish and sea-urchins).

### *Microborers*

Microborers are common inhabitants of carbonate substratum of tropical marine environments (Golubic et al. 1975; Perkins and Tsentas 1976; Budd and Perkins 1980; Golubic and Scheneider 2003), colonizing a multitude of carbonate substrates. Such substrates include skeletons of live and dead corals (Le Campion-Alsumard et al. 1995a), coralline algae (Tribollet and Payri 2001), mollusk shells (Radtke 1993; Mao Che et al. 1996; Carreiro-Silva et al. 2005), limestone rocks (Scheneider and Turunski 1983), and loose carbonate sediment grains (Tudhope and Risk 1985). Experimental work in tropical settings has demonstrated that microborers are important but often overlooked agents of bioerosion (e.g., Chazottes et al. 2002; Carreiro-Silva et al. 2005; Tribollet et al. 2002, 2005), representing a significant destructive force in a reef's calcium carbonate budget. In addition to the erosion caused by their boring activity, microbial endoliths reinforce bioerosion of carbonate substrates by facilitating recruitment by macroborers (worms, sponges, and mollusks) and by making the substrate attractive for grazers as a source of food (Chazottes et al. 1995; Pari et al. 1998).

Grazers and microborers effects are synergistic in that microborers provide a renewable food source for grazers and facilitate the process of grazing by weakening the superficial substratum layers (Le Campion-Alsumard 1995; Chazottes et al. 1995, 2002). Conversely, the constant removal of substratum by grazers extends the depth to which algae can bore. Under conditions of intense grazing, however, the penetration of algae

into the substratum is not rapid enough to compensate for the removal of substratum by grazers, resulting in an underestimate of microboring rates (Chazottes et al. 1995, 2002).

**Table 6.1** Bioerosion rates by microborers, length of exposure, substrate exposed, depth of exposure and habitat

Location	Bioerosion rate (g m <sup>-2</sup> y <sup>-1</sup> ) (as reported by authors)	Length of exposure	Substrate exposed	Depth of exposure (m)	Habitat	Reference
<b>Caribbean</b>						
Black Rock	259	383 days	Biomimetic substrates	2	Limestone bank	Hoskin et al. (1986)
Southwest Little Bahama Bank						
Lee Stocking Island, Bahamas	800	3 months	Limestone	2-30	Windward reef	Kiene et al. (1995)
	160		<i>Strombus</i> sp. shell			
	4		Calcite crystals			
	<4		Limestone, <i>Strombus</i> sp. shell, Calcite crystals	100-275	Windward deep slope	
Lee Stocking Island, Bahamas	520	6 months	Micritic Limestone	6	windward reef	Vogel et al. (2000)
	135			30		
	1-2		Micritic Limestone	275	Windward deep slope	
Glovers Atoll, Belize	40 ± 15 - 590 ± 36	49-56 days	<i>Strombus gigas</i> shell	2	Lagoon patch reef	Carreiro-Silva et al. (2005); Chapters 2,4
<b>Western Pacific</b>						
French Polynesia, Moorea Island,	570	2 months	Coral substrates	1.5	Reef flat	Peyrot-Clausade et al. (1995a) and Chazottes et al. (1995)
	200	24 months				
<b>Great Barrier Reef, Australia</b>						
Daves Reef	350	358 days	Sediment particles	5	Lagoon patch reef	Tudhope and Risk (1985)
One Tree Island	20 -30	5 months	<i>Tridacna</i> shell	2	Lagoon patch reef	Kiene (1997)
Northern Great Barrier Reef (cross-shelf transect)	120 ± 60 - 1340 ± 740	1 year	<i>Porites</i> sp blocks	7-10	Lagoon patch reef	Tribollet et al. (2002)
	230 ± 6 – 1420 ± 65	3 years	<i>Porites</i> sp blocks	7-10		Tribollet and Golubic (2005)
<b>Indian Ocean</b>						
Reunion Islands	24± 0.4 – 69 ± 3	24 months	<i>Porites lobata</i> blocks	2	Inner and outer reef flat and back reef	Chazottes et al. (2002)
Kenya and Northern Tanzania	157± 36 -468 ± 12	3 months	<i>Lambis chirarga</i>	1-2	Lagoon patch reef	This dissertation (chapter 5)
	139 ± 10 - 378 ± 26	6 months				



Microbial endolithic organisms colonize substrata more rapidly than any other group of bioeroders, representing the first bioerosion process to occur (within 4 to 9 days) on newly exposed carbonate substrata (Golubic et al. 1975; Perkins and Tsentas 1976; Kobluk and Risk 1977; Tudhope and Risk 1985; Vogel et al. 2000). Because of the rapid colonization of substrata by microborers, the response of these endoliths to elevated nutrients has been suggested as a potentially valuable early indicator of declining water quality in reef environments that results from eutrophication (Kiene et al. 1995; Carreiro-Silva et al. 2005).

The importance of nutrients as a control of microbioerosion has been demonstrated in a series of three experiments in Belize (Carreiro-Silva et al. 2005, Chapters 3 and 4). These studies showed that fertilization with inorganic nitrogen and phosphorus, in the absence of herbivores, enhanced microbial endolithic colonization and bioerosion rates in experimental substrates made of *Strombus gigas* by a factor of 8 to 15 ( $450\text{-}590 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$  in fertilized treatments compared with  $40\text{-}60 \text{ gCaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$  in control treatments, Table 6.1). In addition, Carreiro-Silva (2005) showed that the inclusion of herbivores reduced observed bioerosion rates by half, demonstrating that herbivores can decrease measurable bioerosion rates, potentially masking nutrient effects on microbioerosion.

Results of these experiments contrast with the results of the ENCORE fertilization experiment (Great Barrier Reef, Australia, Kiene 1997; Koop et al. 2001). Kiene (1997) did not record any significant effects of nutrient additions on bioerosion rates by microbial endoliths in their experimental *Tridacna* sp. shells. However, this study lacked

a control for herbivory, which may have contributed to the difficulty in estimating the nutrient effect. High herbivory could dampen the influence and ability to detect nutrient effects and this could explain the reported differences. Another factor potentially contributing to reported differences is that in the Belize experiments the fertilizer was placed under the substrata whereas it was applied periodically to the water column above the experimental substrata in the ENCORE experiment. Slow-release fertilizer placed under the substrata is less likely to be carried away quickly by currents. It is likely that this continuous input produced higher nutrient concentrations around the substrata compared to the ENCORE experimental method. Maximum recorded bioerosion rates in the ENCORE study were 20 to 30 g CaCO<sub>3</sub>m<sup>-2</sup> y<sup>-1</sup> (Table 6.1). These rates are lower than the rates obtained in the control treatments in Belize, which suggests a poor fertilization effect with ENCORE.

Field studies on the relationship between water chemistry conditions and microbioerosion rates have given variable results (Zubia and Peyrot-Clausade 2001; Chazottes et al. 2002; Tribollet et al. 2002; Tribollet and Golubic 2005; Chapter 5 of this dissertation). Zubia and Peyrot-Clausade (2001) and Chazottes et al. (2002) found higher bioerosion rates by microborers in reefs subjected to eutrophication compared to more oligotrophic reefs. Chazottes et al. (2002) recorded high microbioerosion rates in association with low grazing, while Zubia and Peyrot-Clausade (2001) found higher infestation levels at heavily grazed sites. Tribollet et al. (2002) and Tribollet and Golubic (2005) found the lowest microbioerosion rates to occur in inshore waters of the Great Barrier Reef subjected to high terrigenous inputs; highest bioerosion rates were recorded in oligotrophic waters in the outer barrier or oceanic reefs (Table 6.1).

Microbioerosion studies in Kenya and Northern Tanzania (East Africa, Chapter 5) did not find a significant correlation between nutrient levels in reefs and bioerosion rates. High bioerosion rates were recorded on both nitrified reefs and “pristine” reefs. Instead, total percent cover of bioeroders and bioerosion rates were negatively correlated with current speed and abundance of benthic crustose coralline algae. These results, together with observations of higher epilithic coralline algae cover in samples with lower bioerosion rates, suggest that coralline algae may have negatively influenced colonization by microborers by reducing light conditions within substrates, even in reefs that experienced high nutrient concentrations. In contrast, Chazottes et al. (2002) found highest bioerosion rates by microborers in substrates covered by coralline algae and exposed for one year. However, in this case the major bioeroder was *Ostreobium quekettii*, which is a late-colonizer, low-light specialist, and did not have time to become abundant in our shorter study (6 months).

Divergent results of these studies suggest that several physical, chemical, and ecological processes in reefs interact in determining rates of microbioerosion. For example, high sediments in terrestrial run-off may reduce light levels, thus restricting colonization of microborers even in the presence of high nutrients (Tribollet et al. 2002; Tribollet and Golubic 2005). Likewise, epilithic algae cover may influence the development of endolithic microflora by reducing light levels.

### *Macroborers*

The relationship between nutrients and macroborers is arguably the best-documented relationship in the literature (Rose and Risk 1985; Sammarco and Risk 1990; Risk et al. 1995; Holmes 1997; Edinger et al. 2000; Holmes et al. 2000). Several studies

have shown increases in the abundance of filter and detritus feeders, which include macroborers like sponges, sabellid and spionid polychaetes, sipunculans, and bivalves, in organically polluted waters (Risk and McGeaky 1978; Smith et al. 1981; Brock and Smith 1983; Rose and Risk 1985; Risk et al. 1995). Nutrient availability, particularly of utilizable forms of nitrogen and phosphorous, controls phytoplankton abundance. Increased food supply should enhance survival of planktivorous larvae, i.e., many polychaetes, bivalves, echinoids, and fish (Birkeland 1988). Growth rates of planktivorous post-larvae of boring sponges, polychaetes, and bivalves may also be stimulated by abundant food supplies.

Several studies in the Great Barrier Reef show that the rate of internal bioerosion by macroborers is higher in inshore areas with high loads of nutrients and particulate matter than in nutrient-poor clear oceanic waters (Table 6.2; Sammarco and Risk 1990; Risk et al. 1995; Tribollet et al. 2002; Tribollet and Golubic 2005). In French Polynesia, Pari et al. (2002) found higher macrobioerosion rates in high islands subjected to terrestrial run-off as compared to more oligotrophic atoll reefs. Similar studies exist from Indonesia, where coral biodiversity decreases and bioerosion rates increase with increasing water pollution (Edinger et al. 2000; Holmes et al. 2000).

Generally, the macroborer community changes with time, from initial colonization by polychaetes and sipunculans, to greater abundance of bivalves and sponges after more than two years of substrate exposure (Kiene and Hutchings 1994; Hutchings and Peyrot-Clausade 2002; Pari et al. 2002; Tribollet and Golubic 2005). However, macroborer species composition may be altered in areas of high sedimentation. For example, macrobioerosion studies in Discovery Bay, Jamaica, showed that while

**Table 6.2** Bioerosion rates by macroborers, length of exposure, substrate exposed, depth of exposure and habitat

Location	Bioerosion rate (kg m <sup>-2</sup> y <sup>-1</sup> ) (as reported by authors)	Length of exposure	Substrate exposed	Depth of exposure (m)	Habitat	Reference
<b>Caribbean</b>						
Bermuda	23* 7*	?	Limestone blocks	1-3	Lagoon patch reef	Neumann (1966) Rützler (1975) Reaka-Kudla et al. (1996)
<b>Eastern Pacific</b>						
Galapagos Islands, Ecuador (Champion Island)	2.6 0.6	14.8 months	<i>Porites lobata</i> blocks Limestone blocks	5-13	Lagoon and fore reef slope	
Uva Island, Panama	3.7 -8.0	1 year	<i>Pocillopora</i> spp.	5-15	Reef flat, back reef, fore reef and reef base	Eakin (1996)
<b>Western Pacific</b>						
French Polynesia	0.02 ± 0.02 - 0.26 ± 0.51	6 months	<i>Porites lutea</i> blocks	1-2	Lagoon patch reef	Pari et al. (1998)
	0.02 ± 0.02 - 0.14 ± 0.25	1 year				
	0.02 ± 0.02 - 1.04 ± 0.41	5 years				Pari et al. (2002)
<b>Great Barrier Reef, Australia</b>						
Lizard Island, (cross reef transect)	0.06 ± 0.03 - 0.24 ± 0.14	7-9 years	<i>Porites lutea</i> blocks	1-20	Reef flat, lagoon patch reef, lagoon channel, leeward and windward slopes	Kiene and Hutchings (1992)
One Tree Island	0.005 - 0.026	26 months	<i>Porites lutea</i> blocks	2	Lagoon patch reef	Kiene (1997)
Northern Great Barrier Reef (cross shelf transect)	0.01 ± 0.01 - 0.09 ± 0.15	1 year	<i>Porites</i> sp blocks	7-10	Lagoon patch and back reef zone	Tribollet et al. (2002)
	0.03 ± 0.06 - 0.28 ± 0.36	2 years	<i>Porites</i> sp blocks	7-10	Lagoon patch and back reef zone	Osorno et al. (2005)
	0.15 ± 0.08 - 1.08 ± 0.66	4 years	<i>Porites</i> sp blocks	7-10	Lagoon patch and back reef zone	
	0.12 ± 0.29 - 1.16 ± 0.32	3 years	<i>Porites</i> sp blocks	7-10	Lagoon patch and back reef zone	Tribollet and Golubic (2005)
<b>Indian Ocean</b>						
Reunion Island	5.7 ± 1.3 - 47 ± 21	1 year	<i>Porites lobata</i> blocks	2	Reef flat and back reef zone	Chazottes et al. (2002)

\* Bioerosion by sponges only; ? indicates that no information was provided by the author

worms and bivalves were most common in turbid nutrient-rich inshore areas, sponges were more abundant in deeper clearer waters in the fore-reef (McDonald and Perry 2003). A lower abundance of bioeroding sponges in high turbidity and sedimentation areas has been reported for other reefs (Rützler 2002) and may be related to the clogging of internal canals of sponges with sediment particles.

Another important factor influencing species composition and succession of the macroborer community is grazing pressure (Risk and Sammarco 1982; Sammarco et al. 1987; Kiene and Hutchings 1994; Risk et al. 1995). These authors suggest that grazing and associated removal of the surface substrata limits full ecological succession. Consequently, the newly exposed substratum is colonized by early boring colonists (such as polychaetes and bivalves) and not by larger and slower colonizing macroborers (e.g., bivalves and sponges).

Among the different macroborer taxa, clionid sponges are the most important framework bioeroders, particularly in the Caribbean (Neumann 1966; Hein and Risk 1975; Rützler 1975; Stern and Scoffin 1977), with boring sponges accounting for >90% of total borings in most live and dead coral heads (Glynn 1997), and eroding as much as  $23 \text{ kgCaCO}_3\text{m}^{-2} \text{ y}^{-1}$  (Table 6.2). In areas of reduced sedimentation, clionid sponges respond strongly to nutrient and organic matter content of reef waters (Rose and Risk 1985; Holmes 2000; Ward-Paige et al. 2000). Rose and Risk (1985) found that the density of *Cliona delitrix* at Grand Cayman was positively correlated with the number of water-column fecal bacteria. Holmes (2000) found that the abundance of clionid sponges in pieces of coral rubble increased along an eutrophication gradient (inorganic nutrients and organic matter) in Barbados. Likewise, a recent study in the Florida Reef Tract

(Ward-Paige et al. 2005) found the greatest abundance and size of boring clionid sponges to occur at sites with the highest levels of total nitrogen, ammonium, and  $\delta^{15}\text{N}$ , suggesting sewage contamination.

### *Sea-urchins*

Sea-urchins are often the major agent of bioerosion in areas where population densities are high, and their grazing can lead to rapid framework loss (Hutchings 1986; Glynn 1997). Sea-urchins scrape epilithic and endolithic algae with their highly evolved jaw apparatus (Aristotle's lantern) that includes a protrusible masticatory organ consisting of five radially arranged calcified teeth. Sea-urchin spines also assist in bioerosion when they are employed in the enlargement of burrows.

High densities of sea-urchins are often present in areas experiencing overfishing. Studies in the Caribbean (Hay 1984) and along the Kenyan coast (Indian Ocean, McClanahan and Shafir 1990) presented evidence suggesting that sea-urchin abundances are controlled by finfish predators. In Kenya, increased densities of sea-urchins in heavily fished reefs are related to the overfishing of triggerfish, predators of sea-urchins, especially the red-line triggerfish *Balistapus undulatus* (McClanahan and Muthiga 1989; McClanahan and Shafir 1990; McClanahan 1995). A study comparing echinoid bioerosion rates in protected (unfished) reefs with unprotected (overfished) in Kenya, recorded echinoid bioerosion rates 20 times greater in fished versus unfished reefs (Carreiro-Silva and McClanahan 2001, Table 6.3). In fished reefs, sea-urchins eroded 45% of the gross calcium carbonate deposition, as compared with only 1 % in marine parks (Table 6.3). Recent echinoid bioerosion studies in Belize (Brown-Saracino et al.

**Table 6.3** Size of sea-urchins used in bioerosion studies, abundance, rates of bioerosion and bioerosion rates as percentage of calcium carbonate gross production at different locations. Modified from Carreiro-Silva and McClanahan (2001).

Locality	Echinoid species	Size of urchins (mm)	Borer abundance (ind m <sup>-2</sup> )	Bioerosion rate (Kg m <sup>-2</sup> y <sup>-1</sup> ) (as reported by authors)	Bioerosion rate as percentage of CaCO <sub>3</sub> gross production	Habitat	Reference
<b>Caribbean</b>							
St Croix, Virgin Is.	<i>Diadema antillarum</i>	?	9	4.6	?	Patch reef	Odgen (1977)
Barbados		?	12	2.9	96	Fringing reef	Scoffin et al (1980)
Curaçao							Bak et al (1984)
St Croix, Virgin Is.	<i>Echinometra lucunter</i>		100	3.9	?	Algal ridge	Odgen (1977)
Belize	<i>Echinometra viridis</i>	13-34	1-40	0.2-2.6	?	Reef lagoon	Brown-Saracino (2007)
Mesoamerican Barrier Reef							
<b>Eastern Pacific</b>							
Panama							
Gulf of Chiriqui	<i>Diadema mexicanus</i>	1.2-3.0	50-150	3.47-10.4	34.7-100	Seaward slope	Glynn (1988)
Uva Island		?	1.5-48	1-3.65	2-105	Seaward reef	Eakin (1996)
Galapagos Islands	<i>Eucidaris thoursii</i>	42-62	30.8	22.3	200	Reef flat	Glynn (1988)
Floreana Is.							
<b>Western Pacific</b>							
Eniwetak Atoll	<i>Echinometra mathaei</i>	19-22	2-7	0.08-0.33	2-8	Limestone rock	Russo (1980)
	<i>Echinostrephus aciculatus</i>	17-24	1.05				
French Polynesia							
Moorea Island	<i>Echinometra mathaei</i>	0-39	7.8			Reef lagoon	Bak (1990)
	<i>Diadema savignyi</i>	10-69	4.8	4.6	216		
	<i>Echinothrix diadema</i>	55-89	0.6				
French Polynesia			4.8	3.3			Peyrot-Clausade et al. (1995)
Moorea Island	<i>Echinometra mathaei</i>						
	<i>Diadema savignyi</i>		0.7	0.8			
	<i>Echinothrix diadema</i>		7.4	0.4			
	<i>Echinostrephus molare</i>		0.2	0.04			



Table 6.3 continued

Locality	Echinoid species	Size of urchins (mm)	Borer abundance (ind m <sup>-2</sup> )	Bioerosion rate (Kg m <sup>-2</sup> y <sup>-1</sup> ) (as reported by authors)	Bioerosion rate as percentage of CaCO <sub>3</sub> gross production	Habitat	Reference
<b>Indian Ocean</b>							
Reunion Isl.	<i>Echinometra mathaei</i>	12-50	3.8-73.6	0.4 - 8.3	?-67	Back reef to reef flat	Conand et al (1997)
Gulf of Aqaba/Eilat	<i>Echinometra mathaei</i>	18-28	3.7-10.5	0.5 - 0.9	7-22	Reef flat and	Mokady et al (1996)
	<i>Diadema setosum</i>	22-37	0.1-6.4			slope	
Kenya						Reef lagoon	This study
	<i>Echinometra mathaei</i>		0.03-5.6				
	<i>Diadema savignyi</i>		0-0.1	0.05-1.2	1-45		
	<i>Diadema setosum</i>		0.01-0.4				
	<i>Echinothrix diadema</i>		0.02-0.07				
Arabian Gulf	<i>Echinometra mathaei</i>	37	30	9.9 - 15.3	?	Reef flat	Downing & El-Zahr (1987)

2007) have also recorded bioerosion rates 13 times greater in fished reefs as compared with reefs within marine protected areas, emphasizing the vital role marine protected areas play in coral reef conservation.

The role of echinoids in the reef's calcium carbonate budget has been calculated for the Caribbean (Ogden 1977; Scoffin et al. 1980; Bak et al. 1984) and the Indo-Pacific (Glynn 1988; Bak 1990; Peyrot-Clausade et al. 1995; Mokady et al. 1996; Conand et al. 1997). In all cases, sea urchin grazing was an important factor in the carbonate budget, generally eroding more than 20% of the calcium carbonate accreted (Table 6.3). In some cases, however, the balance between coral accretion and erosion was negative, with echinoid erosion greater than coral accretion (Glynn 1988; Bak 1990; Eakin 1996).

Negative calcium carbonate budgets in these reefs were a combination of both high sea-urchin population densities and low coral cover.

The relationship between nutrients and sea-urchin abundance is not as clear, or well documented, as the relationship between sea-urchin abundance and invertivore fishes. In many instances, such as in French Polynesia (Pari et al. 1998), sea-urchin bioerosion is high in areas experiencing both over-fishing and eutrophication. Here, it is likely that both factors have contributed to the high abundance of sea-urchins. Lack of predators may have increased survival of sea-urchins while high nutrients may have promoted the abundance of algae as a food source for sea-urchins. Unfortunately, investigators may not report physical-chemical conditions and the levels of resource exploitation of the reefs under investigation (e.g., Russo 1980; Bak 1990; Mokady et al. 1996), making it difficult to ascertain the causes for high sea-urchin abundance and high bioerosion rates.

Glynn (1988) suggests that the high number of sea-urchins recorded and high bioerosion rates in the Eastern Pacific (Panama, Galapagos, and Costa Rica) may be a result of high coral mortality from the 1982-83 ENSO event and high water productivity as a result of upwelling of nutrient-rich waters in that area. High plankton production could favor echinoid larval development, or high benthic algal production could favor recruitment and survival of young echinoids, or both factors may be in play. In the Galapagos Islands, vacant holes of boring bivalves in corals, where echinoid larvae would settle, could have also contributed to increased echinoid recruitment and survival (Reaka-Kudla et al. 1996).

## *Fishes*

Numerous fish species erode the reef substrata while grazing on epilithic and endolithic algae. Surgeonfishes (Acanthuridae) and parrotfishes (Scaridae) are the principal grazing groups. Erosion and excavation are accomplished through the use of well-developed jaw muscles and tooth armature (Hutchings 1986). Grazing fish can cause significant amounts of bioerosion, particularly in areas where their population densities are high. For example, the parrotfish *Bolbometopon muricatum* is the dominant bioeroder on the outer shelf of the Great Barrier Reef, where their numbers can attain 50 individuals  $\text{ha}^{-1}$  and where they are responsible for bioerosion rates of  $32.3 \text{ kg m}^{-2} \text{ y}^{-1}$  (Bellwood et al. 2003, Table 6.4). Parrotfish *Chlororus* spp. are another major bioeroder on the Great Barrier Reef, but because of their smaller body size (70 cm compared with 120 cm body length for *B. muricatum*); they erode less carbonate ( $9 \text{ kg m}^{-2} \text{ y}^{-1}$ , Table 6.4).

In the Caribbean, where the major eroders are the parrotfish *Scarus ventula* and *Sparisoma viride*, maximum bioerosion rates can attain  $7 \text{ kg m}^{-2} \text{ y}^{-1}$  (Table 6.4). However, scarid bioerosion rates in the Great Barrier Reef are generally higher than in the Caribbean (Bellwood 1995; Bruggemann et al. 1996, Table 6.4). Bruggemann et al. (1996) suggests that this difference may be related to differences in excavating power by individual taxa that may reflect a different evolutionary history between Indo-Pacific and Atlantic coral reefs.

Studies on bioerosion by fish in relation to different levels of eutrophication seem to suggest that such bioerosion may be more important in oligotrophic reef areas. For example, several studies on bioerosion patterns across reefs in the Great Barrier Reef Australia (Kiene 1988; Risk 1990; Kiene and Hutchings 1994; Risk et al. 1995; Tribollet

et al. 2002; Tribollet and Golubic 2005) recorded higher fish bioerosion in offshore, more oligotrophic reef waters than in more nutrient-rich inshore waters. Low rate of grazing inshore as compare to offshore appeared to be related to the low availability of preferred food of grazers (epilithic turf and endolithic microalgae) in inshore sites that were subjected to high sedimentation. In addition, low rates also reflected the smaller average size of inshore fish compared to those offshore (juvenile fish are more common inshore whereas most adults live offshore: Bellwood and Choat 1990). In most instances, bioerosion studies are done in reef areas experiencing both overfishing and eutrophication, which makes it difficult to ascertain the relationship between nutrient enrichment and fish bioerosion.

**Table 6.4** Size of parrotfish, abundance, and bioerosion rates at different locations.

Locality	Fish species	Size of fish (mm)	Fish abundance (ind ha)	Bioerosion rate (Kg m <sup>-2</sup> y <sup>-1</sup> )  (as reported by authors)	Reference
<b>Caribbean</b>					
Bermuda	<i>Sparisoma viride</i>	?	?	0.04	Gygi (1969)
		350	151	0.21	Gygi (1975)
Barbados	<i>Sparisoma viride</i>	?	?	0.07	Stearn and Scoffin (1977)
		?	15-133	0.04-0.17	Frydl and Stearn (1978)
Panama	<i>Scarus inserti</i>	?	?	0.5	Odgen (1977)
St. Croix	Scarids – indirect estimate	?	?	0.02	Hubbard et al. (1990)
Bonaire, Netherland Antilles	<i>Scarus ventula</i>	150-400	549	0.1-2.4	Bruggemann et al. (1996)
	<i>Sparisoma viride</i>	150-400	292	0.6-5.4	
<b>Great Barrier Reef, Australia</b>					
Lizard Island	Scarids – indirect estimate	?	?	0 – 9.1	Kiene and Hotchings (1992)
Lizard Island	<i>Chlororus gibbus</i>	350-400	29	0.4 - 5.5	Bellwood (1995) <sup>a</sup>
	<i>Chlororus sordidus</i>	150-200	127	0.1 – 0.5	
Heron Island	<i>Chlororus gibbus</i>	350-400	21	1.0-3.3	Bellwood (1995) <sup>b</sup>
	<i>Chlororus sordidus</i>	150-200	262	0.3-1.0	
Yonge, Day and Hicks reefs	<i>Chlororus microrhinus</i>	70	?	9	Bellwood et al. (2003)
	<i>Bolbometopon muricatum</i>	120	?	28-32	
Cross-shelf transect	Scarids – indirect estimate	?	?	0.28 -2.8	Tribollet et al. (2002)
		?	?	0.3 – 5.4	Tribollet and Golubic (2005)

\* Indirect estimates indicates that bioerosion rates were estimated from calcium carbonate removed from experimental coral blocks, as opposed to direct estimates made on individual fish species

? indicates that no information was provided by the author

<sup>a</sup> Abundance data from Choat and Bellwood (1985)

<sup>b</sup> Abundance data from Choat and Robertson (1975)

## Geographic patterns of bioerosion

### *Caribbean*

There have been several studies of calcium carbonate budgets in the Caribbean. These studies were undertaken from 1970 to 1980 and used a process-based approach, with quantifications of gains and losses of calcium carbonate within the reef system; different methods were used for the quantification of carbonate accretion and erosion. Although studies were carried out in different parts of the Caribbean, e.g., Discovery Bay, Jamaica (Land 1979), Bellair Reef, Barbados (Stearn and Scoffin 1977; Scoffin et al. 1980), the main agent of erosion at all these locations was the sea-urchin *Diadema antillarum*, which removed as much as 80% of the gross calcium carbonate production (Scoffin et al. 1980).

The high abundance of this sea-urchin was related to the over-fishing of their predators in many areas of the Caribbean (Hay 1984). However, *Diadema antillarum* was almost completely eliminated from the Caribbean in 1983 as a result of a waterborne pathogen (Lessios et al. 1984). In addition, during the 1980s and 1990s the Caribbean suffered from region-wide declines in coral cover caused by a combination of hurricanes, “white-band disease” that that resulted in a near total die-off of corals *Acropora palmata* and *Acropora cervicornis* (Brown 1997), and a bleaching event in 1998. These effects on construction and destruction of reefs have likely changed the processes that contribute to calcium carbonate budgets.

A recent study in Rio Bueno, Jamaica (Mallela and Perry 2007), and its comparison with a previous budget from the same area (Land 1979), illustrates some of the changes in coral carbonate production and erosion. Differences consisted of a

reduction in live coral cover and a change from fast growing branching species to slower growing dome-shaped and platy species. These differences contributed to a reduction in carbonate accretion rates, with a reduction in external bioerosion mainly by *D. antillarum* and an increase in borer abundance and bioerosion rates due to a reduction in predation by grazing organisms. Although carbonate accretion rates in Rio Bueno were less than half rates at Discovery Bay in the 1970s (Land 1979), because bioerosion was also lower, reefs at this location were still able to maintain a positive net calcium carbonate budget.

Although the above example of how a reef was able to maintain a positive calcium carbonate balance in spite of reduced levels of accretion offers some reason for optimism, the reality in other parts of the Caribbean is likely to be less encouraging. *Diadema antillarum* was the major herbivore in Caribbean coral reefs, mainly because of over-fishing of herbivorous fishes, and their death in combination with a reduction in coral cover resulted in phase-shifts from coral to algae-dominated coral reefs in many reefs in the area (Gardner et al. 2003).

Furthermore, there have been recent reports of increased abundance in bioeroding clionid sponges in the Caribbean (Holmes 2000; Ward-Paige et al. 2000; Rützler 2002). These increases in sponge abundance have been associated with increased terrestrial run-off near coastal areas (see section 2.2). Because sponges are one of the major bioeroders of coral framework, their role in the carbonate budget of reefs is likely to be significant (Holmes 2000).

The combination of low coral cover, low coral recruitment, and increases in internal bioerosion by clionid sponges is likely to contribute to negative carbonate

budgets in many parts of the Caribbean. More research is needed to determine the extent of internal bioerosion in the Caribbean and its role in the calcium carbonate budget of reefs.

### *Eastern Pacific*

The Eastern Pacific provides an example of one of the most extreme cases of biological destruction of coral framework caused by population outbreaks of sea-urchins (Glynn 1988; Eakin 1996; Reaka-Kudla et al. 1996). In 1982-1983, during a severe El Nino Southern Oscillation event (ENSO), extensive bleaching resulted in mass mortalities of corals around Costa Rica, Panama, Columbia and Galapagos Islands (Glynn 1984). Coral death increased the availability of inert substrate for colonization of infaunal organisms and grazing surfaces for sea-urchins and fish. Abundance of echinoid species increased, with *Diadema mexicanus* increasing from 3 to 80 individuals  $m^{-2}$  in Panama and *Eucidaris thouarsii* increasing from 5 to 30 individuals  $m^{-2}$  in the Galapagos (Glynn 1988, Glynn 1997). Comparisons between bioerosion rates (10-20  $kg\ m^{-2}\ y^{-1}$  in Panama and 20-45  $kg\ m^{-2}\ y^{-1}$  in Galapagos, Table 6.3), and carbonate production at the same locations ( $< 10\ kg\ m^{-2}\ y^{-1}$ ) revealed that coral reefs experienced net erosion (Glynn 1997). Bioerosion studies by Reaka-Kudla et al. (1996) in other location of the Galapagos Islands found similarly high rates of bioerosion (25.4  $kg\ m^{-2}\ y^{-1}$ , Table 6.3), highlighting the rapid erosion of coral frameworks at this location. A calcium carbonate budget for Uva Island, Panama (Eakin 1996) demonstrated that the reef was eroding at a rate of 4,800  $kg\ y^{-1}$  ( $-0.19\ kg\ m^{-2}\ y^{-1}$ ), whereas the reef showed a net deposition of 8,600  $kg\ y^{-1}$  ( $0.34\ kg\ m^{-2}\ y^{-1}$ )



prior to the 1982-1983 ENSO. A carbonate budget of Uva Island after another ENSO event in 1997-1998 (Eakin 2001) revealed that although coral mortality was not as severe as after the 1992-1983 ENSO event, the reef has remained Erosional, with rates varying from -3,000 to -18,000 kg y<sup>-1</sup> ((-0.12 to -0.71 kg m<sup>-2</sup> y<sup>-1</sup>).

#### *Western Pacific and Southeast Asia*

Long-term bioerosion studies (5 years) undertaken in sites in French Polynesia exposed to varying levels of anthropogenic impact illustrate variations in bioerosion rates within a geographical region and with time (Peyrot-Clausade et al. 1995; Pari et al. 1998, 2002). These authors showed that rates of bioerosion on experimental substrates made of *Porites lutea* increased with time, mainly because of an increase in rates of internal bioerosion, as densities of borers increased and boring sponges began to colonize the experimental substrates. These reports also showed that high bioerosion rates can be obtained in affected and pristine sites, although main agents of bioerosion differ. High rates of bioerosion were obtained at an inshore fringing reef site (Faa) exposed to terrestrial run-off from a nearby river and to heavy fishing. Here, high densities of the sea-urchin *Echinometra matheii* were the major agent of bioerosion. High bioerosion rates were also found at one pristine atoll lagoon (Tikehau), but in this case scarid fish in one site and sponges in another site were the main agents of bioerosion. These studies suggest that anthropogenic effects are only some of the factors controlling bioerosion rates.

Case studies from south Sulawesi and Indonesia illustrate the effects of terrestrial run-off on internal bioerosion by macroborers and their effect on calcium

carbonate budgets (Edinger et al. 2000; Holmes et al. 2000). Holmes et al. (2000) studied polluted and unpolluted reefs and found that bioerosion by macroborers in both live corals and coral rubble was correlated with a gradient in eutrophication. Edinger et al. (2000), working on the same reefs, documented how coral growth (linear extension) rates and net reef accretion (accretion – bioerosion) become decoupled in polluted compared with unpolluted reefs. They found that while growth rates of massive corals on polluted reefs were not different from coral growth in unpolluted reefs, live coral cover was low and bioerosion rates were high, leading to a negative carbonate budget on polluted reefs. Their approach in their study has been criticized (e.g. Tribollet and Golubic 2005) because it does not include estimates of other bioerosion processes in reefs (microbioerosion and grazing) and bioerosion was only measured in live corals. Nevertheless, the study shows how detrimental macrobioerosion can be to reef health of reefs experiencing eutrophication. That is, macrobioerosion can be so intense that it outpaces coral accretion rates.

Coral reefs in Southeast Asia are the most biodiverse reefs in the world, but unfortunately are also some of the most threatened by humans (Roberts et al. 2002). Threats to these reefs include destructive fishing practices and over-fishing, as well as pollution and sediments from dredging, deforestation, and industries. More studies on other reef areas and on other agents of bioerosion are needed to determine how important bioerosion processes are in this region.

### *Great Barrier Reef, Australia*

Numerous studies on the Great Barrier Reef have examined bioerosion levels across shelf transects (Kiene 1988; Sammarco and Risk 1990; Kiene and Hutchings 1994; Risk et al. 1995; Tribollet et al. 2002; Tribollet and Golubic 2005). Sammarco and Risk (1990) and Risk et al. (1995) measured macrobioerosion as the percentage area bored by macroborers in coral branches. All other studies measured bioerosion rates by borers and grazers in experimental coral blocks, but only Tribollet et al. (2002) and Tribollet and Golubic (2005) measured microbioerosion rates.

All of these studies demonstrate higher colonization and bioerosion rates by macroborers in inshore areas characterized by higher levels of terrestrial inputs when compared with more offshore oligotrophic areas. Microbioerosion followed the inverse pattern, with higher microbioerosion rates in offshore areas when compared with inshore areas (Tribollet et al. 2002; Tribollet and Golubic 2005).

Bioerosion by grazers in experimental coral blocks was the major bioerosion process in outer shelf reef areas in all the above studies (Kiene et al. 1988; Kiene and Hutchings 1994; Tribollet et al. 2002; Tribollet and Golubic 2005), with measured bioerosion rates from grazers representing more than 60% of total bioerosion. Direct measurement of bioerosion rates by individual species of parrotfish by Bellwood et al. (1995) and Bellwood et al. (2003) support these findings (see section 2.3).

Bioerosion rates by parrotfish can reach  $32.3 \text{ kg m}^{-2} \text{ y}^{-1}$  in reef crest areas, approaching rates of calcification in these areas ( $35 \text{ kg m}^{-2} \text{ y}^{-1}$ ; Bellwood et al. 2003). Although bioerosion in these reef areas almost totally counterbalances calcium carbonate deposition, Bellwood et al. (2003, 2004) argue that this steady-state reef

accumulation is essential for the functioning of the ecosystem. Bioeroding fishes remove dead corals, exposing the hard reef matrix for settlement of coralline algae and corals. There is evidence that reduced rates of bioerosion by parrotfishes have increased mortality of corals, thus increasing the prevalence of large tracts of dead coral skeletons (Wilkinson 2002). This interaction may in turn affect coral settlement by providing a physically fragile or unstable foundation for settlement by coral larvae, resulting in abbreviated recovery and a shift to opportunistic coral species.

In addition, the loss of parrotfish bioerosion may result in structural instability as erosional activity becomes dominated by either physical processes, with periodic disruption caused by storms, or by invasive erosion by echinoids (Bellwood et al. 2003). Echinoids are more destructive bioeroders than fish. Only a few species of parrotfish erode significant volumes of reef carbonate when feeding, and they feed primarily on dead corals and other protuberances while avoiding flat surfaces. In contrast, echinoids burrow and erode the reef matrix (see section 2.3).

In the Great Barrier Reef, high rates of erosion by grazing scarids are regarded as normal and indicate that fish populations were not depleted by over-fishing. Hence, high bioerosion rates are a poor indicator of coral reef “health” in this region.

### *Indian Ocean and Red Sea*

There is currently limited information on the degree of bioerosion of coral reefs in this region, because such studies have only been undertaken in Kenya, Northern Tanzania (Carreiro-Silva and McClanahan 2001; this dissertation), Reunion Island (Conand et al. 1997; Chazottes et al. 2002), Gulf of Eilat, Red Sea (Mokady et

al. 1996), and Chagos Islands (Sheppard et al. 2002). More information is urgently needed, since Indian Ocean coral reefs were the most severely affected by bleaching and mortality during the 1998 bleaching event, when 40-90% of coral died in most reefs (Goreau et al. 2000). As a result, significant negative effects of bioerosion are expected.

Gulf of Eilat, Red Sea, Kenya, and Reunion studies suggest that echinoids are the major agent of bioerosion. In all these studies high sea-urchin abundances appeared to be more related to fishing pressure than to nutrient levels. These studies demonstrate how detrimental sea-urchins can be to the coral reef framework, eroding up to 67% of the gross calcium carbonate production (Table 6.3). In addition, studies in Kenya show other ecological changes in reefs caused by intense grazing by sea-urchins populations, such as exclusion of herbivorous fishes, reduced benthic cover and diversity, and low coral settlement (McClanahan and Mutere 1994).

Chagos Islands bioerosion studies consisted of a short-term assessment of the degree of internal bioerosion by macroborers of corals three years after a 1998 bleaching event (Sheppard et al. 2002). Coral mortality was > 90% in many reef areas. Although this reef does not experience any terrestrial pollution, internal bioerosion was severe in all sampling sites, resulting in the framework structure collapse in some areas. Recovery around Chagos is expected to be slow because substrate available for coral larvae settlement consists mainly of unstable unconsolidated coral rubble. This study underscores how mass-mortality events like coral bleaching can rapidly tip the balance from reef growth to reef erosion even in areas not affected by pollution.

A recent effort in Eilat, Red Sea (Wielgus et al. 2006) has reported infestations of live coral with boring spionid worms in an area receiving waste water from an aquaculture facility. Spionid polychaetes are common bioeroders, but are found most frequently in dead coral substrates. Wielgus et al. (2006) study demonstrate that coral exposure to nutrient rich waters may cause live corals to become vulnerable to infestation by boring spionid polychaetes, resulting in coral skeleton aberrations and increased susceptibility to storm damage.

### **Bioerosion and the carbon cycle of coral reefs**

Coral reefs play an important role in calcium carbonate cycling through two key biological processes (Sorokin 1995): (1) metabolism of organic carbon (photosynthesis and respiration) characterized by rapid turnover and (2) metabolism of inorganic carbon (calcium carbonate precipitation and dissolution), which in “pristine” coral reefs is characterized by a net accumulation of calcium carbonate (Kinsey 1985; Hubbard et al. 1990). Bioeroder organisms play an important role in the inorganic carbon cycle by converting coral framework into sediments that accumulate in the reef and producing dissolved calcium carbonate that is used again by corals and other calcifying organisms.

These two main biological processes of organic and inorganic carbon represent two pathways of fixed carbon within coral reefs: the bioconstructional and trophic pathways (*sensu* Done et al. 1996). In the bioconstructional pathway, carbon is fixed by corals and coralline algae into the reef framework and part of this calcium

carbonate is converted to sediments by bioeroders and physical disturbance. In the trophic pathway, producer/consumer interactions are linked to decomposer/detrital guilds.

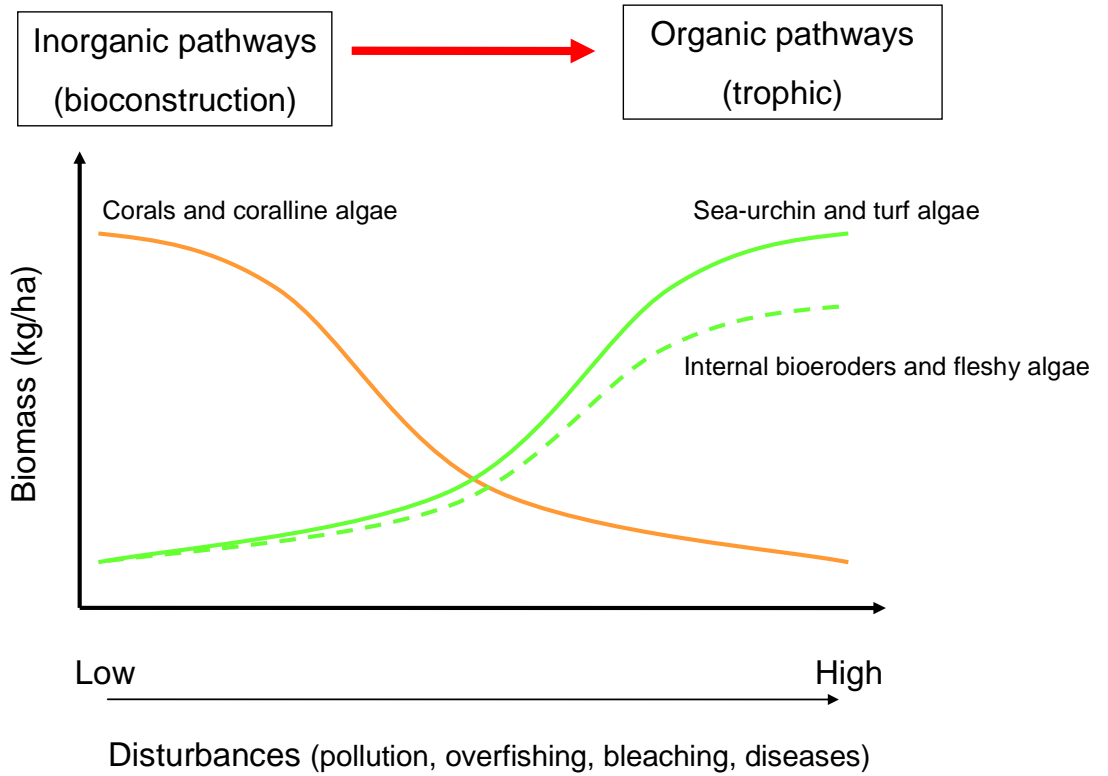
In a *healthy* reef, both carbon pathways are balanced and the reef is able to perform critical ecosystem-level functions of photosynthesis, respiration, and calcification (Done et al. 1996). When a reef is *degraded*, it fails to produce sustained protein yield (fisheries productivity) and structural integrity.

In general, increasing disturbance in reefs (e.g., pollution, overfishing, bleaching, and diseases) will reduce the abundance of live corals and coralline algae (major framework producers) and will increase the abundance of bioeroders and algae, changing the balance between organic and inorganic carbon pathways in favor of a greater production of organic carbon (McClanahan et al. 2002; Fig. 6.1). Within the organic pathway, the lack of herbivorous fish and sea-urchins will produce an accumulation of macroalgae, part of which will be transported away from the reef by currents and wave action and part will be used by the decomposer/detritivore guild. This is true for many Caribbean reefs, which have been affected by overfishing, mass mortality of sea-urchin *Diadema antillarum*, hurricanes, disease, and bleaching events that reduced live coral cover (section 3.1). In many instances, this degradation is further exacerbated by increased availability of nutrients. This increase further promotes the abundance of algae, reduces coral calcification and recruitment, and enhances the abundance of bioeroding species such as worms, sponge and bivalves, all of which seem to be increasingly abundant in inshore areas of the Caribbean (Holmes 2000; Rützler 2002; Ward-Paige 2002; McDonald and Perry 2002). In areas

with high sediments, such as in Indonesia (section 3.3) and in Rio Bueno, Jamaica (section 3.2), macroalgae may not develop although macroborers are abundant.

In overfished coral reefs where sea-urchins have become the major herbivore, such as in Kenya, East Africa (McClanahan and Kurtis 1994, section 3.5), there is no accumulation of macroalgae. However, the high densities of sea-urchins results in significant amounts of bioerosion (Carreiro-Silva et al. 2001). In addition, these reefs have poor coral recruitment because substrate is grazed too frequently for newly settled larvae to survive. Because of the low density and diversity of herbivorous and predator fishes in these reefs, most of the net primary productivity is channeled through sea urchins by grazing and is made available to the reef community mainly thorough their feces (Carreiro-Silva et al. 2001). Relatively low dietary absorption by urchins (25%) results in energy-rich faecal detrital matter (Mamelona and Pelletier 2005), which is used by the decomposer/detritivore guild. Therefore, degraded reefs will experience a decrease in reef framework growth and a shift in a fish-based food-web to detritus-based food web.

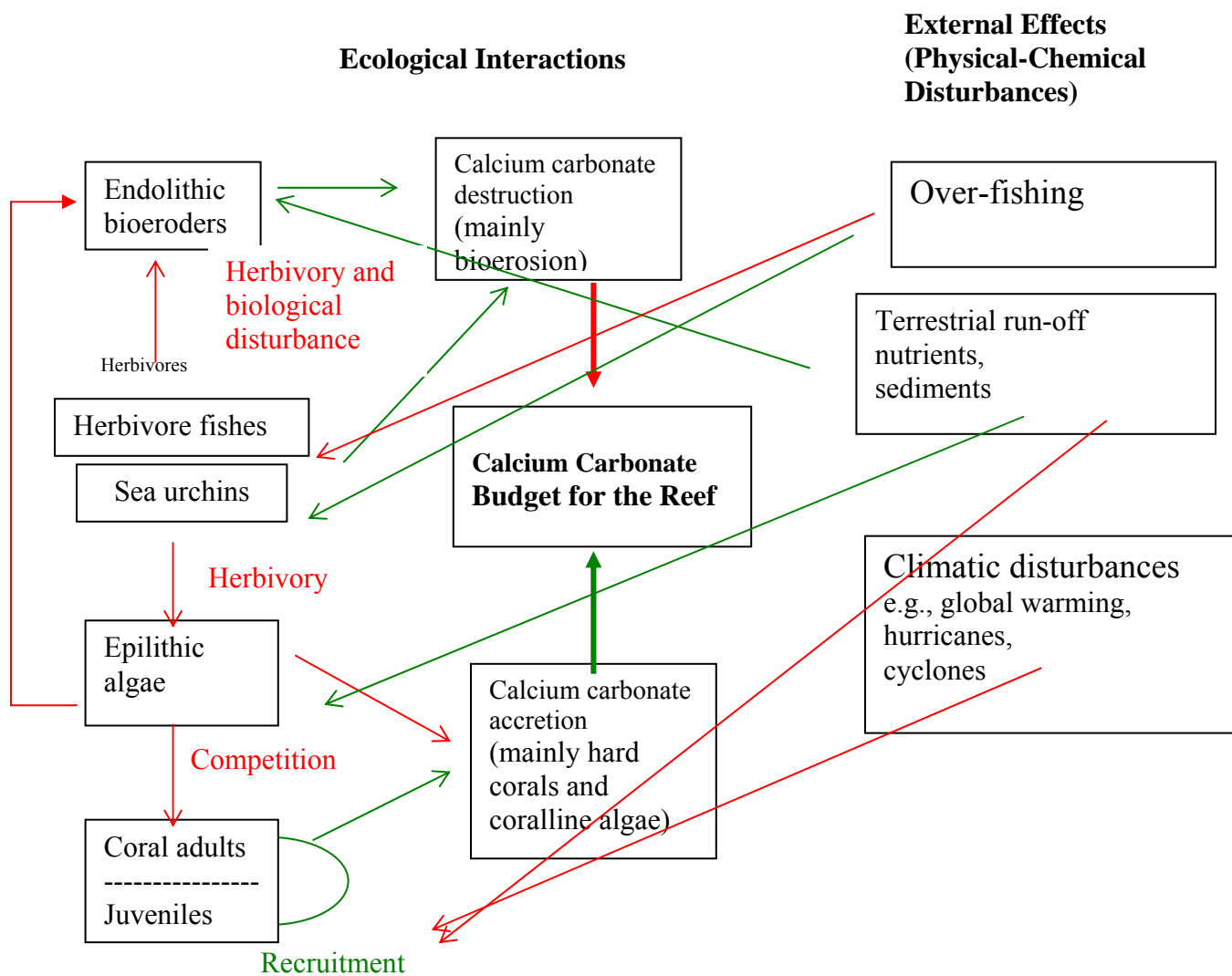




**Figure 6.1** Diagram representing the relationship between disturbances in coral reefs with changes in coral reef community structure and carbon pathways.

## **An integrated conceptual model of the factors controlling bioerosion and the calcium carbonate budget of coral reefs**

The evidence presented above can be used in a conceptual model representing the ecological interactions that influence calcium carbonate budget of reefs and how these are affected by anthropogenic (fishing, pollution) and climatic disturbances (global warming, hurricanes) (Fig. 6.2). Two major anthropogenic disturbances on nearshore coral reefs are over-fishing and terrestrial runoff (Wilkinson 1999; McClanahan 2000). Increased inorganic nutrients and organic matter associated with terrestrial run-off have the potential to increase growth rates of fast-growing algae, microborers (bacteria, algae and fungi), and heterotrophic suspension-feeding organisms, such as many endolithic bioeroders (e.g., mollusks, polychaetes, and sponges) relative to the growth of hard corals (McClanahan 2000). Climatic disturbances to reefs such as storms (cyclones, hurricanes) and increased seawater temperature from global warming may interact with anthropogenic disturbances by killing coral, thereby increasing dead coral substrate that can be rapidly colonized by algae and endolithic bioeroders.



**Figure 6.2** Conceptual model showing the ecological interactions influencing the calcium carbonate budget of coral reefs and how these are affected by external disturbances. Red arrows indicate negative effects (inhibition) and green arrows indicate positive effects (enhancement).

Herbivorous fish and sea urchins have the potential to control the growth and biomass of algae and the development of endolithic bioeroders, and at the same time, the physical abrasion of the substrate during grazing may also increase rates of reef destruction by bioerosion. Over-fishing can reduce the level of herbivorous fishes but it also can, by removing invertivorous fishes, promote sea urchin populations (McClanahan and Shafir 1990).

Among the relationships illustrated in Fig 6.2, there is enough supporting evidence from the literature that macroborers are generally associated with increased terrestrial run-off (section 2.2). However, the composition of the boring community may change with the level of sediments in terrestrial run-off. Worms and bivalves are generally more indicative of turbid waters whereas sponges are more abundant in nutrient-rich but less turbid waters (McDonald and Perry 2002, Ward-Paige et al. 2005). There are a few cases, however, where sponges are abundant in oligotrophic waters (e.g., French Polynesia: Pari et al. 2002; Great Barrier Reef: Osnorno et al. 2005; Tribollet and Golubic 2005). Although the reason for this abundance is unclear, it may be related with grazing pressure within the reef, or with local patterns of recruitment (Hutchings et al. 2005). The interaction among grazers and macroborers also seems to be well documented, with grazers generally arresting macroborers in an early successional stage (Kiene and Hutchings 1994; Tribollet and Golubic 2005).

There is also much evidence that over-harvesting of sea-urchin predators is a major control of sea-urchin abundance and therefore of their bioerosion rates (see section 2.3). However, coral mortality may exacerbate the problem by providing grazing substrate for sea-urchins (e.g., Galapagos Islands, section 3.2). Within the same context,

nutrient enrichment by increasing algae growth can also indirectly promote sea-urchins. The potential effect that nutrient enrichment may have on sea-urchin larvae, as suggested by Glynn (1988), remains speculative but deserves further research.

High numbers of grazing fish, and high bioerosion rates by fish, are generally common in healthy reefs (e.g., Great Barrier Reef, section 3.4). However, it is not clear how herbivorous fish are affected by nutrient enrichment of reef waters because most studies compare pristine reefs (no pollution, no fishing) with degraded reefs (pollution and fishing). Studies in reef areas that experience no fishing but that are exposed to terrestrial run-off (e.g., Malindi and Watamu MNP in Kenya) would help understand the relationship between nutrients and herbivorous fish.

The relationship between nutrients and microborers is well supported by fertilization experiments in Belize (section 2.1). However, field studies have produced variable results, with high bioerosion levels recorded in both eutrophic and pristine reefs. High microbioerosion rates in pristine reefs may be related with the availability of nutrients reef in framework and coral sand as nutrient reservoirs, and this hypothesis deserves further research. In addition, studies in Eastern African reefs suggest that epilithic algae cover, specifically by crustose coralline algae, may affect bioerosion rates. In this case, crustose coralline algae by introducing shading and protection from grazing would limit the progression of microbial boring and further destruction of the substrate. More research is needed to fully understand this interaction between epilithic and endolithic algae.

## **Implications for reef science and management**

Factors contributing to increased bioerosion rates can be divided into two general categories (Glynn 1997): (1) conditions that cause coral death (e.g., increased water temperature, diseases, nutrients, organic matter, sedimentation); (2) conditions that give a growth advantage to bioeroders in relation to calcifying organisms (e.g., nutrients and organic matter, overfishing). Therefore, any management strategies that aim at minimizing the impact of bioerosion on coral reefs should target these two sets of conditions.

Global warming and increased seawater temperature require global level policies, and are unlikely to be reduced in the near future. However, overfishing and terrestrial run-off of nutrients and sediments can be addressed at regional levels. Increased incidence of coral diseases has been linked to anthropogenic impacts (Bruno et al. 2003) such as eutrophication and therefore any policies that reduce terrestrial organic and inorganic run-off will also reduce the incidence of disease.

Establishing marine protected areas is an important tool in preventing population outbreaks of major reef bioeroders such as sea-urchins by protecting their predators from fishing (McClanahan 2000). However, such areas cannot protect reefs from pollution. Therefore, their enactment should be incorporated within an integrated management plans. Managing adjacent coastal areas will control for pollution run-off, drainage of wetlands, and other sources of non-point pollution and land development in adjacent coastal areas that can negatively affect marine protected areas.

Because global warming is not likely to be reduced in the near future, it is expected that coral bleaching and mortality will continue to take place. Since any dead coral

substrate will be invaded by borers and grazers, bioerosion can never be totally prevented. The best we can do is to control for local factors that reduce cover by live coral and increase bioeroders, and promote global policies that enhance the re-colonization of the reefs with new coral larvae, such as international reserve networks.

Based on the evidence presented in previous sections, it appears that changes in the abundance of macroborers and sea-urchins best reflect anthropogenic effects in reefs. Macroborers respond directly to increases in nutrients and organic matter, and may therefore represent an appropriate indicator of eutrophication in reef waters, as suggested by Holmes et al. (2000). High abundance of sea-urchin populations may represent an indicator of reef degradation associated with over-fishing.

Estimates of sea-urchin abundances are already part of monitoring protocols in coral reefs (Hill and Wilkinson 2004). However, the degree of colonization of coral framework by macroborers that has been suggested as a reliable indicator of eutrophication by several researchers (Holmes et al. 2000; Risk et al 2001) is still not part of current monitoring protocols. Given the amount of evidence presented in this review, there should not be any doubt on the value of macrobioerosion as an indicator of changes in reef waters. The “rubble technique” reported by Holmes et al. (2000) is an easy method that can be done with little training. This method is based on measurements of levels of infestation of coral rubble by macroborers, recorded in a scale from 0 to 5.

Edinger et al. (2000) have suggested that levels of erosion, together with estimates of carbonate production, can provide a rapid assessment of the “health” of the reef. Although their method for estimating calcium carbonate budget received some criticism (Tribollet and Golubic 2005, see section 3.3), they (Edinger et al. 2000) demonstrate that

reef health is best considered at the scale of reef growth and carbonate budgets. A reef unable to maintaining a positive carbonate budget will not be able to survive.

The importance of considering bioerosion rates together with carbonate accretion rates is best illustrated with some of the study cases considered above. These studies show that bioerosion rates can be high in both degraded and healthy reefs (e.g., French Polynesia: Pari et al. 2002; Great Barrier Reef: Tribollet and Golubic 2005; discussed in Osorno et al. 2005). For example, high densities of echinoids and high levels of erosion by grazing in some reefs in Kenya and Reunion Island appear to be the result of over-fishing, whereas in French Polynesia the increase in sea-urchin abundance was linked to both over-fishing and high algal coverage caused by increased levels of nutrients. At the Galapagos, a large percentage of corals bleached and died during the 1982-1983 ENSO event and this, together with high water productivity, led to a proliferation of echinoids and algae (Glynn 1988; Reaka-Kudla et al. 1996). In these cases, high bioerosion rates are related to poor reef health. However, high rates of erosion by grazing scarids in the Great Barrier Reef are regarded as normal, indicating that fish populations were not depleted by over-fishing (Osorno et al. 2005). Comparisons of fish bioerosion with reef accretion rates show that, under normal coral cover conditions (no bleaching and mortality), fish bioerosion does not exceed coral accretion rates (Bellwood 2003). Therefore the health of the reef is best described by comparing rates of bioerosion by different groups of organisms only when bioerosion rates with accretion rates within the same reef.

In conclusion, evidence reviewed here supports the contention that nutrient enrichment is a major control of bioerosion, especially of internal borers. The direct



effect of nutrients on microbioerosion is sometimes difficult to determine in field studies, because of interactions with grazing and epilithic algae cover. However, there is enough evidence from controlled experiments to suggest that bioerosion by microborers increased with nutrient enrichment. Although microbioerosion is a self-stabilizing process when acting alone, it can contribute to high levels of bioerosion in reefs that have low coralline algae cover and high grazing. For example, reefs that experience a combination of high nutrient input and high coral mortality from coral bleaching and overfishing can have extremely high bioerosion rates. In this case, high nutrients and available dead coral framework will stimulate microbial endolithic algae, which in turn represent a source of food for sea-urchins.

Macroborers have the most consistent response to increased eutrophication, particularly in areas with low grazing where bivalves and sponges become the dominant eroders. Because of this consistent response, the abundance of macroborers represents a valuable indicator of nutrient change in reef waters that should be used in reef monitoring surveys.

Sea-urchins can rapidly become major eroders in reefs experiencing overfishing, high coral mortality, and eutrophication. High sea-urchin abundance can lead to rapid framework loss, exclusion of herbivorous fish, reduced benthic diversity, and low coral larval settlement (McClanahan and Kurtis 1994), and therefore their abundance can be an important indicator of reef health. High bioerosion rates by grazing fish are considered normal in coral reefs. Not very much is known about the effects of nutrients on bioerosion rates by grazing fish

**Appendix 1.** Data used in the cluster analysis of study sites based on physical-chemical measurements in reef water, during 2002 and 2005. Variables used were mean and maximum nutrient contents, total particulate matter, particulate organic matter, chlorophyll a, temperature, and current speed in different reefs. Data for Chumbe was obtained from Horrill et al. (2000). However, Chumbe was not included in the analysis because data for most variables were not available. Variables are presented for two different seasons: northeast monsoon (NEM) and southeast monsoon (SEM); n= 35 per reef for nutrient concentrations; n= 45 for total particulate matter, particulate organic matter, and chlorophyll *a*; n= 10 for temperature; and n= 20 current speed.

Reef	Nitrate + Nitrite ( $\mu\text{M}$ )			Ammonia ( $\mu\text{M}$ )			Phosphate ( $\mu\text{M}$ )			Temperature ( $^{\circ}\text{C}$ )			Current Speed (m/s)			Total Particulate Matter (mg/l)			Particulate Organic Matter (mg/l)			Chlorophyll <i>a</i> ( $\mu\text{g/l}$ )		
	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max	NEM	SEM	Max
<b>Watamu</b>	0.28	0.32	0.40	3.24	1.75	4.12	0.42	0.53	1.05	27.5	26.8	29.7	7.95	8.16	13.8	15.1	13.3	20.6	3.05	2.91	4.30	0.20	0.45	0.75
<b>Malindi</b>	0.32	0.25	0.54	2.65	3.60	4.71	0.5	0.75	1.26	27.3	26.0	29.7	6.70	8.88	11.7	17.3	13.4	23.4	3.88	1.90	7.30	0.18	0.44	0.80
<b>Kanamai</b>	0.25	0.33	0.54	3.24	1.92	4.12	0.66	0.80	0.95	27.9	27.4	30.3	5.66	4.93	6.50	15.7	12.5	25.1	4.03	2.62	8.50	0.15	0.44	1.14
<b>Mombasa</b>	0.25	0.39	0.52	3.79	1.66	4.71	0.70	0.37	1.16	27.1	26.9	29.8	8.44	7.72	12.1	16.7	13.4	25.9	3.04	2.83	5.30	0.20	0.36	0.77
<b>Ras Iwatine</b>	0.23	0.29	0.40	3.75	2.29	5.29	0.57	0.80	1.47	28.0	29.5	29.9	7.18	7.67	10.7	15.2	12.7	24	2.87	2.46	4.70	0.25	0.52	0.77
<b>Diani</b>	0.21	0.30	0.40	3.04	2.08	4.12	0.42	0.47	0.95	27.9	27.0	29.9	9.63	7.51	15.2	16.1	13.1	21	3.11	2.60	5.0	0.23	0.34	0.97
<b>Kisite</b>	0.23	0.42	0.56	3.13	2.87	4.12	0.40	0.62	0.74	27.4	26.6	29.4	9.45	6.33	12.3	15.3	12.9	19	2.50	2.70	3.60	0.15	0.26	0.43
<b>Mpunguti</b>	0.24	0.27	0.37	3.24	2.35	4.12	0.37	0.44	0.95	ND	ND	ND	7.74	7.74	8.13	17.0	12.5	20.7	3.11	2.48	4.60	0.18	0.28	0.56
<b>Chumbe</b>	UD	UD	UD	UD	UD	UD	UD	UD	UD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: UD= undetectable concentrations  
 ND = no data

**Appendix 2** Percent cover of microboring traces (*ichnotaxa*) and their producers (*bio-species*) in experimental substrata made from *Lambis chiragra* shells in different reefs and exposed to different grazing levels (exposed (G) or not exposed (UG) to grazers) and time periods, (a) 3 months and (b) 6 months exposure.

Values are Mean (Standard deviation).

(a)	Malindi		Watamu		Kanamai		Mombasa		Ras Iwatine		Mpunguti		Kisite		Chumbe	
	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G
<i>Ichnotaxa = Bio-species</i>																
<b>Cyanobacteria</b>																
<i>Scolecia filosa = Plectonema terebrans</i>	44.1 (12.1)	4.3 (5.5)	8.9 (4.7)	6.5 (8.1)	31.4 (21.9)	14.8 (3.5)	15.6 (20.9)	8.2 (12.1)	7.5 (10)	2.0 (1.8)	5.1 (5.7)	–	19 (26.3)	35.8 (15.4)	14.3 (4.3)	15.4 (7.7)
<i>Fascichnus dactylus = Hyella caespitosa</i>	0.7 (1.0)	2.3 (2.4)	1.0 (0.9)	1.2 (0.7)	5.3 (4.8)	2.2 (0.4)	2.0 (0.7)	6.5 (9.2)	–	–	0.8 (1.4)	1.4 (1.3)	1.4 (1.4)	–	0.2 (0.3)	0.8 (1.2)
<i>Fascichnus frutex = Hyella gigas</i>	1.0 (1.4)	–	–	–	–	–	–	0.1 (0.2)	–	0.6 (0.8)	–	–	0.8 (1.2)	–	2.0 (2.8)	0.2 (0.3)
<i>Fascichnus parvus = Hyella pyxis</i>	0.1 (0.2)	–	0.06 (0.08)	0.06 (0.08)	–	0.5 (0.7)	0.6 (0.9)	–	–	0.07 (0.1)	–	–	–	–	0.1 (0.2)	0.07 (0.1)
<i>Fascichnus acinosus = Hyella balani</i>	–	–	–	–	–	–	–	–	0.3 (0.4)	–	–	–	0.7 (1.0)	–	–	0.8 (0.1)
<i>Eurygonum nodosum = Masticoleus testarum</i>	5.5 (1.2)	3.1 (1.2)	5.3 (2.8)	3.0 (0.4)	5.1 (7.3)	0.2 (0.3)	7.1 (10.0)	9.2 (4.2)	16.1 (12.1)	14.9 (21.0)	27.5 (24.9)	7.3 (12.6)	7.6 (9.6)	5.3 (0.2)	28.3 (20.0)	13.4 (2.2)
<i>Planabola isp. = Cyanosaccus piriformis</i>	–	–	0.07 (0.09)	–	–	4.3 (0.6)	0.2 (0.3)	–	–	4.7 (6.7)	–	–	–	0.1 (5.9)	–	0.2 (0.3)
<b>Total cyanobacteria</b>	<b>51.4 (10.3)</b>	<b>9.6 (9.1)</b>	<b>15.1 (3.1)</b>	<b>10.8 (9.3)</b>	<b>41.9 (10)</b>	<b>22 (2.8)</b>	<b>25.2 (10.9)</b>	<b>24.4 (8.5)</b>	<b>18.3 (10.4)</b>	<b>22.2 (13.6)</b>	<b>33.5 (27.1)</b>	<b>8.64 (13.5)</b>	<b>29.6 (14.9)</b>	<b>41.3 (13.2)</b>	<b>44.9 (21.0)</b>	<b>22.2 (5.8)</b>

**Appendix 2 a) Three months exposure (Cont.)**

Ichnotaxa = Bio-species	Malindi		Watamu		Kanamai		Mombasa		Ras Iwatine		Mpunguti		Kisite		Chumbe	
	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G
<b>Green algae</b>																
<i>Fascichnus grandis</i> = <i>Acetabularia</i> rizhoid	0.2 (0.3)	0.1 (0.2)	–	0.4 (0.5)	1.0 (1.0)	0.3 (0.5)	5.4 (6.5)	–	1.81 (2.6)	–	5 (2.5)	0.8 (0.4)	1.25 (1.3)	2.1 (2.9)	0.1 (0.3)	1.4 (0.5)
<i>Cavernula pediculata</i> = <i>Gomontia polyrhiza</i>	0.07 (0.09)	–	–	–	–	0.4 (0.3)	4.0 (1.5)	–	0.1 (0.2)	0.6 (0.8)	0.4 (0.7)	–	–	–	0.8 (0.2)	0.9 (1.3)
<i>Ichnoreticulina elegans</i> = <i>Ostreobium quekettii</i>	7.1 (5.1)	1.7 (0.9)	1.1 (0.1)	0.6 (0.8)	0.8 (1.2)	0.2 (0.3)	–	1.9 (3.4)	–	1.5 (1.5)	–	–	–	11.0 (14.1)	8.8 (1.6)	6.7 (7.7)
<i>Rhopalia</i> <i>catenata</i> = <i>Phaeophila</i> sp.	10 (1.8)	19.2 (6.5)	27.8 (1.2)	29.1 (2.0)	32.6 (5.9)	32 (4.0)	24.6 (16.5)	23.7 (13.9)	22.1 (3.3)	0.3 (0.4)	19.7 (1.6)	22.9 (2.9)	25.1 (1.1)	17.6 (11.3)	11.6 (5.0)	12.6 (9.3)
<b>Total green algae</b>	<b>17.2</b> <b>(3.6)</b>	<b>21</b> <b>(5.78)</b>	<b>29</b> <b>(2.2)</b>	<b>30.1</b> <b>(1.7)</b>	<b>34</b> <b>(7.8)</b>	<b>32.8</b> <b>(3.7)</b>	<b>30</b> <b>(23)</b>	<b>25.6</b> <b>(15.7)</b>	<b>24.0</b> <b>(5.7)</b>	<b>21.7</b> <b>(7.0)</b>	<b>24.7</b> <b>(3.9)</b>	<b>23.8</b> <b>(2.6)</b>	<b>26.5</b> <b>(1.0)</b>	<b>30.4</b> <b>(1.5)</b>	<b>21.1</b> <b>(6.2)</b>	<b>21.6</b> <b>(17.9)</b>
<b>Fungi</b>																
<i>Saccomorpha sphaerula</i> = <i>Lithophyllum gangliiforme</i>	–	–	–	–	–	–	–	0.03 (0.05)	–	–	–	0.06 (0.1)	–	–	–	–
<i>Saccomorpha clava</i> = <i>Dodgella priscus</i>	–	0.07 (0.09)	–	–	–	–	0.3 (0.4)	–	–	–	–	–	–	–	–	0.8 (1.1)
<i>Orthogonum fusiferum</i> = <i>Ostracoblabe implexa</i>	1.6 (1.6)	3.9 (0.3)	8.1 (1.8)	7.7 (7.6)	11.3 (1.7)	10.1 (0.1)	4.1 (3.0)	5.1 (4.0)	7.2 (4.1)	3.0 (2.8)	1.7 (2.4)	3.8 (4.0)	7.7 (2.1)	0.3 (0.5)	3.7 (3.6)	0.4 (0.6)
<i>Orthogonum lineare</i> = Unknown heterotroph	0.5 (0.6)	0.2 (0.3)	1.6 (0.9)	4.7 (6.6)	–	–	0.2 (0.3)	0.06 (0.1)	1.8 (1.7)	1.3 (1.9)	–	0.06 (0.1)	0.6 (0.4)	1.5 (2.6)	0.5 (0.7)	0.2 (0.3)
<b>Bacteria *</b>	–	–	–	–	1.6 (0.8)	1.7 (2.4)	–	–	–	0.7 (0.6)	–	–	–	1.1 (1.9)	0.3 (0.2)	–
<b>Cocoid form = unknown producer</b>																
<b>Total heterotrophs</b>	<b>2.1</b> <b>(2.2)</b>	<b>4.2</b> <b>(0.06)</b>	<b>9.7</b> <b>(2.7)</b>	<b>12.4</b> <b>(1.0)</b>	<b>12.9</b> <b>(2.4)</b>	<b>11.8</b> <b>(2.5)</b>	<b>4.6</b> <b>(2.4)</b>	<b>5.2</b> <b>(3.9)</b>	<b>9.0</b> <b>(2.3)</b>	<b>5.1</b> <b>(1.5)</b>	<b>1.7</b> <b>(2.4)</b>	<b>3.9</b> <b>(3.9)</b>	<b>8.2</b> <b>(1.8)</b>	<b>2.9</b> <b>(2.7)</b>	<b>4.3</b> <b>(4.4)</b>	<b>1.4</b> <b>(1.4)</b>
<b>Total</b>	<b>70.7</b> <b>(11.7)</b>	<b>34.8</b> <b>(3.2)</b>	<b>53.7</b> <b>(5.8)</b>	<b>53.2</b> <b>(6.5)</b>	<b>88.7</b> <b>(2.3)</b>	<b>66.4</b> <b>(3.01)</b>	<b>59.8</b> <b>(36.3)</b>	<b>55.3</b> <b>(24.1)</b>	<b>51.4</b> <b>(2.4)</b>	<b>51.3</b> <b>(4.7)</b>	<b>59.9</b> <b>(31.9)</b>	<b>36.3</b> <b>(19.8)</b>	<b>63.2</b> <b>(15.8)</b>	<b>74.6</b> <b>(10.8)</b>	<b>70.3</b> <b>(10.3)</b>	<b>45.2</b> <b>(22.3)</b>

\* Information from Dr S. Golubic and Dr G. Radtke (personal communication) that I received after the dissertation defense date suggest that what I considered to be coccooid bacteria traces may be early stages of cyanobacteria in the order Pleurocapsales.

**Appendix 2 b) Six months' exposure**

<i>Ichnotaxa = Bio-species</i>	Malindi		Watamu		Kanamai		Mombasa		Ras Iwatine		Diani		Mpunguti		Kisite		Chumbe	
	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G
<b>Cyanobacteria</b>																		
<i>Scolecia filosa = Plectonema terebrans</i>	56.7 (27.6)	41.4 (30.9)	13.1 (14.1)	0.1 (0.2)	41.8 (20.4)	39.9 (26.1)	11.7 (5.5)	3.0 (4.3)	18.5 (14.7)	4.2 (2.9)	38.5 (5.8)	22.1 (9.5)	12.8 (12.5)	–	34.6 (4.5)	14.5 (18.6)	47.1 (29.1)	35.6 (11.5)
<i>Fascichnus daetylus = Hyella caespitosa</i>	1.1 (1.9)	0.8 (1.4)	7.0 (2.1)	4.1 (3.4)	8.6 (7.6)	4.0 (4.0)	2.2 (2.8)	4.4 (1.5)	4.7 (4.8)	13.9 (12.1)	4.6 (2.6)	0.07 (0.1)	–	–	5.3 (4.7)	3.8 (5.3)	2.9 (4.1)	1.5 (0.5)
<i>Fascichnus frutex = Hyella gigas</i>	–	4.2 (3.6)	0.4 (0.6)	0.3 (0.4)	–	–	–	–	–	–	–	–	–	–	0.4 (0.6)	–	–	4.2 (5.9)
<i>Fascichnus parvus = Hyella pyxis</i>	0.3 (0.5)	1.7 (2.2)	–	–	–	5.1 (8.9)	1.0 (0.9)	–	–	–	–	–	–	–	–	–	–	–
<i>Fascichnus acinosus = Hyella balani</i>	–	–	1.9 (2.7)	0.3 (0.4)	–	–	–	0.4 (0.5)	–	–	–	0.2 (0.3)	–	–	–	–	–	–
<i>Eurygonum nodosum = Masticoleus testarum</i>	0.4 (0.7)	–	9.7 (4.8)	6.9 (6.4)	3.9 (6.7)	10.3 (12.5)	22.4 (0.2)	7.8 (2.8)	18.3 (9.4)	14.4 (10.4)	6.5 (6.6)	4.3 (1.0)	19.9 (15.7)	3.3 (5.1)	13.9 (2.8)	10.5 (9.53)	0.8 (1.2)	1.4 (0.3)
<i>Planabola isp. = Cyanosaccus piriformis</i>	–	–	0.03 (0.04)	0.3 (0.5)	–	–	0.2 (0.3)	–	–	–	0.07 (0.09)	–	–	–	–	–	–	0.07 (0.1)
<b>Total cyanobacteria</b>	<b>58.5 (26.6)</b>	<b>48.1 (25.9)</b>	<b>32.0 (10.9)</b>	<b>11.4 (26.0)</b>	<b>54.3 (13.9)</b>	<b>59.3 (11.0)</b>	<b>33.6 (9.3)</b>	<b>15.5 (8.0)</b>	<b>41.4 (10.7)</b>	<b>30.3 (23.6)</b>	<b>49.7 (1.8)</b>	<b>26.6 (10.2)</b>	<b>32.6 (6.8)</b>	<b>3.3 (5.1)</b>	<b>54.2 (3.1)</b>	<b>24.3 (27.3)</b>	<b>50.8 (26.2)</b>	<b>39.7 (17.0)</b>

**Appendix 2 b) Six months' exposure (cont)**

Ichnotaxa = Bio-species	Malindi		Watamu		Kanamai		Mombasa		Ras Iwatine		Diani		Mpunguti		Kisite		Chumbe	
	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G	UG	G
<b>Green algae</b>																		
<i>Fascichnus grandis</i> = <i>Acetabularia rizhoid</i>	6.7 (5.8)	–	6.81 (6.2)	1.5 (1.1)	0.1 (0.2)	1.0 (1.7)	5.83 (1.2)	2.1 (0.8)	5 (4.4)	7.5 (1.7)	1.1 (0.4)	3.0 (1.8)	0.4 (0.7)	–	4.3 (3.9)	1.9 (0.9)	5.6 (2.7)	1.6 (0.5)
<i>Cavernula pediculata</i> = <i>Gomontia polyrhiza</i>	–	–	0.8 (1.2)	0.1 (0.2)	0.1 (0.2)	–	4.2 (1.2)	0.8 (1.2)	0.6 (1.0)	2.8 (0.7)	–	0.07 (0.1)	0.3 (0.5)	–	0.1 (0.2)	–	2.1 (0.6)	3.0 (4.0)
<i>Ichnoreticulina elegans</i> = <i>Ostreobium quekettii</i>	1.4 (2.4)	–	2.3 (2.4)	0.8 (0.4)	–	3.6 (3.4)	4.5 (5.0)	0.5 (0.7)	16.7 (28.9)	0.4 (0.1)	8.8 (2.6)	4.1 (1.6)	1.7 (2.9)	1.1 (1.0)	10.7 (14.9)	0.4 (0.6)	17.9 (11.0)	18.1 (4.6)
<i>Rhopalia catenata</i> = <i>Phaeophila</i> sp.	7.2 (7.6)	23.5 (23.3)	27.8 (2.8)	16.2 (2.0)	24.5 (5.7)	19.2 (10.8)	14.4 (6.2)	38.6 (0.9)	18.5 (16.0)	18.8 (12.6)	8.8 (5.6)	20.4 (11)	14.9 (11.1)	41.0 (18.0)	17.9 (1.8)	23.4 (24.4)	14.1 (11.0)	12.6 (9.6)
<b>Total green algae</b>	<b>15.3</b> <b>(4.6)</b>	<b>23.5</b> <b>(23.3)</b>	<b>37.7</b> <b>(10.7)</b>	<b>18.6</b> <b>(3.5)</b>	<b>24.6</b> <b>(5.8)</b>	<b>23.8</b> <b>(15.4)</b>	<b>26.3</b> <b>(4.8)</b>	<b>42.0</b> <b>(0.4)</b>	<b>40.7</b> <b>(16.8)</b>	<b>29.2</b> <b>(8.3)</b>	<b>18.7</b> <b>(3.4)</b>	<b>27.5</b> <b>(10.8)</b>	<b>17.0</b> <b>(8.6)</b>	<b>42.1</b> <b>(17.1)</b>	<b>33.1</b> <b>(6.3)</b>	<b>24.9</b> <b>(8.1)</b>	<b>39.6</b> <b>(25.6)</b>	<b>34.7</b> <b>(1.3)</b>
<b>Fungi</b>																		
<i>Saccomorpha sphaerula</i> = <i>Lithophyllum gangliiforme</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Saccomorpha clava</i> = <i>Dodgella priscus</i>	–	–	–	–	–	–	–	–	–	–	–	–	0.3 (0.5)	–	–	–	–	–
<i>Orthogonum fusiferum</i> = <i>Ostracoblabe implexa</i>	1.3 (1.8)	1.5 (1.7)	10.1 (4.8)	4.6 (1.0)	8.2 (3.6)	2.9 (2.7)	8.9 (3.7)	11.0 (4.8)	14.9 (17.7)	8.4 (7.3)	1.9 (2.7)	6.2 (3.9)	5.9 (6.5)	2.7 (3.8)	3.3 (1.7)	6.5 (9.2)	5.0 (4.4)	2.7 (3.4)
<i>Orthogonum lineare</i> = Unknown heterotroph	1.1 (1.6)	–	0.14 (0.2)	–	–	–	0.3 (0.4)	0.1 (0.2)	–	–	–	0.1 (0.2)	–	–	–	–	–	–
<b>Bacteria *</b> <b>Coccoid form =</b> unknown producer	–	–	–	–	–	<b>0.5</b> <b>(0.9)</b>	–	–	–	–	–	–	–	–	–	<b>0.8</b> <b>(1.2)</b>	–	–
<b>Total heterotrophs</b>	<b>2.4</b> <b>(2.0)</b>	<b>1.5</b> <b>(1.7)</b>	<b>10.2</b> <b>(4.6)</b>	<b>4.6</b> <b>(1.0)</b>	<b>8.2</b> <b>(3.6)</b>	<b>3.4</b> <b>(1.9)</b>	<b>9.17</b> <b>(4.1)</b>	<b>11.2</b> <b>(5.0)</b>	<b>14.9</b> <b>(17.7)</b>	<b>8.4</b> <b>(7.3)</b>	<b>1.9</b> <b>(2.7)</b>	<b>6.9</b> <b>(3.4)</b>	<b>6.1</b> <b>(6.2)</b>	<b>2.7</b> <b>(3.8)</b>	<b>3.3</b> <b>(1.7)</b>	<b>7.3</b> <b>(8.0)</b>	<b>5.0</b> <b>(4.4)</b>	<b>2.7</b> <b>(3.4)</b>
<b>Total</b>	<b>76.1</b> <b>(21.2)</b>	<b>73.1</b> <b>(7.5)</b>	<b>79.7</b> <b>(26.2)</b>	<b>34.6</b> <b>(29.5)</b>	<b>87.1</b> <b>(7.9)</b>	<b>86.5</b> <b>(9.9)</b>	<b>69.1</b> <b>(4.0)</b>	<b>68.6</b> <b>(3.4)</b>	<b>88.7</b> <b>(11.6)</b>	<b>67.8</b> <b>(36.0)</b>	<b>70.3</b> <b>(4.3)</b>	<b>61.0</b> <b>(4.0)</b>	<b>55.8</b> <b>(8.3)</b>	<b>48.2</b> <b>(18.8)</b>	<b>90.5</b> <b>(8.0)</b>	<b>55.7</b> <b>(11.3)</b>	<b>95.4</b> <b>(3.9)</b>	<b>77.0</b> <b>(12.3)</b>

**Appendix 3** Correlations between microbial endolith percent cover (cyanobacteria, green algae, heterotrophs) and bioerosion rates against physical-chemical variables in different study sites and for different times of exposure (3 months and 6 months). Percent cover and bioerosion rates after 3 month's exposure were correlated with physical-chemical data collected during the southeast monsoon, and percent cover and rates after 6 month's exposure were correlated with physical-chemical data collected during the northeast monsoon. Analysis excluded Chumbe Sanctuary because of a lack of data. Analysis for 3 month's exposure also excluded Diani because experimental cages were lost. N=7 for analysis of 3 month's exposure data and N=8 for analysis of 6 month's exposure data. For significant correlations, p-value is given inside brackets; NS = non-significant

	Nitrate + Nitrite ( $\mu\text{M}$ )	Ammonia ( $\mu\text{M}$ )	Phosphate ( $\mu\text{M}$ )	Chlorophyll a ( $\mu\text{g l}^{-1}$ )	Total Particulate Matter ( $\text{mg l}^{-1}$ )	Particulate Organic Matter ( $\text{mg l}^{-1}$ )	Current Speed ( $\text{m s}^{-1}$ )	Water Temperature ( $^{\circ}\text{C}$ )
<b>Cyanobacteria (3 month's exposure)</b>	0.1748 NS	-0.61741 NS	-0.2460 NS	0.1215 NS	0.6817 NS	-0.1804 NS	-0.3753 NS	-0.19412 NS
<b>Cyanobacteria (6 month's exposure)</b>	-0.2892 NS	-0.0813 NS	-0.1885 NS	-0.2338 NS	-0.1529 NS	0.2021 NS	0.1539 NS	0.4520 NS
<b>Green Algae (3 month's exposure)</b>	0.16529 NS	0.1401 NS	0.26824 NS	-0.0432 NS	-0.2854 NS	-0.2003 NS	-0.5312 NS	0.0820 NS
<b>Green Algae (6 month's exposure)</b>	0.1869 NS	0.0598 NS	0.3120 NS	0.1206 NS	0.010 NS	-0.1099 NS	-0.2406 NS	0.0290 NS
<b>Heterotrophs (3 month's exposure)</b>	0.0365 NS	0.3386 NS	0.4775 NS	0.3258 NS	-0.4030 NS	-0.2173 NS	-0.5361 NS	0.2770 NS
<b>Heterotrophs (6 month's exposure)</b>	0.1774 NS	0.4061 NS	0.6182 NS	0.3236 NS	0.2567 NS	-0.4024 NS	-0.6101 NS	0.0915 NS
<b>Total cover (3 month's exposure)</b>	0.2396 NS	-0.4658 NS	0.1453 NS	0.2324 NS	0.4332 NS	-0.32723 NS	<b>-0.7724 (0.0418)</b>	-0.1342 NS
<b>Total cover (6 month's exposure)</b>	0.0155 NS	0.0006 NS	0.2634 NS	-0.1559 NS	0.0177 NS	-0.0558 NS	-0.2323 NS	0.3615 NS
<b>Bioerosion Rate (3 month's exposure)</b>	0.5291 NS	0.4761 NS	-0.2724 NS	-0.1652 NS	0.6958 NS	-0.3802 NS	<b>-0.7654 (0.0449)</b>	0.0683 NS
<b>Bioerosion Rate (6 month's exposure)</b>	0.0639 NS	0.6591 NS	0.4532 NS	0.0182 NS	-0.0026 NS	-0.6964 NS	-0.0578 NS	-0.2159 NS

**Appendix 4** Correlations between microbial endolith percent cover (cyanobacteria, green algae, heterotrophs), bioerosion rates, and sea-urchin and herbivorous fish biomass in different reefs. N=8 for 3 months exposure and N=9 for 6 months exposure; all correlation coefficients were non-significant.

	<b>Biomass</b>			
	<b>Sea-urchin</b>	<b>Acanthuridae</b>	<b>Scaridae</b>	<b>Herbivorous fish (Acanthuridae + Scaridae)</b>
<b>Cyanobacteria (3 month's exposure)</b>	0.0595	-0.4330	0.2974	0.0084
<b>Cyanobacteria (6 month's exposure)</b>	-0.1861	-0.1943	0.4474	0.2181
<b>Green Algae (3 month's exposure)</b>	0.2409	-0.1943	-0.6738	-0.4779
<b>Green Algae (6 month's exposure)</b>	-0.0460	-0.0096	-0.5847	-0.4080
<b>Heterotrophs (3 month's exposure)</b>	0.1910	0.0776	-0.5926	-0.3917
<b>Heterotrophs (6 month's exposure)</b>	-0.1228	-0.1764	-0.5388	-0.4541
<b>Total cover (3 month's exposure)</b>	0.1909	-0.3885	-0.0865	-0.2480
<b>Total cover (6 months exposure)</b>	-0.1485	-0.2345	-0.2112	-0.2552
<b>Bioerosion Rate (3 month's exposure)</b>	0.6801	-0.5250	-0.2781	-0.4519
<b>Bioerosion Rate (6 month's exposure)</b>	-0.1871	-0.1879	-0.0447	-0.1185



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