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Protozoan epibionts on the prop roots of the Red Mangrove Tree, *Rhizophora mangle*

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Summary

Despite the importance of understanding carbon flow in mangrove systems, relatively little research has focused on the grazing protozoan populations inhabiting these sensitive areas. A conspicuous feature of the mangrove tree is the array of emergent aerial roots that are covered in epibiont film. This study is the first to consider the numbers of protozoa inhabiting this dense surface film layer. No obvious temporal pattern was detected throughout a one-year sampling program; numbers of protozoa were variable between sampling events but always abundant. Heterotrophic flagellates averaged 2.7 x 10^3 g^{-1} dry film, amoebae were the next most abundant group at 7.7 x 10^3 g^{-1} dry wt and ciliates averaged 4.8 x 10^3 g^{-1} dry wt. In this non-limited substrate environment, bacteria were numerous averaging 6.9 x 10^9 cells g^{-1} dry wt as were the other possible prey items, the cyanobacteria (9.8 x 10^6 cells g^{-1} dry wt) and pennate diatoms (2.4 x 10^6 cells g^{-1} dry wt). It was estimated that the combined protozoan population was only removing about 3% of bacteria carbon d^{-1}, suggesting that micrograzers are not major regulators of bacterial carbon in this specialized habitat. Some preliminary trials comparing the growth of protozoa (as generation time) on tightly and loosely associated bacteria suggest that amoebae are more capable of removing tightly-associated bacteria than the other micrograzers. Since these attached bacteria are likely to be involved in the degradation of mangrove carbon, this observation suggests a possible important ecological role for amoebae in the film community.

Key words: temporal variation, amoebae, ciliates, flagellates, cyanobacteria, diatoms, abundance

Introduction

Mangroves are marine tidal forests that cover about 180,000 km^2 of the globe's surface in subtropical and tropical regions. Through their productivity, mangroves contribute significantly to coastal ecosystems (Steinke, 1995). They play a vital role in coastal protection and fisheries production by soaking up nutrients that would otherwise pollute coastal waters and by providing

breeding grounds for juvenile marine animals. Exported mangrove litter carbon is also a major source of detritus to the subtidal benthos in adjacent coastal waters helping to keep these areas rich in fauna and productive. Despite the importance of mangroves, they are being destroyed at an alarming rate (Fondo and Martens, 1998), and it has become a matter of some urgency to understand trophic interactions and material flow within these ecosystems.

Within the microbial assemblage of mangroves, protozoa have been poorly studied. Alongi (1988, 1990) reported that the protozoan community of mangrove litter deposits was dominated by ciliates and flagellates and amoebae were rare (Alongi, 1986). However, his enumeration methods may have been inappropriate for amoebae since a more recent study has shown the planktonic waters are rich in these protists containing up to 1.0 x 10^5 amoebae l^{-1} (Rogerson and Gwaltney, 2000). This discrepancy merely serves to highlight gaps in our knowledge about the relative importance of protozoa in this organically rich location.

The nutrient rich environment offered by the submerged prop root of mangrove trees allows for the continuous trapping of allochthonous material. Thus, carbon is not only being collected on the prop root by the release of dissolved carbon from the root but is also being accumulated from the water column that is rich in decomposing litter and other mangrove debris. The rich film of material and attendant biota on the roots explains why prop root communities have been shown to have some of the highest rates of oxygen uptake within the mangrove ecosystem (Gocke et al., 1981).

Despite the possible importance of this epibiont community, in terms of ecosystem function, research to date has concentrated on the macrofaunal communities (Perry, 1988; Ellison and Farnsworth, 1992; Bingham, 1992; Bingham and Young, 1994; Farnsworth and Ellison, 1996). The lack of research on grazing microfauna (i.e. protozoa) is surprising since both bacterial and macrobiological communities are well represented in the surface film. It follows that there may be a functional connection between the two groups, thus making the understanding of the biotic interactions within these prop root films a priority.

Material and methods

Study sites and sampling methods

Samples were collected from two adjacent sites in John U. Lloyd State Park, Fort Lauderdale, Florida, U.S.A. (Fig. 1). This mangrove rich park is located off the Port Everglades Inlet, in the south east of the state. The east site (Fig 1 E) was in a sheltered channel (26°04′ 28.74"N 80°06′ 40.79"W) while the western site (Fig. 1 W) was more exposed opening into the Port Everglades Intracoastal Waterway (26° 04′ 28.66" 80° 06′48.62"W). Both sites were surrounded by dense stands of red mangrove trees, *Rhizophora mangle,* all with prop roots covered in rich, attached epibiont films (Fig. 2). This film was composed of a heterogenous mix of trapped mangrove material with accompanying biota. It had high mineral grain content and resembled loose, detritus-rich surface sediment. Presumably, loose benthic sediments become entrapped in the film during tidal movements. On each sampling occasion, nine epibiont samples were collected from randomly chosen prop roots. Samples were taken by scraping a 1 cm^2 patch of film into sterile 15 ml collecting tubes. All material was collected from just above the water line approximately 1 h before low tide to ensure that only moist film was collected (see arrow in Fig. 2). Samples were taken monthly or bimonthly between June, 1999 and May, 2000. On each outing, O_2 levels, temperature, salinity and pH were recorded. For these measurements water was tested *in situ* using a YSI 556 Environmental Multi-Probe System (Yellow Springs, OH, USA). Exact sample dates (sampling events in Figs. 3 and 4) were 28/6/99, 20/7/99, 9/8/99, 31/8/99, 13/10/99, 28/10/99, 11/11/99, 6/12/99, 20/12/99, 6/1/00, 8/2/00, 17/3/00, 5/4/00, 27/4/00, 25/5/00.

Enumeration methods

No single counting method is appropriate for all groups of microbes, hence several different methods were employed. Each of the nine samples collected were subdivided into identical aliquots to facilitate the different counting methods. All epibiont film material was diluted with a known volume of filtered sterile seawater and vigorously shaken for 1 min (using a vortex shaker) to dislodge biota. This method disrupted the flocculent material and randomly distributed the protists.

To enumerate heterotrophic flagellates, samples were fixed in 2% gluteraldehyde and stained with the DNA specific fluorochrome DAPI (4'6-diamidino-2-phenyl indole). A 2 ml aliquot of sample was filtered through a 0.2 µm Nuclepore filter with an 8.0 µm Millipore backing filter to ensure even distribution of cells on the filter surface. Using epifluorescence microscopy with UV light illumination and an overall magnification of x 600, the number of flagellates in 50 random fields of view was counted.

Ciliates were counted by taking diluted, well-shaken, epibiont material and adding 1 ml to a Sedgewick Rafter counting chamber. The chamber was searched using an inverted microscope and live ciliates counted. Because of the amount of detrital material in

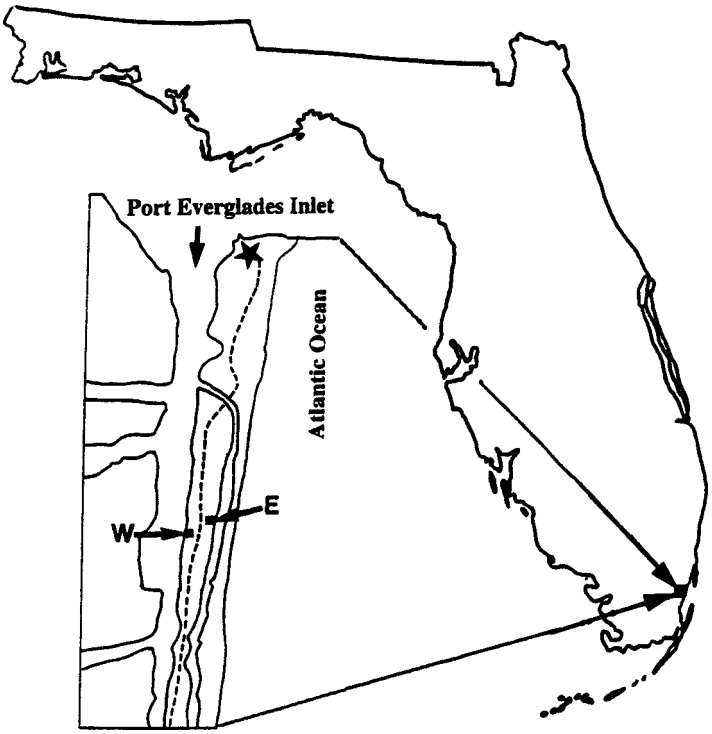

Fig. 1. Location of mangrove sampling site in the south west corner of Florida, U.S.A. The sites (east, E and west, W) were located in the John Lloyd State Park on a peninsula with the Intracoastal waterway to the west and the Atlantic ocean to the east. The sites were within 1 mile of the Oceanographic Center (see star).

Fig. 2. Prop root of the mangrove tree, *Rhizophora mangle*, with heavy covering of epibiont film. Samples were taken from close to the water at low tide to ensure that only moist film was collected (see arrow).

the samples, it was easier to search for live cells rather than fixed cells. Given the small amount of material scanned, and the probably patchy distribution of ciliates in the film, it is likely that these counts are underestimates of true ciliate abundance.

Naked amoebae were counted by the aliquot enrichment method described by Anderson and Rogerson (1995). Sample aliquots (20 µl) of water containing dislodged amoebae (typically from approximately 0.1 g wet weight biofilm material suspended in 5 ml sterile filtered mangrove water) were added to the wells of 24 well tissue culture plates each containing 1 ml of sterile sea water and a small fragment (approximately 1 mm^3) of sterile rice grain. The rice grain provided nourishment for attendant bacteria and any amoebae present in the inoculum multiplied as bacterial prey became abundant. By using a small inoculum (i.e. 20 µl) of the appropriately diluted sample, around half of the wells were positive for amoebae. This allowed us to assume that a population of amoebae in a well (of each species) developed from a single cell. However, this is a false assumption since some aliquots may have contained more than one amoeba of a single species leading to an underestimate of total abundance. After inoculation, cultures were incubated and examined for the presence of amoebae. The estimate of abundance is further underestimated because not all amoebae are amenable to laboratory cultivation. However, Rogerson and Gwaltney (2000) did show good agreement between counts based on the aliquot enrichment method and direct counting by epifluorescence, at least in the case of planktonic amoebae from a mangrove.

Densities were calculated from: $D = [N \times (V/I) \times W]/S$, where, D is the density of amoebae, N is the number of wells positive for amoebae, V is the total volume of suspension inoculated (480 µl per replicate), I is the aliquot size added to each well (20 µl), W is the total number of wells inoculated (24) and S is the quantity of biofilm used to prepare the suspension (g dry wt).

To provide an indication of numbers of potential prey in the epibiont film, the total numbers of bacteria and cyanobacteria were counted. Pennate diatoms were also abundant within the film and were also counted. These counts were all performed using epifluorescent microscopy. All samples containing dislodged biota were fixed in 2% glutaraldehyde and stained with DAPI. For the bacterial samples, epibiont film was vortexed

with filtered seawater containing 0.1% sodium duodecyl sulfate (SDS). The presence of this detergent helped release the surface associated bacteria. Bacteria were collected on 0.2 μm black membranes with an 8.0 μm backing filter. Heterotrophic bacteria were counted at x 900 magnification using UV epifluorescence (DNA fluoresced blue) while cyanobacteria were counted at x 600 magnification under blue light epifluorescence (cyanobacterial pigments fluoresced orange). Diatoms were dislodged by shaking without SDS, filtered onto a black membrane and counted at x 600 magnification under blue light illumination (chlorophyll fluoresced red).

To estimate how many of the bacteria in the film were tightly associated to the surface, as opposed to loosely associated or free, a sample of surface film was removed from the root and dipped repeatedly into 10 ml sterile, filtered seawater contained in a Petri dish . The loosely associated bacteria were released into the dish and were counted by DAPI staining after collecting on a 0.2 μm filter. The remaining bacteria on the film (the tightly associated bacteria) were removed by vortexing with 0.1% SDS (mild detergent) as described above. Released bacteria were DAPI stained and counted by epifluoresence microscopy. This experiment was replicated 5 times.

In all cases, counts were converted to numbers of cells g^{-1} dry wt. To allow these conversions, the dry wt of individual epibiont films processed was determined by drying the film to constant weight on a pre-weighed filter.

Growth trials

The enumeration experiments showed that microbes were abundant in the epibiont film, and that attached bacteria were particularly common (ca. 50% of total count). Thus growth trials of three film protozoa were conducted in the presence of predominately 'free' bacteria and 'tightly attached' bacteria.

Three protozoan isolates were obtained from the epibiont film of the mangrove roots and cloned by serial dilution. The protozoa comprised a small, unidentified bodonid flagellate (4 μm in length), an unidentified ciliate (30 μm in length), and the naked amoeba, *Vannella* sp. (20 μm in length). All three protozoa were routinely maintained in seawater with an added rice grain to nourish attendant bacterial prey.

Growth experiments used a mixture of unidentified 'free bacteria' and unidentified 'attached bacteria'. These prey bacteria were isolated from a mixed culture isolated from the mangrove root film. After enrichment, aliquots of culture containing suspended bacteria were removed and inoculated into fresh Petri dishes containing sterile seawater and rice. Firmly attached bacteria were isolated after rinsing the mixed culture repeatedly with sterile seawater. After washing away free bacteria, small portions of tightly attached bacterial film were inoculated into fresh Petri dishes containing sterile seawater and rice. This procedure was repeated until culture dishes containing either predominately free-swimming bacteria or an attached bacterial film. Palatability of the prey was not determined, however, it can be assumed that since the bacteria were mixed, some of the population were palatable to the grazers. Also, while the extent to which selection occurs in bacterivory is unknown at this time (Strom, 2000) observations on amoebae feeding on a wide range of bacteria (unpublished) suggests that the majority of bacteria amenable to laboratory culture are palatable.

Growth experiments were conducted with an abundance of bacterial prey to promote maximum growth rates. To remove contaminants, all protozoa were washed prior to experimentation by repeated gentle centrifugation (2000 rpm) and rinsing in sterile, filtered seawater. A few drops of washed protozoa (ca. 50 cells) were added to dishes (n = 3) containing the respective prey. Counts were made using an inverted phase contrast microscope. Every 12 h, the numbers of protozoa in 10 random fields of view were counted and more prey was added as required. These experiments were only continued for a few days to ensure that the growth of contaminant bacteria was minimized. All determinations were at 20°C in the dark. Growth curves were drawn and regressions calculated for the exponential phase of growth. The slopes were used to compute the growth rate constant (k) according to the formula given by Stanier et al. (1976).

$$K = \frac{\log_{10}N_t - \mathrm{Log}_{10}N_o}{0.301t}$$

where N_t is the final number of cells, N_o is the initial number of cells, and t is the time in h. The generation time in hours was calculated as 1/k.

Results

Throughout the sampling program, no significant differences (T test, $p > 0.05$) were found between prop-root associated biota of the west and east sites, thus abundance data throughout the year for these sites was pooled. Likewise, temperature and salinity were similar on each sampling occasion at the two sites. Temperature of the water varied between 22°C and 28°C, and salinity varied between 24.5 ppt (after heavy rain) and 33.5 ppt. The only significant differences between sites were found in the case of pH levels and oxygen concentra-

tions. Both of these parameters were lower at the sheltered eastern site. Here, oxygen levels generally varied between 4.0 and 9.0 mg l^{-1} compared with 5.8 to 11.0 mg l^{-1} at the west site. The pH values were also lower at the east site (7.4 to 8.1) relative to the west site (7.8 – 8.4). Even so, these differences were not sufficient to affect the numbers of heterotrophic and autotrophic microbes enumerated in this study.

Numbers of protozoa varied markedly over the sampling period (Fig. 3). High numbers of ciliates and flagellates were found in June (2.2 x 10^4 cells and 8.8 x 10^5 cells g^{-1} dry wt, respectively). It is possible that these high counts were related to the very high bacterial count at this time (7.2 x 10^{10} bacteria g^{-1} dry wt; Fig. 3, sample 1). In the case of the heterotrophic flagellate and bacterial counts throughout the year, a Pearson's r correlation analysis showed a high positive correlation (r = 0.80). There was also a peak in abundance for all protozoa in October and November (Fig 3; samples 5 – 7; up to 1.0 x 10^4 ciliates, 2.4 x 10^4 amoebae and 3.3 x 10^5 flagellates g^{-1} dry wt) that corresponded to the highest cyanobacterial and diatom counts. Cyanobacteria (Fig. 4) were most abundant in the warmer months, peaking in the fall (October; sample 5). Pennate diatoms showed a clear peak in the winter months of November and December (Fig. 4; samples 7 - 10). The extent to which these autotrophs were influenced by grazing protozoa is unknown, although ciliates and amoebae are both capable of consuming cyanobacteria and diatoms. However, any tight coupling between autotrophs and heterotrophs was not maintained over the entire year. Pearson's r correlation analyses between protozoa (flagellates, ciliates and amoebae) and cyanobacteria were weak (flagellates, r = -0.07; ciliates, r = 0.08; amoebae, r = 0.15), suggesting that grazing by protozoa was not a strong regulating factor in the case of cyanobacteria. Similarly, correlations between ciliates and flagellates with diatoms across the year were low (ca. zero) although a modest positive correlation was found in the case of amoebae (r = 0.44). Low numbers of protozoa were found in August for all three groups of protozoa, and in February and March for amoebae and February for flagellates and ciliates. Likewise, at this time, bacterial and cyanobacterial prey were at their lowest density. Overall, there were on average 4.8 x 10^3 ciliates g^{-1} dry wt, 7.7 x 10^3 amoebae g^{-1} dry wt, and 2.7 x 10^5 heterotrophic flagellates g^{-1} dry wt surface material. In the case of potential prey, average numbers throughout the study period were 6.9 x 10^9 bacteria g^{-1} dry wt, 9.8 x 10^6 cyanobacteria g^{-1} dry wt, and 2.4 x 10^6 diatoms g^{-1} dry wt. Clearly, this microhabitat within the mangrove ecosystem harbors a rich abundance of both prokaryotic and eukaryotic microbes.

The ability of a representative amoeba, flagellate and ciliate to grow on attached and unattached bacteria

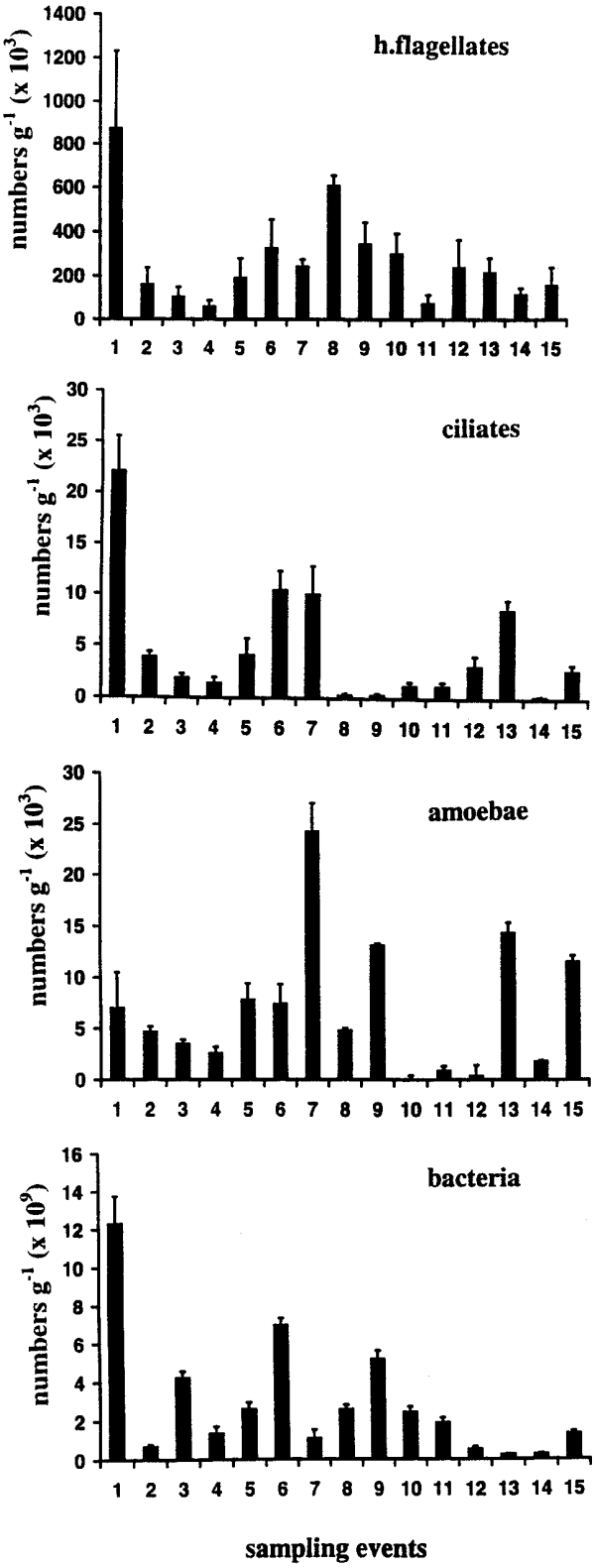

Fig. 3. Number of heterotrophic flagellates, ciliates, amoebae and bacteria g^{-1} dry weight of mangrove epibiont film. Sampling events between June 1999 and April 2000 (see materials and methods for exact dates). Data pooled from east and west sites as means with SE in parentheses (n=18).

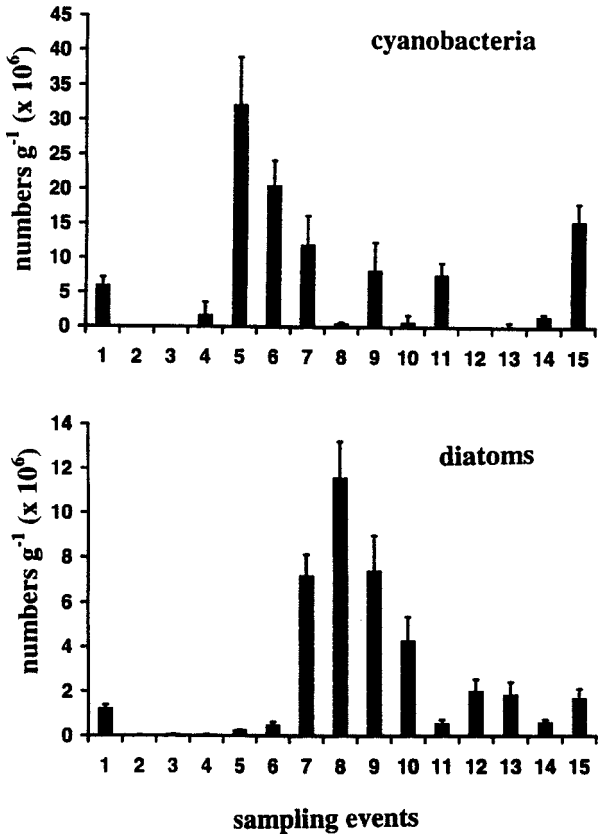

Fig. 4. Number of cyanobacteria and diatoms g^{-1} dry weight of mangrove epibiont film. Sampling events between June 1999 and April 2000 (see materials and methods for exact dates). Data pooled from east and west sites as means with SE in parentheses (n = 18).

was investigated since results showed that 50% of all the bacteria counted in the epibiont layer were 'attached' bacteria requiring vortexing with 0.1% SDS to remove them. The specific growth rate was computed for the exponential phase of growth using between 4 and 6 data points. Overall, R^2 values for the regressions defining exponential growth ranged between 0.7034 and 0.9609. Since these results only included one morphotype from each group, the ecological interpretations can only be considered preliminary at this time. However, they are interesting since positive growth (measured as generation time) was not found in all the treatments. When the bacterial prey was predominately 'unattached', all three protozoans were capable of rapid growth. Under these conditions, *Vannella* had a generation time of 16.0 h (±3.4 S.D.), and the unidentified ciliate and flagellate generation times of 14.2 h (9.9) and 3.0 h (1.8), respectively. On the other hand, when the same protozoa were cultured with predominately 'attached' bacteria, only the amoebae managed to sustain positive growth dividing in 31.3 h (4.0).

Discussion

From June 1999 to May 2000, the numbers of heterotrophic protozoa inhabiting the surface film of mangrove aerial roots was investigated. The most striking feature was the high counts of protozoa in this microhabitat. Heterotrophic flagellates ranged from 6.2 x 10^4 to 8.8 x 10^5 cells g^{-1} dry wt, amoebae ranged from 9.2 x 10^2 to 2.4 x 10^4 cells g^{-1} dry wt, while ciliates were numerically the least abundant ranging from 1.7 x 10^2 to 2.2 x 10^4 cells g^{-1} dry wt. These high densities are undoubtedly linked to the high bacterial counts (mean 6.9 x 10^9 bacteria g^{-1} dry wt) since all these protozoa consume bacteria and thus should generally fall under the same optimal growth conditions as bacteria (Finlay and Esteban, 1998). High protist counts may also have been linked to the high levels of dissolved organic carbon (DOC) in mangroves, although the contribution of osmotrophy in the nutrition of protists remains unclear at this time.

Cyanobacteria and pennate diatoms showed clear peaks in October and November/December, respectively. Whether these autotrophic peaks influenced the protozoan populations is unclear, however, there is some evidence to suggest that the high numbers of amoebae and ciliates around November were linked to these blooms. The different morphologies of cyanobacteria in the film can impose limits on what protozoa are able to consume them. The root film was dominated by coccoid forms (approx. 3 μm diameter) and filamentous forms up to 400 μm in length but ciliates and amoebae have both been shown to consume a broad range of cyanobacteria from coccoids to filamentous forms (Finlay et al., 1988; Rogerson 1991; Anderson and Rogerson, 1995; Finlay and Esteban, 1998). Likewise, the high diatom numbers in the winter may have been prey to ciliates and amoebae at this time. Although the diatom frustule has been demonstrated to be effective against attacks (Kuhn, 1997), ciliates have been shown to be the dominant predators of diatoms in the plankton (Finlay and Esteban, 1998) and amoebae can consume diatoms either by ingesting them whole, or by inserting their pseudopodia through the girdle region of diatom frustules (Kuhn, 1997).

Bacterial abundances in the epibiont film ranged from 2.0 x 10^8 cells to 7.2 x 10^{10} cells g^{-1} dry weight (Fig. 3). These high numbers are in accord with those reported from mangrove sediments of Cape York Peninsula, Australia where numbers ranged from 2.0 x 10^8 cells to 3.6 x 10^{10} cells g^{-1} dry weight (Boto et al., 1989; Alongi 1994). Similarly, in another study close to a mangrove forest, high bacterial abundances were found (up to 2.1 x 10^{11} cells g^{-1} dry wt) and it was postulated that these high counts were due to the 25,000 tons of carbon that were exported annually (Alongi,

1990). These mangrove bacterial densities are high when compared to sediments elsewhere. For example, Nickell (1992) found approximately 6.0 x 10^8 to 8.0 x 10^9 bacteria g^{-1} dry sediment in mud from a Scottish sea-loch.

Alongi (1986, 1990) reported that numbers of ciliates and flagellates in tropical sediments were relatively low compared to those in temperate sediments and that naked amoebae were rare in tropical mangrove sediments (Alongi, 1986). However, the present study suggests that the epibiont layer on roots is rich in protozoa, perhaps because it is more oxygenated relative to the mangrove benthic sediment. Mean densities of 4.8 x 10^3 ciliates, 7.7 x 10^3 amoebae and 2.7 x 10^5 heterotrophic flagellates g^{-1} dry sediment corresponding to around 9.4 x 10^3 ciliates, 1.5 x 10^4 amoebae and 5.3 x 10^5 flagellates cm^{-3} (1 cm^3 dried epibiont film weighed 0.506 g). These numbers are comparable to levels found in productive marine sediments from temperate regions (Butler and Rogerson, 1995). Moreover, the epibiont film was concentrating the protozoa, as evidenced particularly in the case of the amoebae. A recent study of the mangrove water yielded an average of 35.4 cells ml^{-1} (Rogerson and Gwaltney, 2000) some 400 times less than in the film.

With so many protozoa in the film, it is of interest to determine whether they are making any significant impact on the bacterial population. Such a calculation involves many assumptions and is at best an 'order of magnitude' estimate, but is nonetheless informative. Using the density data for the year, an estimate of the magnitude of daily grazing on the total bacterial population was calculated for each of the three protozoan groups. It was assumed that bacterial biovolume was 0.11 μm^3 $cell^{-1}$ and that the carbon equivalent was 3.5 x 10^{-13} g C μm^{-1} (Bjørnsen, 1986). It was also assumed that ciliates ingested 5.25 pg C $cell^{-1}$ h^{-1} (Kemp, 1988), heterotrophic flagellates consumed 27 bacteria h^{-1}, and amoebae consumed 0.131 bacteria h^{-1} μm^{-3} (Butler and Rogerson, 1996) and that the average cell biovolume of amoebae was 828 μm^3 (Rogerson et al, 1993). Employing these conversions, ciliates removed approximately 0.2% of bacterial standing crop per day, amoebae 0.3% and the numerically abundant flagellates consumed 2.5% of bacterial carbon per day. While these are just 'best-estimates', based on several assumptions, not the least of which is the lack of adequate consumption rate data, the numbers do indicate that protozoa are not major regulators of bacterial biomass in the epibiont film. The magnitude of these estimates has some support in the literature. For example, when heterotrophic flagellates are present in the ratio of 1 : 1000 bacteria, they are often considered major regulators of bacterial biomass (Fenchel, 1986). However, in this bacterial rich mangrove habitat, their abundance ratio was only 1: 25,500 bacteria. Similar ratios have been shown for mangrove sediments (Alongi, 1986) and for saltmarsh sediments (Kemp, 1988), suggesting that the role of heterotrophic flagellates in such habitats is not straightforward.

Preliminary experiments comparing the growth of ciliates, flagellates and amoebae on mixtures of indigenous 'attached' bacteria and 'free' bacteria suggested that there are differences in the effectiveness of these groups at removing attached prey. Only the amoebae, which are in close association with the substrate, were capable of growing on tightly attached bacteria, although their rate of growth was reduced relative to the division rate when grown with free bacteria. These results, however, are preliminary and merely highlight an important area for additional study. No attempt was made to test the palatability of the different strains, or standardize the concentrations of prey used. Moreover, the results are based on just three species isolated from the film, which may, or may not, accurately reflect the grazing groups in question. Even so, the results are intriguing and suggest that amoebae may occupy a unique niche in aquatic systems. This is an exciting result that warrants additional research using improved methods since, until now, the ecological role of amoebae has been largely overlooked. Although considerably less abundant than flagellates, they were almost twice as common as ciliates and they alone, seemed capable of growth on tightly attached bacteria. In short, their grazing impact might have important implications for the turnover of mangrove carbon since grazing is often thought to stimulate the degradation of organic materials (Rogerson and Berger, 1983).

Acknowledgements

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References

Alongi D.M.1986. Quantitative estimates of benthic protozoa in tropical marine systems using a silica gel: a comparison of methods. Estuar. Coast. Shelf Sci. 23, 443-450.

Alongi D.M. 1988. Bacterial productivity and microbial biomass in tropical mangrove sediments. Microb. Ecol. 15, 59-79.

Alongi D.M. 1990. Abundances of benthic microfauna in relation to outwelling of mangrove detritus in a tropical coastal region. Mar. Ecol. Prog. Ser. 63, 53-63.

Alongi D.M.1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiol. 285, 19-32.

Anderson O.R. and Rogerson A. 1995. Annual abundances and growth potential of gymnamoebae in the Hudson Estuary with comparative data from the Firth of Clyde. Europ. J. Protistol. 31, 223-233.

Bingham B.1992. Life histories in an epifaunal community coupling of adult and larval processes. Ecology 73, 2244-2259.

Bingham B. and Young C.M. 1994. Stochastic events and dynamics of a mangrove root community. Mar. Ecol. 16: 145-163.

Bjørnsen P.K. 1986. Automatic determination of bacterioplankton biomass by image analysis. Appl. Environ. Microbiol. 51, 1199-1204.

Boto K.T., Alongi D.M. and Nott A. 1989. Dissolved organic carbon-bacteria interactions at sediment-water interface in a tropical mangrove system. Mar. Ecol. Prog. Ser. 51, 243-251.

Butler H. and Rogerson A. 1995. Temporal and spatial abundance of naked amoebae (gymnamoebae) in marine benthic sediments of the Clyde Sea area, Scotland. J. Eukar. Microbiol. 42, 724-230.

Butler H. and Rogerson A. 1996. Growth potential, production efficiency and annual production of marine benthic amoebae (gymnamoebae) inhabiting sediments of the Clyde Sea area. Aquat. Microb. Ecol. 10, 123-129.

Ellison A.M. and Farnsworth E.J. 1992. The ecology of Belizean mangrove-root fouling communities: patterns of epibiont distribution and abundance, and effects on root growth. Hydrobiologia. 247, 87-98.

Farnsworth E.J. and Ellison A.M. 1996. Scale-dependent spatial and temporal variabilityin biogeography of mangrove root eipibiont communities. Ecol. Monographs. 66, 45-66.

Fenchel T. 1986. The ecology of heterotrophic microflagellates. Adv. Microb. Ecol. 9, 57-97.

Finlay B.J. and Esteban G.F. 1998. Freshwater protozoa: biodiversity and ecological function. Biodiversity and Conservation. 7, 1163-1186.

Finlay B.J., Clarke K.J., Cowling A.J., Hindle R.M and Rogerson A. 1988. On the abundance and distribution of protozoa and their food in a productive freshwater pond. Europ. J. Protistol. 23, 205-217.

Fondo E.N. and Martens E.E. 1998. Effects of mangrove deforestation on macrofaunal densities, Gazi Bay, Kenya. Mangroves and Salt Marshes. 2, 75-83.

Gocke K., Vitola M. and Rojas G. 1981. Oxygen consumption patterns in a mangrove swamp on the Pacific coast of Costa Rica. Re. Vista de Biologia Tropical. 29, 143-154.

Kemp P.F. 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. Mar. Ecol. Prog. Ser. 49, 163-169.

Kuhn S.F. 1997. *Rhizamoeba schnepfii* sp. nov., a naked amoeba feeding on marine diatoms (North Sea, German Bight). Arch. Protistenkd. 147, 277-282.

Nickell L.A. 1992. Deep bioturbation in organically enriched marine sediments. Ph.D. thesis, University of London, UK.

Perry D.M. 1988. Effects of associated fauna on growth and productivity in the red mangrove. Ecology. 69, 1064-1075.

Rogerson A. 1991. On the abundance of marine naked amoebae on the surfaces of five species of macroalgae. FEMS Microb. Ecol. 85, 301-312.

Rogerson A. and Berger J. 1983. The stimulatory role of ciliates in the microbial degradation of crude petroleum. J. Gen. Appl. Microbiol. 29, 41-50.

Rogerson A. and Gwaltney C. 2000. The numerical importance of naked amoebae in the planktonic waters of a mangrove stand in southern Florida. J. Eukar. Microbiol. 47, 235-241.

Rogerson A., Butler G.H. and Thomason J. 1993. Estimation of amoeba cell volume from nuclear diameter and its application in protozoan ecology. Hydrobiologia. 284, 229-234.

Stanier R.Y., Adelberg E.A. and Ingraham J. 1976. The Microbial World. Prentice-Hall inc. New York.

Steinke T.D. 1995. A general review of the mangroves of South Africa. In: Wetlands of South Africa (Ed. Cowan G.I.). Dept. of Environment Affairs and Tourism, Pretoria.

Strom L.S. 2000. Bacterivory: interactions between bacteria and their grazers. In: Microbial Ecology of the Oceans (Ed. Kirchman D.L.). Wiley-Liss, Inc. New York. pp. 351-386.

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