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CHANGES IN ABUNDANCE AND BIOMASS OF THE ATTACHED BACTERIAL COMMUNITY THROUGHOUT THE DECOMPOSITION OF THREE SPECIES OF AQUATIC MACROPHYTES

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Resumo

Variação na abundância e biomassa da comunidade de bactérias perifíticas durante a decomposição de três espécies de macrófitas aquáticas. Com o objetivo de acompanhar a variação de abundância e biomassa da comunidade bacteriana aderida durante a decomposição de material vegetal, foram selecionadas três espécies de macrófitas aquáticas como substrato: *Eleocharis interstincta* (emergente), *Potamogeton stenostachys* (submersa), e *Nymphaea ampla* (folhas flutuantes). Estas espécies foram selecionadas por ocuparem habitats diferentes no corpo d'água e apresentarem diferentes composições químicas. Para o acompanhamento da comunidade bacteriana durante o processo de decomposição, amostras foram acondicionadas debaixo d'água a uma pequena profundidade, permitindo o acesso da luz solar. em garrafas de plástico vazadas lateralmente, com a utilização de mangueiras de borracha como um substrato controle. A composição química do substrato (concentrações de C, N, e P), e a taxa de perda de peso seco foram estimadas a partir de amostras separadas, acondicionadas em litter bags. A comunidade bacteriana apresentou valores diferentes de cada variável analisada (densidade, biovolume, biomassa, produtividade secundária) em cada substrato. O substrato artificial sempre exibiu os menores valores de densidade, biomassa e produtividade secundária. *N. ampla* foi o substrato mais favorável à colonização bacteriana no que diz respeito à densidade e biomassa desta comunidade, seguida de *P stenostachys* e *E. interstincta*. Este padrão corresponde também ao padrão da perda de peso do detrito destas espécies, indicando uma relação entre o processo de decomposição e a colonização bacteriana. Não foi encontrada nenhuma relação entre a composição química (C, N e P) e as variáveis bacterianas os valores das variáveis químicas e bacterianas. Os valores de densidade e biomassa da comunidade bacteriana aderida ao detrito foram aproximadamente 10 vezes maiores do que os registrados para a comunidade bacteriana da coluna d'água, em comparação de área x volume (cm^2 x cm^3). O acúmulo de biomassa bacteriana na superfície do detrito provavelmente resultou no aumento da qualidade nutricional deste material, com implicações para os níveis tróficos superiores.

Palavras-chave: Decomposição, bactérias perifíticas, composição química, dinâmica bacteriana, lagoas tropicais.

Abstract

Aiming at observing the variation in abundance and biomass of the attached bacterial community throughout the decomposition of plant material, three species of aquatic macrophytes were selected as substrate: *Eleocharis interstincta* (emergent), *Potamogeton stenostachys* (submersed), and *Nymphaea ampla* (floating leaves). These species were selected since they occupy different habitats in the aquatic environment and exhibit different chemical compositions. Samples were set up under water at a small depth, allowing for the passage of light, in plastic bottles with lateral openings, and rubber hoses were used as a control substrate. The chemical composition of

the substrates (concentration of C, N and P) as well as the rate of dry weight loss were estimated from separate samples, incubated in litter bags. The bacterial community exhibited different values of each analyzed variable (density, biovolume, biomass, secondary productivity) in each substrate. The artificial substrate always exhibited lower values of density, biomass and secondary productivity. *N. ampla* was the best substrate for the bacterial community regarding its density and biomass, followed by *P. stenostachys* and *E. interstincta*. This pattern was also found in the curves of dry weight loss of the detritus of these species, indicating a relationship between the process of decomposition and the bacterial colonization. No relationship was found between the chemical (concentrations of C, N, and P) and bacterial variables. The values of density and biomass of the attached bacterial community were approximately 10 times greater than those registered in the bacterial community of the water column, in a comparison of area x volume (cm^2 x cm^3). The increase of bacterial biomass in the surface of the detritus probably results in an improvement of its nutritional quality, with implications for the higher trophic levels.

Key-words: Decomposition, attached bacteria, chemical composition, bacterial dynamics, tropical lagoons.

Introduction

Coastal lagoons are among the most productive aquatic environments of the planet, with values of primary production close to those of estuaries, approximately 280 gC m^{-2} $year^{-1}$ (Knoppers, 1994). They are, by and large, shallow environments, parallel to the ocean shore and separated from it by a sandbar, with a predominance of deposition and small hydrological fluctuations in natural conditions (Kjerfve, 1994). Some of these morphometric characteristics (high perimeter/volume ratio, absence of pronounced fluctuations in the water level) favor the development of the community of aquatic macrophytes, bestowing upon the littoral region an important role in the nutrient and carbon cycling within the aquatic environment (Wetzel, 1993).

The community of aquatic macrophytes is an active participant in several processes in aquatic environments. For instance, some species are able to remobilize the large nutrient stock of the sediments, otherwise inaccessible in the short run, since they have access to this stock through their roots (Pomeroy, 1970; Carpenter, 1980). Besides that, they provide substrate for the growth of periphytic organisms and shelter for benthic and nektonic animals (Hutchinson, 1975). Some of these species are among the most productive yet studied (Wetzel, 1990; Piedade *et al.*, 1991; Enrich-Prast, 1998).

Most of the biomass produced by the community of aquatic macrophytes is not as exposed to the effects of herbivory as observed in other plant communities, probably due to their low nutritional quality (Polunin, 1984). Therefore, the biomass and accumulated nutrients, both in dissolved and particulate state, are returned to the ecosystem, mainly, by the detritus chain (Pieczynska, 1993), based on the bacterial activity (Benner *et al.*, 1988, Moran & Hodson, 1989).

The bacteria are able to process the particulate, more refractory fraction of the plant detritus as well as the soluble fraction (Valiela *et al.*, 1985, Benner *et al.*,

1986, Findlay *et al.*, 1986). The decomposition of this fraction is fundamentally a microbial process, regulated mainly by factors such as temperature, oxygen and nutrient availability, and quality and size of the particles (Godshalk & Wetzel, 1978). Other factors were underlined by several authors as being of importance in the process, such as the pH, redox potential, and species of decomposing organisms (Boon *et al.*, 1982; Bianchini Jr. & Toledo, 1988).

Some recent articles focus on the dynamics of the attached bacterial community in live aquatic macrophytes (Thomaz & Esteves, 1997; Thiel-Nielsen & Søndergaard, 1999). In spite of that, and considering the great number of articles on the chemical variation of the detritus of aquatic macrophytes throughout the process of decomposition (p. ex., Howard-Williams & Junk, 1976; Hietz, 1992; Emery & Perry, 1996), there are no articles aiming to relate the chemical variation of the detritus and the dynamics of the attached bacterial community. Most of these articles were performed in temperate environments, and studies on bacterial dynamics in tropical regions are rare.

For this research, three species of aquatic macrophytes found in the Cabiúnas Lagoon were selected – *Eleocharis interstincta* (VAHL) Roemer et Schults, *Potamogeton stenostachys* K. Schum., and *Nymphaea ampla* DC. The three species were chosen because they belong to different ecological groups, according to the classification proposed by Esteves (1998). *E. interstincta* is an emergent macrophyte, with photosynthetic structures above the water level and rooted to the sediment. *N. ampla* is an aquatic macrophyte with floating leaves, rooted to the sediment and with leaves floating on the surface of the water. *P. stenostachys* is a submersed macrophyte, rooted to the sediment and with no structures above the water level. These differences in the habitat of each species are reflected in different chemical compositions due to their structural material, since the role of the water in the support of the plant is different in each case (different stress among the ecological groups). These differences influence the rates of dry weight loss in the detritus of the three species in the course of time, as observed by Bianchini Jr. (1997).

The hypothesis guiding this research was that these differences, reflected in different concentrations of C, N and P in the detritus in the course of time, would be reflected in the variables of the bacterial community (density, biomass, secondary productivity), with detritus presenting better nutritional conditions (lower C:N:P ratios) exhibiting higher values of the bacterial variables. The aim of this work was to describe the changes in the attached bacterial community during this period, looking for relationships between the bacterial and chemical variables.

Study area

This study was executed in Cabiúnas Lagoon (22° and 22° 30' S; 41° 30' and 42° W), in the municipality of Macaé, inside the National Park of the Restinga de Cabiúnas, in the north of the State of Rio de Janeiro, between November 1998

and January 1999 (see map in Chapter 6). It is a shallow lagoon (maximum depth of 4 m), with an area of 0.34 km^2 (Panosso *et al.*, 1998) and dendritic shape, which represents a greater perimeter/volume ratio and enhances the colonization by aquatic macrophytes. In the region, it is the lagoon displaying the greatest diversity of aquatic macrophytes, with 15 identified species (Henriques *et al.*, 1988). The climate in the region is hot and humid, with a yearly average temperature of 26.6 °C, the yearly total precipitation reaching 1,164 mm, with a fairly pronounced dry season (Henriques *et al.*, *op. cit.*).

According to Esteves *et al.* (1983), Cabiúnas Lagoon may be classified as a dark water lagoon, due to the high concentrations of humic compounds which are a result of the decomposition of the organic matter produced in great amounts in its draining area and by the marginal vegetation.

Material and methods

Observation of the chemical variables

For the study of the process of decomposition of *E. interstincta*, *P. stenostachys* and *N. ampla*, the litter bag method was used. Green parts of the three chosen species were collected and dried at 70 °C, in the case of *E. interstincta* and *P. stenostachys*, and 60 °C, in the case of *N. ampla*, until constant weight was reached. Prior to the beginning of the experiment, some material was ground up, and its concentration of organic carbon (EMBRAPA, 1994), organic nitrogen (Allen *et al.*, 1974) and phosphorus (Fassbender, 1973) determined, these concentrations being considered as pertaining to day "0" of the experiment. Approximately 5 g (dry weight) of the material was placed in each bag. The bags, with a 5 mm mesh size, allowed for the passage of most organisms associated to the decomposition process (Gonçalves Jr. *et al.*, 1998).

For each kind of detritus, after 1, 2, 4, 7, 11, 15, 30, 60, 81 and 95 days, the bags were withdrawn from the lagoon, with three replicates being collected for each species under study. The material was taken to the laboratory and placed on absorbing paper for two hours. After this step, the samples were dried at 70 °C until constant weight was reached.

At each sampling, the temperature (thermistor YSI 30/10 FT), pH (pHmeter Analion PM608) and concentration of dissolved oxygen (Winkler method, modified by Golterman *et al.*, 1978) were determined. Besides that, samples of water from the site were also taken for analyses of total nitrogen and reactive phosphorus (ortho-phosphate).

After constant weight was reached, the material was weighed and ground up for chemical analyses. The concentrations of organic nitrogen were obtained by the Kjeldahl method (Allen *et al.*, 1974), of phosphorus, by the method proposed by Fassbender (1973), and of organic carbon, by the method of oxidation with potassium dichromate, described in EMBRAPA (1994).

The data of dry weight loss from the detritus was adjusted to a double decay exponential model of first order (Bianchini Jr., 1997), assuming the existence of two kinds of particulate material (labile and refractory) at the beginning of the experiment.

Observation of the bacterial variables

All of the containers used were autoclaved or washed with HCl 10% (v/v). The solutions were made with distilled, autoclaved and filtered water (membrane of 0.22 μm of porosity). The radioactive leucine ([^{3}H] leu, 135 Ci mmol^{-1}) was provided by Amershan Co.

The biovolume, density, biomass and secondary productivity were determined from incubations of green parts of the three selected species. To accomplish this, the leaves were collected in a branch of the Cabiúnas Lagoon, where they were later incubated along with an artificial, inert substrate. This inert substrate, composed of rubber hoses, had previously been tested by Enrich-Prast (pers. com.), and did not release detectable amounts of any element (C, N and P) after immersion in water. The plant material and the artificial substrate, in the text, are called "detritus". The detritus was incubated in plastic bottles with lateral and vertical openings, in a spot relatively sheltered from the wind action, at a depth of approximately 10 cm. The incubations in the plastic bottles ran simultaneously with the incubations in the litter bags, and so the abiotic variables were collected as described above. The samplings of material for the estimate of bacterial density, biovolume and biomass occurred after 1, 2, 4, 7, 11, 15, 19, 30, 45, 60 and 95 days of experiment, with three replicates being sampled at each day. For the determination of the bacterial secondary productivity, three replicates were incubated, as described below, on the following days: 2, 7, 11, 15, 30, 45, 95. The results were expressed per area of biofilm, and this area was measured with a digital scanner (Hewlett-Packard ScanJet 4c).

Bacterial secondary productivity

For the determination of secondary productivity, pieces of detritus (1 to 3 cm^2) with intact biofilms were incubated in 20 ml vials, immersed in the water of the lagoon, in the dark, for 15 minutes, with 10 ml of water and [^{3}H] leucine, diluted with cold leucine to reach a final concentration of 400 nM. The activity was interrupted with buffered formaldehyde (3.7%, final concentration). This concentration of leucine was a subsaturation, as demonstrated below.

In the laboratory, the detritus was scraped and later placed in an ultrassonicator (TRANSSONIC 890) for 5 minutes. The efficiency of this method in removing the bacteria is higher than 97% (Thomaz & Esteves, 1997).

The leucine incorporation was measured by extraction with hot trichloroacetic acid (TCA) (Kirchman *et al.* 1986, Simon & Azam 1989). After the

removal of the bacteria from the plant, 3 ml of the solution was placed in a flask and TCA (5%, v/v) was added. The samples were placed in a water bath at 95 °C for 30 minutes. After cooling, the samples were filtered in polycarbonate membranes (Nucleopore, 0.22 μm porosity), which were washed 8 times with 2 ml of TCA 5% solution. The filters containing the incorporated leucine were placed in 10 ml of Aquassol Solution 2 (Du Pont) and radioanalyzed in a Beckman 6800 scintillator.

The incorporated leucine was converted into bacterial secondary productivity with an empirical factor derived from "in vitro" cultures of planktonic bacteria in the exponential phase of growth. These planktonic bacteria were used, since the application of this method to attached bacteria is hazardous, as the increase of density and biomass in this community is a result not only of the production, but also of the immigration and emigration of bacteria from the water (Thomaz & Esteves, 1997). The empirical factor used was determined by Farjalla (1998). For the determination of this factor, Farjalla (1998) used planktonic bacteria from the Cabiúnas Lagoon in laboratory cultures free of predators and enriched with nutrients. Measuring the biomass after determined time intervals, in the exponential phase of growth of the cultures, and measuring the leucine incorporation in these intervals, it was possible to estimate an empirical factor for the conversion of incorporated leucine into produced carbon.

Time interval of incubation and saturation curve

The concentration of leucine and the time interval of incubation for the estimate of bacterial secondary productivity were determined prior to the experiment with intact biofilms collected in Cabiúnas Lagoon, as suggested by Thomaz & Esteves (1997). For the determination of the time interval, incubations with 10 nM of [^{3}H] leu were set up and stopped after 10, 20, 40 and 80 minutes with buffered formaldehyde (3.7%, v/v). The saturation levels were measured with incubations of 10 nM of [^{3}H] leu, and with the addition of cold (non-radioactive) leucine until several concentrations between 10 and 800 nM were reached. The activity of these incubations was stopped after 15 minutes with buffered formaldehyde (3.7%, v/v). The incorporation of [^{3}H] leu by the biofilm was determined as described above for the determination of bacterial secondary productivity.

The experiments for the determination of the time interval of the incubation resulted in linearity up to 40 minutes. Saturation was not reached up to concentrations of 800 nM of leucine, the greatest concentration used. These results were used for the establishment of the time interval and of the concentration of [^{3}H] leu in the experiment for the determination of the bacterial secondary productivity. Therefore, the final concentration of 400 nM, the same concentration used by Thomaz & Esteves (1997) in a study at a nearby coastal lagoon (Imboassica Lagoon) was selected, as saturation was never reached.

Bacterial biovolume, density and biomass

Bacterial biovolume, density, and biomass, were estimated after scraping and sonication for 3 minutes of material sampled in the field and immediately fixed with buffered formaldehyde (3.7%, v/v). 2 ml of the material was stained with a solution of acridine orange, 0.01% (v/v) (Hobbie *et al*, 1977). When necessary the material was diluted with sterilized water, filtered in a polycarbonate membrane with 0.22 μm of porosity. The bacterial cells were counted with a Zeiss Axiovert inverted microscope (1,600x magnification).

The cells were divided into four categories: rods, cocci, spirilla and vibrios. At least 20 fields or 200 cells were counted in each slide. Controls with sterilized and filtered water (0.22 μm) showed that the contamination never reached values higher than 1% of the cells counted in the samples.

The average biovolume of the cells was estimated according to the following equation (Fry, 1990):

$$v=(\pi/4)w^2(l-w/3)$$

where v = volume, w = width and l = length. For the conversion of biovolume into biomass, it was assumed that $1\mu m^3 = 3.5 \times 10^{-13}$ gC (Bjørnsen, 1986).

Results and discussion

Dry weight loss and chemical variables

Table 1: Parameters of the double decay exponential models throughout the decomposition of three species of aquatic macrophytes in the Cabiúnas Lagoon (see Equation 1). The bracketed numbers indicate the adjustment of the model (Pearson r).

	POM_L (%)	k_1	POM_R (%)	k_2	$T_{1/2}$ (days)
E. interstincta (0.95)	17.30	1.5 (*)	82.70	0.0047	147
P. stenostachys (0.96)	20.77	1.5 (*)	79.23	0.024	29
N. ampla (0.93)	27.20	1.5 (*)	72.80	0.1122	6

(*) According to the suggestion of Bianchini Jr. (pers. com.), the coefficients of decay of the labile fraction (k_1) were assumed to be 1.5 day^{-1}.

In the double decay exponential model, the leaching process is accounted for by the estimate of labile material in the detritus. The labile fraction of the detritus of all species presented high coefficients of decay, and the process was finished by the second day of the experiment (Table 1).

The catabolism of the particulate detritus is a microbial process, controlled by characteristics such as oxygenation conditions, temperature, available organisms for the decomposition, amount and quality of the detritus, the pH, and avail-

able nutrients (Bianchini Jr., 1997). The concentration of dissolved oxygen never reached values capable of limiting the activity of aerobic organisms (Table 2), indicating the dominance of the aerobic community, more efficient in the processing of the detritus (Bianchini Jr., *op. cit.*). Furthermore, the temperature was kept at relatively high levels (Table 2) throughout the experiment, enhancing microbial activity. These factors are accountable for the great rates of dry weight loss observed.

Table 2: Average values of temperature, dissolved oxygen, pH, total nitrogen and available phosphorus in the site of the experiment. Bracketed values represent the range of the data (n=12).

Temperature (°C)	Oxygen (mg l^{-1})	pH	Total-N (mg l^{-1})	Available-P (mg l^{-1})
26.19 (23.2 – 29.0)	7.75 (6.6 – 8.7)	6.57 (6.2 – 6.8)	0.407 (0.37 – 0.46)	N.D. (<0.093)

The C:N:P ratio is one of the most important factors explaining the rates of decomposition of detritus. Enriquez *et al.* (1993), in a study examining detritus from several sources, from macroalgae to trees, was able to explain 89% of the variation in the data with the analysis of differences in the C:N:P ratio. As the decomposers usually exhibit high concentrations of nitrogen and phosphorus, i.e., low C:N and C:P ratios, they require a substrate with high concentrations of these elements (Swift *et al.*, 1979). Therefore, balanced bacterial growth requires substrates with an atomic C:N:P ratio close to 106:12:1 (Goldman *et al.*, 1987).

C:N:P ratios close to these values result in fast microbial growth, while ratios higher than this, i.e., excess of carbon, result in microbial activity limited by the lack of nutrients. Within this context, the results (Table 3) indicate that there is a limitation of microbial activity by lack of nutrients in the detritus of the three

Table 3: C:N:P ratio in the detritus of three species of aquatic macrophytes at Cabiúnas Lagoon throughout its decomposition.

Days	*E. interstincta*	*P. stenostachys*	*N. ampla*
0	418:9:1	168:6:1	205:7:1
1	1143:19:1	313:23:1	377:10:1
2	1345:19:1	207:16:1	340:5:1
4	991:17:1	270:16:1	330:15:1
7	660:15:1	236:15:1	
11	678:15:1	280:11:1	
15	1148:26:1	269:11:1	
19	1063:26:1		
30	1043:24:1		
60	962:35:1		

species. As can be seen in Table 3, the detritus of *E. interstincta* was severely limiting as regards nitrogen concentration throughout the decomposition process, while the other two kinds of detritus were not nearly as poor in nitrogen.

Bacterial density, biomass and biovolume

Environmental factors such as temperature and nutrient concentrations (Table 1) are regarded as critical for the fluctuation of bacterial variables such as density, biovolume, and secondary productivity. Several relationships between the nutrient concentrations and the cell size were observed in previous studies, especially in bacterioplankton, ranging from bigger cells in richer environments (Simon, 1985) to bigger cells in poorer environments (Bird & Kalff, 1984) or even the absence of relationship between these factors (Letart & Pinel-Annoul, 1991). One of the explanations for these results is the dominance of biotic interactions in the regulation of the population, such as the selective predation of bigger cells, resulting in a smaller average size of the cells (Abreu *et al.*, 1992).

The absolute values of density, in all substrates, are between 10^6 and 10^8 cells per cm^2 (Fig. 1). These results are within the range found in a recent review of the literature (Wetzel & Søndergaard, 1997). Even the artificial sub-

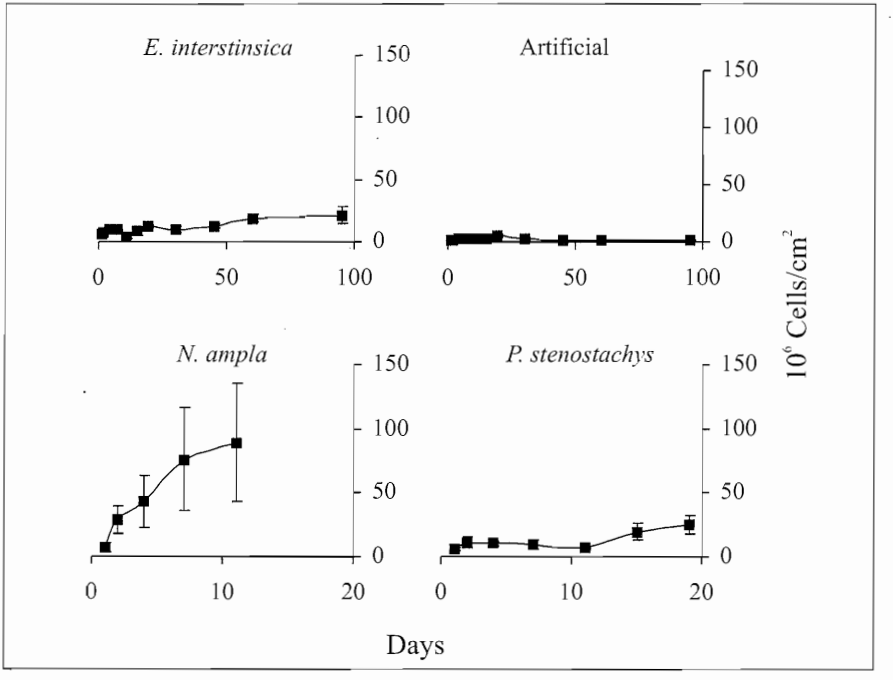

Figure 1: Bacterial density, in cells per cm^2, in the detritus of three species of aquatic macrophytes and an artificial substrate at Cabiúnas Lagoon. Bars indicate the standard deviation (n=3).

strate, inert, exhibited values of the same order of magnitude (10^6 cells) of those attached to submersed macrophytes in the literature. Nonetheless, in the present research, this substrate presented significantly ($p<0.05$) lower values of density and biomass (Figs. 1 and 2) in all samplings, underlining the role of the natural substrates as providers of favorable conditions for the development of the bacterial community. These results point at the importance of the natural substrates as sources of nutrients, as well as providers of physical support for the bacterial populations.

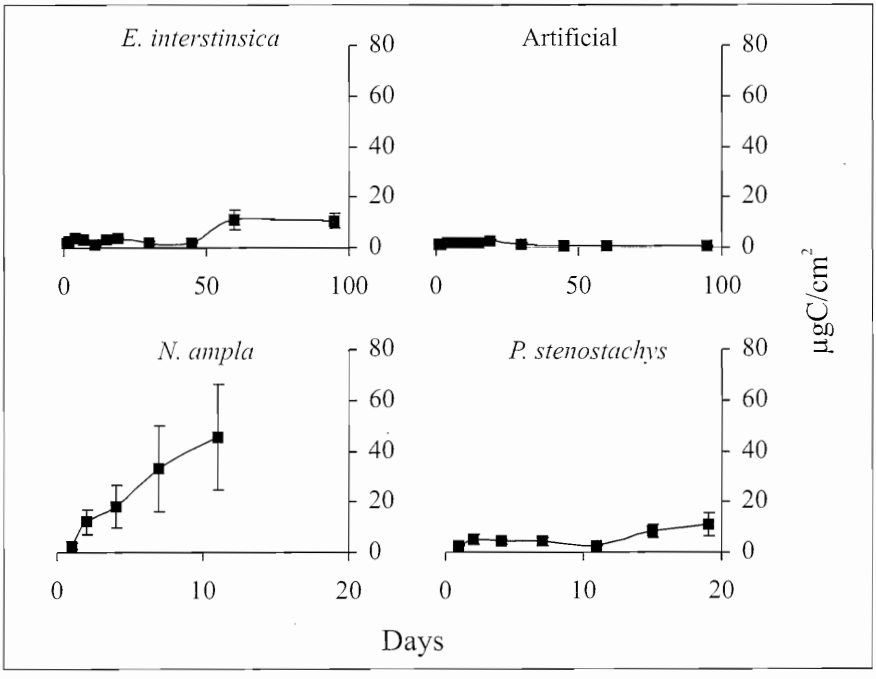

Figure 2: Bacterial biomass, in µg C cm^{-2}, in the detritus of three species of aquatic macrophytes and an artificial substrate at Cabiúnas Lagoon. Bars indicate the standard deviation (n=3).

Twilley *et al.* (1986) used oxygen consumption as an index of microbial activity and observed that the greater availability of nutrients (N, P) favors this community. When we compared bacterial variables in the three substrates, we observed that, although there are differences among them, these differences are not significantly associated to the chemical composition of the substrate (concentrations of organic carbon, nitrogen and phosphorus), as ascertained by linear regressions between each bacterial variable and the chemical variables.

The differences between the density and the biomass of the bacterial community in the different substrates, therefore, may be associated to biotic factors related to "top-down" processes, such as predation, or "bottom-up" factors not observed in the present research, such as the quality of available carbon (ratio of phenolic compounds, concentration of lignin). The biofilm, considered as a unit, is also undertaking a process of succession throughout the decomposition (Fernandes, 1998), which may result in different rates of predation in the course of time.

In every substrate, there was a significant trend towards the increase in bacterial density and biomass in the course of time, the exception being the artificial substrate, with a significant drop after the 19th day (Figs. 1 and 2). This trend was also observed by Blum & Mills (1991), Peduzzi & Herndl (1991) and Belova (1993), in marine and freshwater environments. Peduzzi & Herndl (1991), studying, with electronic microphotographs, the bacterial colonization of the detritus of a species of aquatic macrophyte, observed that, after 25 days, the bacteria had invaded the detritus, being found thereafter not only in the surface but also inside the detritus. Therefore, the densities and biomass found in the present research may underestimate the real values of the bacterial community in the detritus, by neglecting the bacteria found inside the detritus.

While the two more favorable substrates for bacterial colonization, *P. stenostachys* and *N. ampla*, presented and average biovolume similar in the course of the decomposition, the other two substrates, *E. interstincta* and the artificial substrate, exhibited a significant drop ($p<0.05$) of the biovolume in the course of the decomposition (Fig. 3). This trend is reversed, in *E. interstincta*, between the 45th and 60th days, and reappears thereafter. As observed for density and biomass, this result was observed by other authors, such as Blum & Mills (1991) and Thomaz & Esteves (1997), and was explained by the latter as being the result of biotic interactions, such as selective predation on the largest bacteria (Abreu *et al.*, 1992).

Bacterial secondary productivity

The absolute values of secondary productivity (Fig.4), such as those of density, are within the range registered by Wetzel & Søndergaard (1997), who mention values between 0.005 and 4 μg C cm^{-2} h^{-1}. The exception is the artificial substrate, with comparatively lower values. In any case, the values are in the lower range of the literature, being considered low, especially when examined in the light of the values of density and biomass (Figs. 1 and 2). Thiel-Nielsen & Søndergaard (1999), mention values between 0.1 and 3 μg C cm^{-2} h^{-1} in the biofilm of live plants, which release fewer nutrients to the water column than the detritus. These low values are probably a result of the inactivity of a great portion of the attached bacterial community.

The simultaneity of the maximum values of secondary productivity (in all substrates, in the seventh day of the experiment) indicates an underlying factor

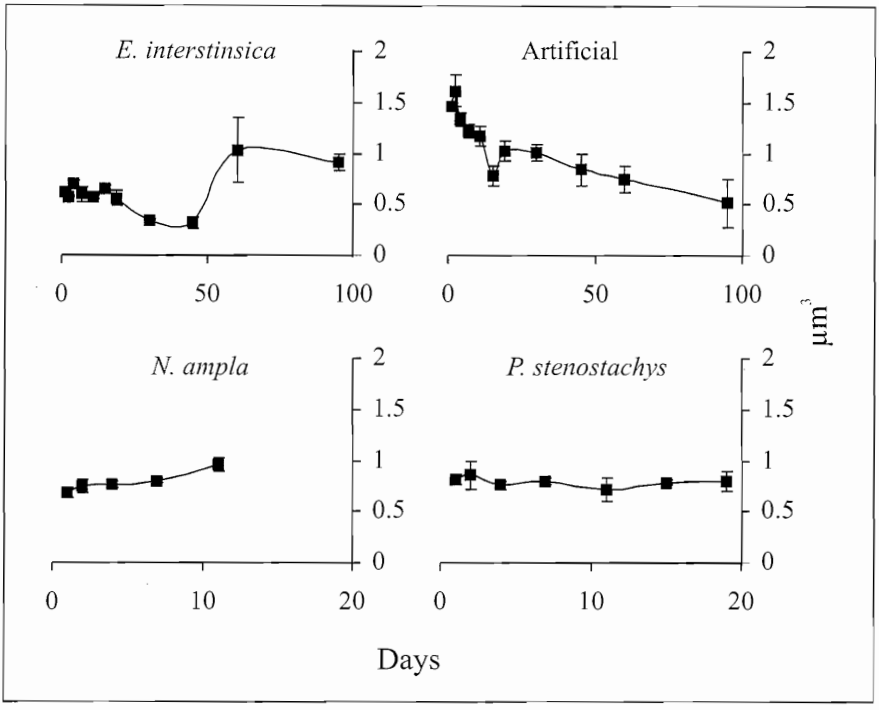

Figure 3: Average bacterial biovolume, in μm^3, in the detritus of three species of aquatic macrophytes and an artificial substrate at the Cabiúnas Lagoon. Bars indicate the standard deviation (n=3).

which is active in all substrates. This could be a methodological artifact, or possibly the result of the colonization of the environment, previously sterilized, by bacteria from the water column, with little or no competition for space, roughly equivalent to the exponential phase of a laboratory culture. This hypothesis would explain the presence of the maximum value on the seventh day even in the artificial substrate, dissociating it from intrinsic factors of the substrate. Another explanation would be the absence or scarcity of predators in the sterilized substrate, at the beginning of the colonization. We could detect the development of a significant biofilm even in the artificial substrate at the end of the experiment. However, several authors associated bacterial secondary productivity to the concentration of nutrients (e.g. Coveney & Wetzel, 1988; Thomaz & Esteves, 1997). These relationships, observed with laboratory experiments, were not confirmed in this field experiment.

Importance of the attached bacterial community

The results obtained in this research add to several previous articles focusing on the importance of the attached bacterial community in the nutrient cycling at

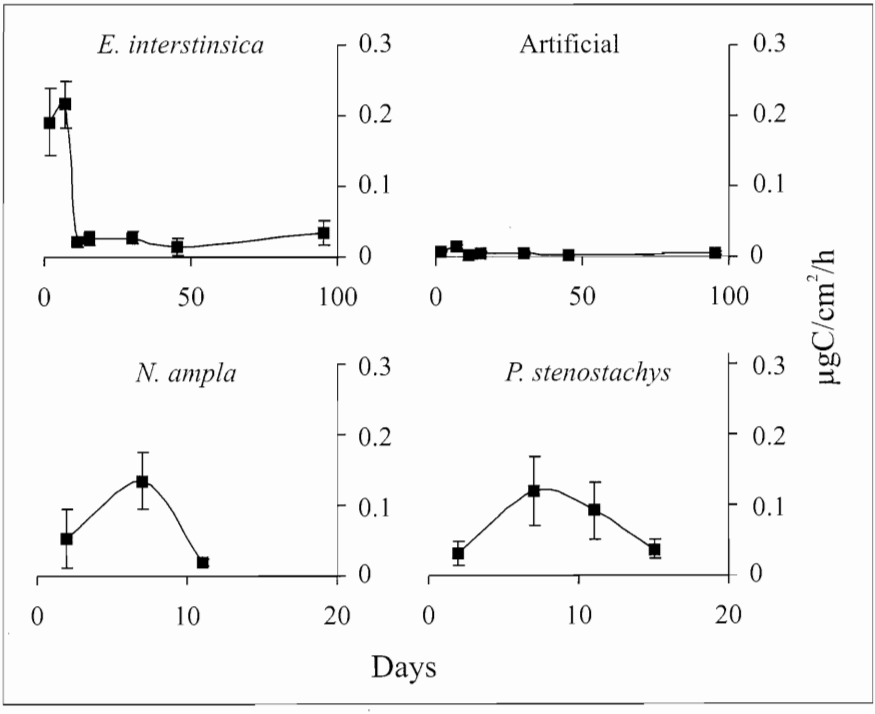

Figure 4: Bacterial secondary productivity, in µgC/cm²/h, in the detritus of three species of aquatic macrophytes and an artificial substrate at Cabiúnas Lagoon. Bars indicate the standard deviation (n=3).

aquatic environments. Thiel-Nielsen & Søndergaard (1999) observed that bacterial production in the biofilm of live plants may be 1,000 times greater (volume x volume) than bacterial production in the water column. Likewise, in a survey of the bacterial productivity in the water column, in the sediments and in the detritus of a freshwater marsh, Moran & Hodson (1992) observed that the total contribution of two bacterial environments (water column and detritus) for the total bacterial production of the environment is approximately the same. When we compare the results of this research with those obtained by Farjalla (1998) for the bacterioplankton of the same lagoon, it can be observed that the bacterial density in the detritus is about 10 times higher than in the water column (area x volume, cm^2 x cm^3). For a direct comparison between the two communities, it is necessary to transform the data of bacterial density of the bacterioplankton, multiplying it by the average depth of the lagoon, 2.37 m, that is, 2,370 cm (Panosso *et al.*, 1998). This direct comparison, however, underestimates the density of the attached bacterial community, since the data of the area of detritus in each square meter of the lagoon is not available. When the great density of the bacterial community is considered in the light of the observation of Cole *et al.* (1988) that the bacterioplankton respiration may reach

and, in some systems, overcome 50% of the phytoplanktonic production, the important role of the attached bacterial community in the nutrient cycling and metabolism of Cabiúnas lagoon is emphasized.

Conclusions

In spite of the unequal behavior of the bacterial community in each kind of substrate, it was not possible to associate the variation in the bacterial dynamics with the chemical composition of the substrate as it changed in the course of time. Possibly biotic interactions or variables that were not measured, related to the structural composition of the detritus (such as the concentrations of lignin and cellulose), have a more profound influence on the bacterial dynamics in these substrates. The bacterial biomass in the detritus of the studied species is approximately 10 times greater than the biomass found in the bacterial community of the water column. Bacterial productivity was relatively low, considering the high values of biomass and density reached. In any case, the increase of bacterial biomass in the detritus influenced the quality of this detritus, making it accessible to higher trophic levels, as the majority of detritivore macroinvertebrates is not able to metabolize the detritus of aquatic macrophytes and feeds mostly on the attached bacterial community.

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References

ALLEN, S.E.: H.M. GRIMSHAW; J.A. PARKINSON & C. QUARMBY 1974. *Chemical analysis of ecological materials*. Oxford, Blackwell Scientific Publications. 565 p.

BELOVA, M. 1993. Microbial decomposition of freshwater macrophytes in the littoral zone of lakes. *Hydrobiologia*, **251**: 59-64.

BENNER, R.; J. LAY; E. K'NEES & R.E. HODSON 1988. Carbon conversion efficiency for bacterial growth on lignocellulose: implications for detritus-based food webs. *Limnol. Oceanogr.*, **33**: 1514-1526.

BENNER, R.; M.A. MORAN & R.E. HODSON 1986. Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: relative contributions of procaryotes and eucaryotes. *Limnol. Oceanogr.*, **31**: 89-100.

BIANCHINI Jr., I. 1997. The degradation process of organic matter in reservoirs. pp. 6-27. In: ROSA, L.P. & M.A. dos SANTOS (eds.) *Hydropower Plants and*

Greenhouse Gas Emissions. Energy Planning Program, COPPE Report / Ed. Tecnológica, Rio de Janeiro.

BIANCHINI Jr., I. & A.P.P TOLEDO 1998. Estudo da mineralização de *Nymphoides indica* (L.) O. Kuntze. *Anais do VIII Sem. Reg. de Ecol.*, **VIII**: 1315-1329.

BIRD, D.F. & J. KALFF 1984. Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. *Can. J. Fish. Aquat. Sci.*, **41**: 1015-1023.

BJØRNSEN, P.K. 1986. Automatic determinations of bacterioplankton biomass by means of image analyses. *Appl. Environ. Microbiol.*, **51**: 1199–1204.

BLUM, L.K. & A.L. MILLS 1991. Microbial growth and activity during the initial stages of seagrass decomposition. *Mar. Ecol. Prog. Ser.*, **70**: 73-82

BOON, J.J.; R.G. WETZEL & G.L. GODSHALK 1982. Pyrolysis mass spectrometry of some *Scirpus* species and their decomposition products. *Limnol. Oceanogr.*, **27**: 839-848.

CARPENTER, S.R. 1980. Enrichment of Lake Wingra, Wisconsin, by submersed macrophyte decay. *Ecology*, **61**(5): 1145-1155.

COLE, J.J.; S. FINDLAY & M.L. PACE 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, **43**: 1-10.

COVENEY, M.F. & R.G. WETZEL 1988. Experimental evaluation of conversion factors for the [^{3}H] thymidine incorporation assay of bacterial secondary productivity. *Appl. Environ. Microbiol.*, **54**: 2018-2026.

EMERY, S.L & J.A. PERRY 1996. Decomposition rates and phosphorus concentrations of purple Loosestrife (*Lythrum salicaria*) and Cattail (*Typha* spp.) in fourteen Minnesota wetlands. *Hydrobiologia*, **323**: 129-138.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) 1994. *Manual de métodos empregados em estudos de microbiologia agrícola*. Brasília, 542 p.

ENRICH-PRAST, A. 1998. *Biometria e concentrações de fósforo, nitrogênio, carbono e teor energético da macrófita aquática* ***Oryza glumaepatula*** *Steud em um lago amazônico impactado por rejeito de bauxita (Lago Batata – PA)*. Dissertação de Mestrado, PPGE-UFRJ, Rio de Janeiro. 70 p.

ENRIQUEZ, S.; C.M. DUARTE & K. SAND-JENSEN 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. *Oecologia,* **94**: 457-471.

ESTEVES, F.A. 1998. *Fundamentos de Limnologia.* 2ª ed. Interciência/Finep. 602 p.

ESTEVES, F.A.; R. BARBIERI; I.H. ISHII & A.F. CAMARGO 1983. Estudos limnológicos em algumas lagoas costeiras do Estado do Rio de Janeiro. *Seminário Regional de Ecologia,* **3**: 25-38.

FARJALLA, V.F. 1998. Nutrientes limitantes ao crescimento do bacterioplâncton nas lagoas Carapebus, Comprida, Cabiúnas e Iodada. pp. 147-157. In: ESTEVES, F.A. (ed.) *Ecologia das lagoas costeiras do Parque Nacional da Restinga de Cabiúnas e do Município de Macaé (RJ).* NUPEM, Macaé.

FASSBENDER, H.W. 1973. Simultane P-Bestimmung im N-Kjeldahl-aufschlu von Bodenproben. *Die Phosphorsäure,* **30**: 44-53.

FERNANDES, V.O. 1998. Variação temporal e espacial na composição da comunidade perifítica na Lagoa Imboassica. pp. 221-236. In: ESTEVES, F.A. (ed.) *Ecologia das lagoas costeiras do Parque Nacional da Restinga de Cabiúnas e do Município de Macaé (RJ).* NUPEM, Macaé.

FINDLAY, S.; L. CARLOUGH; M.T. CROCKER; H.K. GILL; J.L. MEYER & P.J. SMITH 1986. Bacterial growth on macrophyte leachate and fate of bacterial production. *Limnol. Oceanogr.,* **31**: 1335-1341.

FRY, J.C. 1990. Direct Methods and Biomass Estimation. *Met. Microbiol.,* **22**: 41-85.

GODSHALK, G.L & R.G. WETZEL 1978. Decomposition of aquatic angiosperms. I. Dissolved components. II. Particulate components. III. *Zostera marina* L. and a conceptual model of decomposition. *Aquat. Bot.,* **5**: 281-354.

GOLDMAN, J.C.; D.A. CARON & M.R. DENNETT 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.,* **32**: 1239-1252.

GOLTERMAN, H.L.; R.S. CLYMO & M.A.M OHNSTAD 1978. *Methods for physical and chemical analysis of fresh water.* Blackwell, Oxford. 214 p.

HENRIQUES, R.P.B.; D.S.D. ARAÚJO; F.A. ESTEVES & A.C. FRANCO 1988. Análise preliminar das comunidades de macrófitas aquáticas da lagoa Cabiúnas, Rio de Janeiro, Brasil. *Acta Limnol. Brasil.,* **II**: 783-802.

HOBBIE, J.E.; R.J. DALEY & S. JASPER 1977. Use of nucleopore filters for counting bacteria by fluorescent microscopy. *Appl. Environ. Microbiol.*, **33**: 1225-1228.

HOWARD-WILLIAMS, C. & W.J. JUNK 1976. The decomposition of aquatic macrophytes in the floating meadows of a Central Amazonian várzea lake. *Biogeog.*, **7**: 115-123.

HUTCHINSON, G.E. 1975. *A treatise on limnology – limnological botany.* V.III. John Wiley & Sons. New York. 660 p.

KIRCHMAN, D.L.; S.Y. NEWELL & R.E. HODSON 1986. Incorporation versus biosynthesis of leucine: implications for measuring rates of protein synthesis and biomass production by bacteria in marine systems. *Mar. Ecol. Prog. Ser.*, **32**: 47-59.

KJERFVE, B. 1994. Coastal lagoon processes. pp. 1-8. In: KJERFVE, B. (ed.). *Coastal lagoon processes*. Elsevier Oceanography Series n. 60, Amsterdam.

KNOPPERS, B. 1994. Aquatic primary production. pp. 243-286. In: KJERFVE, B. (ed.). *Coastal lagoon processes*. Elsevier Oceanography Series n. 60, Amsterdam.

LETART, Y. & B. PINEL-ANNOUL 1991. Relationships between bacterioplankton production and limnological variables: necessity of bacterial size considerations. *Limnol. Oceanogr.*, **36**: 1208-1216.

MORAN, M.A. & R. HODSON 1989. Bacterial secondary production on vascular plant detritus: relationship to detritus composition and degradation rate. *Appl. Environ. Microbiol.*, **55**: 2178-2189.

MORAN, M.A. & R. HODSON 1992. Contributions of three subsystems of a freshwater marsh to total secondary productivity. *Microb. Ecol.*, **24**: 161-170.

PANOSSO, R.F.; J.L. ATTAYDE; D. MÜEHE & F.A. ESTEVES 1998. Morfometria de quatro lagoas costeiras fluminenses: implicações para seu funcionamento e manejo. pp. 91-108. In: ESTEVES, F.A. (ed.). *Ecologia das lagoas costeiras do Parque Nacional da Restinga de Cabiúnas e do Município de Macaé (RJ)*. NUPEM, Macaé.

PEDUZZI, P. & G.J. HERNDL 1991. Decomposition and significance of seagrass leaf litter (*Cymodosa nodosa*) for the microbial food web in coastal waters (Gulf of Trieste, Northern Adriatic Sea). *Mar. Ecol. Prog. Ser.*, **71**: 163-174.

PIECZYNSKA, E. 1993. Detritus and nutrient dynamics in the shore zone of lakes: a review. *Hydrobiologia*, **25**: 49-58.

PIEDADE, M.T.F.; W.J. JUNK & S.P. LONG 1991. The productivity of the C_4 *Echinocloa polystachya* on the Amazon Floodplain. *Ecology*, **72**(4): 1456-1463.

POLUNIN, N.V.C. 1984. The decomposition of emergent macrohytes in fresh water. pp. 115-166. In: MACFAYDEN, A. & E.D. FORD (eds.) *Advances in Ecological Research*. Vol. 14.

POMEROY, L.R. 1970. The strategy of mineral cycling. *An. R. Ecol. Syst.*, **1**: 71-90.

SIMON, M. 1985. Specific uptake rates of amino acids by attached and free-living bacteria in a mesotrophic lake. *Appl. Environ. Microbiol.*, **49**: 1254-1259.

SIMON, M. & F. AZAM 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* **51**: 201-213.

SWIFT, M.J.; D.W. HEAL & J.M. ANDERSON 1979. *Studies in Ecology – Decomposition in Terrestrial Ecosystems*. Blackwell, Oxford. 371 p.

THIEL-NIELSEN, J. & M. SØNDERGAARD 1999. Production of epiphytic bacteria and bacterioplankton in three shallow lakes. *Oikos*, **86**: 283-292.

THOMAZ, S.M. & F.A. ESTEVES 1997. Bacterial dynamics in periphyton from different regions of a tropical coastal lagoon. *Arch. Hydrobiol.*, **139**(4): 495-507.

TWILLEY, R.R.; G. EDJUNG; P. ROMARE & W. MICHAEL KEMP 1986. A comparative study of decomposition, oxygen consumption and nutrient release for selected aquatic plants occurring in an estuarine environment. *Oikos*, **47**: 190-198.

VALIELA, I.; J.M. TEAL; S.D. ALLEN; R.V. ETTEN; D. GOEHRINGER & S. VOLKMANN 1985. Decomposition in salt marsh ecosystem: the phases and major factors affecting disappearance of above-ground organic matter. *J. Exp. Mar. Biol. Ecol.*, **89**: 29-54.

WETZEL, R.G. 1990. Land-water interfaces: Metabolic and limnological regulators. *Verh. Internat. Verein. Limnol.*, **24**: 6-24.

WETZEL, R.G. 1993. *Limnologia*. Fundação Calouste Gulbenkian, Lisboa, 919 p.

WETZEL, R.G. & M. SØNDERGAARD 1997. Role of submerged macrophytes for the microbial community and dynamics of dissolved organic carbon in aquatic ecosystems. pp. 133-148. In: JEPPESEN, E.; M. SØNDERGAARD; M. SØNDERGAARD & K. CHRISTOFFERSEN (eds.) *The structuring role of submerged macrophytes in lakes*. Kluwer Academic Press.

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