

## ***OECOLOGIA BRASILIENSIS***

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# **ECOLOGY OF AQUATIC MICROORGANISMS IN SOUTHERN BRAZIL: STATE OF ART**

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## **Resumo**

Neste trabalho apresentamos uma análise dos resultados de pesquisas passadas e em desenvolvimento sobre a ecologia de microorganismos aquáticos realizadas no Laboratório de Ecologia do Fitoplâncton e de Microorganismos Marinhos do Departamento de Oceanografia - FURG desde 1985. Os estudos foram conduzidos na Lagoa dos Patos (10.360 Km<sup>2</sup>) e seu estuário, e nas regiões de praia e costeira adjacentes. São fornecidas informações sobre a abundância e biomassa dos microorganismos, bem como suas taxas de produção, respiração e decomposição. Conclui-se que os microorganismos representam um componente importante da teia alimentar aquática destes ambientes, seja por sua elevada biomassa, o que representa uma importante fonte alimentar, e por seus elevados níveis de atividade metabólica.

Palavras-chave: bactéria, ecologia microbiana aquática, Sul do Brasil.

## **Abstract**

In this work we accomplish an analysis of the past and ongoing research on aquatic microbial ecology carried out by the Laboratório de Ecologia do Fitoplâncton e de Microorganismos Marinhos, Departamento de Oceanografia - FURG since 1985. The studies were conducted in the Patos Lagoon (10,360 Km<sup>2</sup>), its estuarine sector and adjacent beach and coastal region. We present information about the abundance and biomass of microorganisms, as well as their production respiration and decomposition rates. It is concluded that microorganisms represent an important component of the microbial food web of these environments due to their high biomass, representing an important food source, as well as by their high metabolic activity.

Key-words: bacteria, aquatic microbial ecology, Southern Brazil.

## Introduction

The study of aquatic microbial ecology has grown exponentially in the last 25 years mainly due to new concepts which have pointed out the importance of microorganisms in the aquatic food webs (Pomeroy, 1974; Azam *et al.*, 1983; Hobbie, 1993), as well as the progress of methods and technologies which have allowed for better and more precise quantification of microbial numbers and processes (Hobbie *et al.*, 1977; Fuhrman & Azam, 1982)

In Brazil, there is a paucity of studies on aquatic microorganisms, mainly due to the high costs of new equipment (e.g. epifluorescence microscope) and the lack of theoretical and practical training of aquatic ecologists in this field. However, this scenario is changing rapidly, since many laboratories have been better equipped in recent years and new aquatic microbial ecology concepts have been incorporated in the *curricula* of Graduate courses.

The research on aquatic microbial ecology at Fundação Universidade Federal do Rio Grande, Southern Brazil, began in the middle 80's. Throughout these 15 years, studies have been carried out in the Patos Lagoon, the largest South American coastal lagoon (10,360 Km<sup>2</sup>), the adjacent beach and coastal zones and at the Southwestern Atlantic Ocean, in a region under the influence of Brazil and Malvinas (Falklands) currents (Subtropical Convergence) and the freshwater outflow from the La Plata River and the Patos Lagoon.

In this work, we will accomplish a review of the past and ongoing studies in these regions, providing a general characterization of the microbial food webs components and processes. Besides the abundance and biomass of microorganisms, we will present results on the production, respiration, decomposition and mineralization rates as well as on the biotic and abiotic controlling factors. Specific information on the coastal and oceanic flagellates of Southern Brazil can be found elsewhere in this volume (chapter 12). More details about the environmental characteristics and the biota of the study area are described in the book: Subtropical Convergence Environments - The Coast and Sea in the Southwestern Atlantic (Seeliger *et al.*, 1997).

## Abundance and Biomass

Bacterioplankton is an important component of the aquatic food webs. The contribution of bacteria to the total biomass is highly significant in aquatic ecosystems. Even so, ecological factors which regulate their abundance and biomass are not clearly defined. Among them are temperature, organic and inorganic nutrient supply, grazing pressure exerted by flagellates and/or ciliates, and mortality caused by virus (Pace & Cole, 1996).

Bacterial abundance and biomass were evaluated monthly for one year (December 1987 - December 1988) at 18 fixed stations along the 360 Km main longitudinal axis of the Patos Lagoon. Total (attached and free) bacteria varied

between  $0.68 - 6.27 \cdot 10^6$  cells  $\text{ml}^{-1}$ . Maximum values were measured during austral winter at the northern sector, close to the main freshwater input and to Porto Alegre, the largest city of the state. Roughly, bacterial biomass ( $291-585 \mu\text{g C l}^{-1}$ ) represented 15 to 86 % of total Particulate Organic Carbon ( $467-2432 \mu\text{g C l}^{-1}$ ). Salinity had no effect on the bacterial distribution in the Patos Lagoon. Reviews considering bacterial abundance and activity in limnic and coastal systems also found no influence of salt content on the bacterial distribution on large space and time scales (Hobbie, 1988; Cole *et al.*, 1988). However, short-term studies demonstrated a decrease of bacterial number in high salinity waters (Wright & Coffin, 1983; Painchaud *et al.*, 1995). Abreu *et al.* (1995a), working on an estuarine plume in the coastal region close to the Patos Lagoon mouth, found a decrease in bacterial number and activity (measured with  $^3\text{H}$ -Thymidine) at the sharp interface of estuarine and coastal water, probably due to salt stress on the bacterial community. It is likely that at larger space and time scales, such salt effect is not observed, since the bacterial community is able to rapidly adjust to new environmental conditions.

In this large-scale study of the Patos Lagoon there was no significant relationship between bacteria and chlorophyll *a*, while a stronger correlation was found between bacterial abundance and Phosphate (Abreu & Odebrecht, 1995). In coastal ecosystems, allochthonous material from river runoff and the input of land-based organic matter may provide additional substrates for bacteria, while bacteria in the open sea have been suggested to be mainly dependent on phytoplankton production (Tuomi *et al.*, 1999). The lack of association between bacteria and phytoplankton in this study suggests that, in the Patos Lagoon, bacterial growth is probably sustained by carbon sources other than phytoplankton, probably originated from macrophyte decomposition and/or domestic and industrial sewage. Moreover, high association between bacteria and inorganic nutrient (P) indicates that dissolved organic Carbon is not a limiting factor for bacteria, while Phosphate probably controls their dynamics (Abreu *et al.*, 1992, Abreu & Odebrecht, 1995).

A field study on the microbial food web was conducted in a shallow region of the Patos Lagoon estuary. Samples were collected weekly for one year (March 1989 - March 1990) (Abreu, 1992; Abreu *et al.*, 1992). Free bacterial abundance varied between  $0.09 - 3.59 \cdot 10^6$  cells  $\text{ml}^{-1}$ , while that of the attached form varied from  $0.04$  to  $0.50 \cdot 10^6$  cells  $\text{ml}^{-1}$ . Though present in a smaller number, attached bacteria made up the largest biomass ( $54.95 - 489.09 \mu\text{g C l}^{-1}$ ), and total bacteria biomass exceeded that of phytoplankton ( $80.50 - 390.50 \mu\text{g C l}^{-1}$ ) for several months of the year (Fig. 1). Again, such observations suggest that bacteria complement their organic carbon requirements using other carbon sources, similarly to what has been observed in the large-scale study. The fact that the greatest increase of attached bacterial biomass has occurred during the coldest period, when senescence of macrophytes and macroalgae in this estuary reaches its maximum (Seeliger *et al.*, 1997), indicates that their abundance is probably related to benthic primary producers.

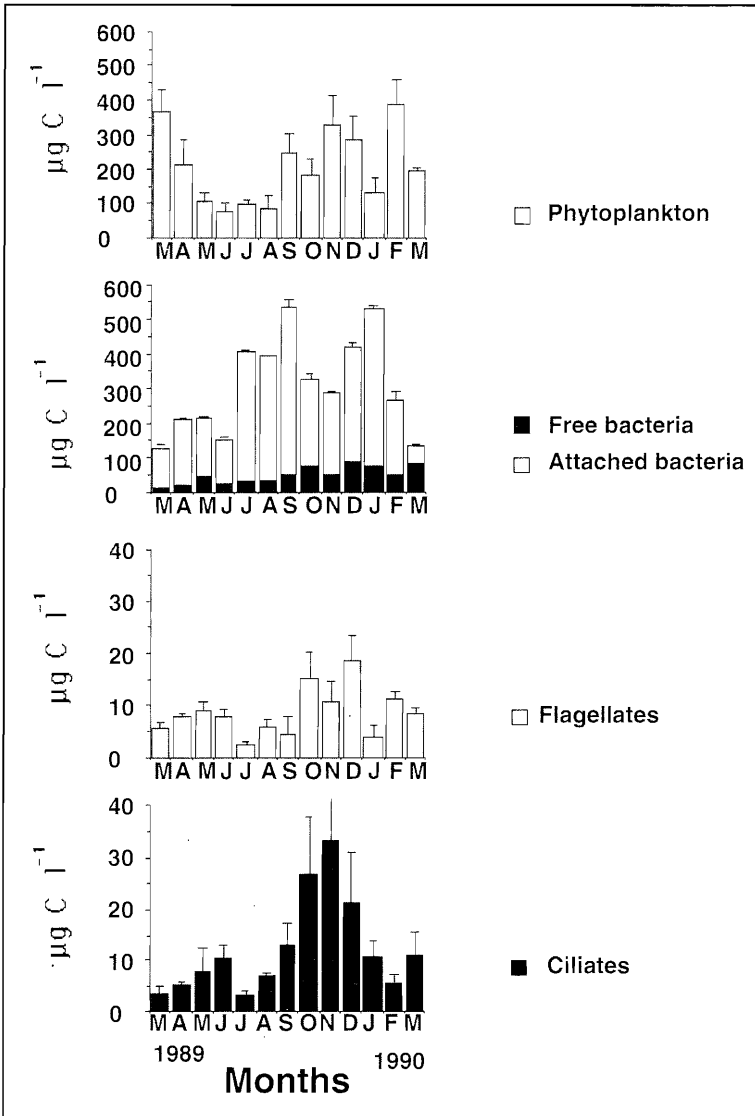


Figure 1. Annual cycle of phytoplankton, bacteria free and attached, heterotrophic flagellates, and ciliates biomass ( $\mu\text{g C liter}^{-1}$ ) (Adapted from Abreu *et al.*, 1992).

Among the physical and chemical parameters analyzed, only Nitrate+Nitrite showed a significant positive correlation with bacterial abundance. This could be the result of a covariance between these parameters and another variable, for in-

stance, salinity, although it could also be interpreted as a direct absorption of Nitrate by bacteria. In this case, competition between bacteria and phytoplankton for nitrogenous nutrients would probably happen during periods of high bacterial biomass. Attached bacteria abundance was poorly correlated with the amount of seston. In addition, forced resuspension of sediments within enclosures connected to the lagoon bed, showed that this was an unlikely mechanism contributing to the increase of attached bacteria abundance in the water column. However, the similarity between the attached bacterial number and the POC/seston ratios, suggests that attached bacteria are not influenced by the seston load nor by resuspension of sediments, but by the quality of particles (Abreu *et al.*, 1992).

It seems that free and attached bacteria respond differently to the activity of the phytoplankton cells. While the free form responds directly to phytoplankton activity, the abundance of attached bacteria was almost constant (Abreu, 1992; Abreu *et al.*, 1992). Preferential grazing on attached bacteria could be a potential explanation for the low number of organisms found bound to particles. Moreover, it was observed that, during the annual cycle, highest abundance of free and attached bacteria were composed of cells with reduced cell volumes. This situation was probably generated by preferential grazing on the larger bacterial cells. Abreu *et al.* (1992) speculated that the reduction in size of the bacteria, concurrent with their increase in number, would represent a response of these organisms to protozoan grazing. In this case, small bacteria, not controlled by grazing, would probably become substrate limited and may represent a pool of organic matter not effectively utilized by higher trophic levels. On the other hand, large bacteria, which are controlled by grazing, will function as an effective link in the carbon flow through the microbial food web of Patos Lagoon estuary.

Possible trophic relationship in the estuarine microbial food web is characterized by similar trends of heterotrophic flagellate and ciliate biomass during the annual cycle and their inverted relationship with attached bacterial biomass for the same period (Abreu *et al.*, 1992). However, heterotrophic flagellate biomass and production rates are not high enough to sustain ciliate biomass and growth. During the annual cycle ciliate biomass was usually higher ( $3.37 - 33.22 \mu\text{g C l}^{-1}$ ) than that of flagellates ( $2.30 - 18.67 \mu\text{g C l}^{-1}$ ), especially during October-December. Similarly, production rates determined during an enclosure experiment showed that flagellate production is much smaller than ciliates. Such discrepancies indicate that ciliates in the Patos Lagoon estuary must exert a grazing pressure on bacteria and/or small autotrophic organisms ( $< 5 \mu\text{m}$ ) in order to supply their carbon demands, characterizing a bypass in the transfer of organic matter through the microbial food web, as suggested by Sherr & Sherr (1988).

The Cassino Beach, near Rio Grande city, is characterized by the frequent occurrence of huge amounts of the diatom *Asterionellopsis glacialis*, concentrated at the beach due to the action of southerly winds (Odebrecht *et al.*, 1995). In a weekly sampling program accomplished at the Cassino Beach between November 1992 and January 1994, bacterioplankton abundance varied between  $0.03$  and  $0.5 \times 10^6$  cells  $\text{l}^{-1}$ , with highest values measured during austral

summer (Abreu *et al.*, 1996; Odebrecht & Abreu, 1997). Samples collected daily after the occurrence of *Asterionellopsis glacialis* blooms showed an increase in bacterial abundance only after 4 to 5 days. Our first hypothesis was that bacterial growth was probably inhibited by an antibiotic substance produced by the algae. However, current studies demonstrated that the quality of dissolved organic Carbon (DOC) exuded by *A. glacialis*, influence the growth and bacterial succession. Actually, experiments show that the production of highly labile DOC by the algae is rapidly absorbed by bacteria which reach a high number in few hours. However, after the decline of bacterial abundance, it remains low for longer periods. On the other hand, when *A. glacialis* do not produce much DOC, the increase in bacterial number is slow but constant, reaching higher number than in the previous situation. Our hypothesis now is that the production of DOC by the algae allows for the increase of fast growing bacteria (*r* type), that take up from the environment not only carbon, but also other inorganic nutrients (N and P). In this case, the slow growing bacteria (*k* type) would not be able to grow and reach a high number afterwards (Reynaldi, *in prep.*).

Between October 1987 and September 1988 the surface water of the southern Brazilian coast was studied in a series of expeditions covering the continental shelf and slope (31° 30' - 34° 30' S). A total of 53 oceanographic stations were sampled in February and July 1987, and September 1988. The number of free bacteria (length 0.5-1.0 µm; width 0.3-0.5 µm) oscillated between 0 and 1.35 10<sup>5</sup> cells l<sup>-1</sup>, whilst the number of attached bacteria (length 1.0-2.0 µm; width 0.3-0.5 µm) varied between 0 to 5.25 10<sup>5</sup> cells l<sup>-1</sup>. Free and attached bacteria were more abundant during spring, and distribution patterns generally follow those of phytoplankton biomass (Chlorophyll *a*) (Ciotti *et al.*, 1995), being highest in Coastal Water and during the influence of Subantarctic Water. Moreover, total bacteria and phytoplankton biomass were of similar magnitude (Abreu, 1997)

Plankton studies were conducted between 30 and 62° S in the Southwestern Atlantic during November 1993, 1994 and 1995 and March and November 1996. The hydrology, nutrient concentration, bacteria, phytoplankton and zooplankton abundance was determined in the upper layer (200 m). The region studied was dominated by a rather stable oceanographic structure, with four hydrological domains: subtropical, mixing zone, subantarctic and Antarctic. A permanent meander of the Brazil-Malvinas confluence was observed around 38° S, with an upwelling of deep, nutrient-rich water. Two zones of high phytoplankton production were identified. The first one was located in the upper euphotic zone of Brazil-Malvinas confluence (36-50° S). High Chlorophyll *a* concentration observed in this region seems to be the result of water column stabilization due to the interplay of both water masses (Brandini *et al.*, 2000). The second phytoplankton maximum zone occurs in the subsurface layer of Antarctic water, near the Polar Front. Bacterial number was relatively low (mean 0.3 10<sup>6</sup> ml<sup>-1</sup>) and highest bacterial biomass (0 - 453 µg C l<sup>-1</sup>) occurred close to the northern and southern borders of the Subtropical Convergence (Abreu *et al.*, 1995b). Though surface bacteria abundance did not

match phytoplankton biomass variation, highest values of these microorganisms normally occurred in the same Chlorophyll *a* maximum regions. Negative relationship with flagellate abundance indicates that bacteria are probably under grazing pressure. In this study bacterial abundance showed a positive relationship with water temperature, and negative with Nitrate and Phosphate. Felip *et al.*, (1996) proposed that, in general, bacterial growth rates are temperature dependent up to a threshold, but that the patterns of change across temperature gradients are resource dependent, so that temperature has little effect on growth in resource-rich (eutrophic) environments but a strong effect in oligotrophic ecosystems.

### **Metabolic processes: Production, Respiration and Decomposition**

The organisms responsible for most organic carbon uptake and respiration in aquatic systems are microbes, especially heterotrophic bacteria (Azam *et al.*, 1983). Measurements of bacterial respiration in association with production can be used to estimate bacterial metabolism and calculate bacterial growth efficiency (Søndergaard & Theil-Nielsen, 1997). However, there are many methodological uncertainties and limitations, which hamper the generalization of the fate of organic carbon in aquatic systems. Principal sources of errors are the use of different methods, conversion factors and the difficulty in separating bacterial respiration from that of other planktonic organisms (Cole & Pace, 1995; Jahnke & Craven, 1995). Despite these uncertainties, Cole & Pace (1995) emphasize that much can be learned with current techniques, and that it is necessary to continue looking forward to less ambiguous methods for measuring both bacterial production and respiration.

Bacterial production rates in the Patos Lagoon estuary were estimated by the frequency of dividing cells (FDC) (Abreu *et al.*, 1992), by changes in cell numbers in microcosms (Abreu *et al.*, 1992; Cesar, 1997) and by the use of <sup>3</sup>H-Thymidine method (Abreu *et al.*, 1995a), showing similar results and trends. Matching variability of FDC (0 - 74%) and phytoplankton dissolved production (0.97 - 89.25  $\mu\text{g C l}^{-1} \text{h}^{-1}$ ) in the Patos Lagoon estuary suggests that bacterial growth is largely stimulated by phytoplankton-released products. However, the important point to know is how much of this primary production is channeled through bacteria to higher trophic levels. In this way, enclosure experiments have shown that bacteria may consume as much as 26% of phytoplankton primary production (Abreu *et al.*, 1992). Similarly, a study conducted along the estuarine plume using <sup>3</sup>H-Thymidine to measure bacterial production, has shown that, in clear coastal waters, bacterial production (0.11 - 6.55  $\text{mg C m}^{-3} \text{h}^{-1}$ ) represented 32% of phytoplankton primary production, while, in estuarine waters, phytoplankton production (<sup>14</sup>C method - 2.91 to 79.02  $\text{mg C m}^{-3} \text{h}^{-1}$ ) highly overcame the bacterial necessities for growth (10 times more). Salinity, Phosphate and Ammonium concentration, as well as predation pressure exerted by protozooplankton seem to be the main controlling factors of bacterial production (Abreu *et al.* 1995a; Cesar, 1997).

Measurements of community respiration conducted with the oxygen method showed that, throughout most of the year, phytoplankton production in the water column is large enough to supply the demand of heterotrophic organisms. Only during the winter (June-August) the estuary presents a dominance of heterotrophic conditions, with higher respiration than production. During this period, heterotrophic organisms probably survive from the benthic production or decomposition of particulate organic matter (Abreu, 1992).

Aquatic macrophytes *Scirpus maritimus*, *Spartina alterniflora* and *Spartina densiflora* are widely distributed throughout the Patos Lagoon estuary and represent the main primary producers of this ecosystem (Seeliger *et al.*, 1997). Though few herbivores may consume these plants directly, most of the produced organic matter flows into the estuarine food web in the form of detritus. Microorganisms play an important role in this process, but there is little information whether microbes respire most of the plant detritus, or incorporate this carbon into new biomass transferring matter and energy to higher trophic levels through the microbial food web.

Some experiments were conducted in the Patos Lagoon estuary in order to answer this question. Changes in the growth (net biomass carbon increase) and respiration (oxygen uptake) of free and attached bacteria were monitored during the decomposition of *S. maritimus* seagrass leaves in laboratory microcosms for a 30 day-period. The beginning of the experiment was characterized by higher bacterial production, respiration and growth efficiency compared to the later period. Approximately 10% of initial plant dry weight was released into the water as dissolved organic Carbon (DOC). Most of the incorporated plant carbon was respired during the first 4 days by free bacteria (66% of total) and a minor part by attached bacteria. Peaks of free and attached bacterial biomass alternated during experiments, indicating a tight coupling between these two communities. Low values of remaining bacterial biomass (ca. 1% plant DOC) suggested that these organisms contribute less to the microbial food web, although they play an important role mineralizing the *S. maritimus* detritus, giving back inorganic nutrients to the estuarine primary producers (Anésio *et al.*, 1995; Brepöhl *et al.*, 1996). Moreover, the presence of attached microorganisms on the detritus probably enhance the quality of the particles since microbes have more N and P per volume and also contain essential nutrients like polyunsaturated fatty acids, sterols and amino acids, essential to the growth of many organisms (Fagerbakke *et al.*, 1996; Stoecker & Capuzzo, 1990).

In this same study (Anésio *et al.*, 1995), an alternation was observed between the maximum of free and attached bacteria throughout the experiment. Our hypothesis is that attached bacteria act directly in the decomposition of the plant, liberating dissolved organic matter that would benefit the development of the free bacteria. In this sense, free bacteria would also participate in the mineralization process by respiring most of the dissolved organic matter. Also in this study, differ-



ences in the predator-prey relationship were observed, i.e., free bacteria are likely to be consumed by flagellates, while attached bacteria are grazed up by ciliates (Brepöhl *et al.*, 1996)

The decomposition rates of organic matter and nitrogen of *Scirpus maritimus*, *Spartina alterniflora* and *Spartina densiflora* were determined in field experiments using litter bags (Hickenbick, 1999). Largest organic matter decline was observed for the *Scirpus maritimus* and *Spartina alterniflora*. After 180 days of exposition, more than 52% of plant initial dry weight had disappeared from the bags, while litter bags containing *Spartina densiflora* still had ca. 60% of the original plant biomass. *Scirpus maritimus* and *Spartina alterniflora* showed very similar decomposition curves, and both were different from *Spartina densiflora*. The reason for this difference is related to the similar morphological characteristics of *Scirpus maritimus* and *Spartina alterniflora* leaves, which are more turgid and present greater surface exposed to microbial attack, while leaves of *S. densiflora* have smaller surfaces (they are coiled) and more lignin (Perazzolo & Pinheiro, 1991). Thus, macrophyte decomposition is more related to morphological characteristics than phylogenetic similarities (Hickenbick, 1999; Abreu & Odebrecht, *in press*).

Nitrogen content in the plants was quite variable, even showing an increase in the first days of experiment. The increase in Nitrogen content could not be related to the attachment of microorganisms, since bacterial number ( $2.85 \cdot 10^7$  -  $3.63 \cdot 10^8$  cells g detritus<sup>-1</sup>) increased toward the end of the experiment probably due to the larger surface/volume rates of small particles. Moreover, it was observed that attached bacteria present in the *S. densiflora* detritus were bigger than those present on the other two species. This was probably related to the shape (coiled) of *S. densiflora* leaves that better protect bacteria from attacks by their predators attack (Hickenbick, 1999).

Recent ongoing research is analyzing the influence of ultraviolet-B (UV-B) radiation on nitrogen remineralization during the microbial decomposition of *Spartina alterniflora* in the Patos Lagoon estuary. Plant decomposition, microbial abundance and Ammonium concentration were measured in outdoor containers having macrophyte leaves and filtered (0.2  $\mu\text{m}$ ) estuarine water. Some containers were covered with light filters avoiding the UV-B penetration (Mylar). Others were covered with the Aclar filter, which allows the passage of this radiation, in order to test the artifact effect. The remaining containers had no cover. Natural estuarine water was used as inoculum. Preliminary results indicate that natural levels of UV-B have already been influencing negatively the Nitrogen remineralization in the Patos Lagoon estuary. Tanks covered with Mylar filter (no UV-B penetration) showed higher bacterial number and Ammonium concentration. It seems that UV-B affects the bacteria responsible for the transformation of proteins and aminoacids into Ammonium (Daniel *et al.*, 1999) (Fig. 2).

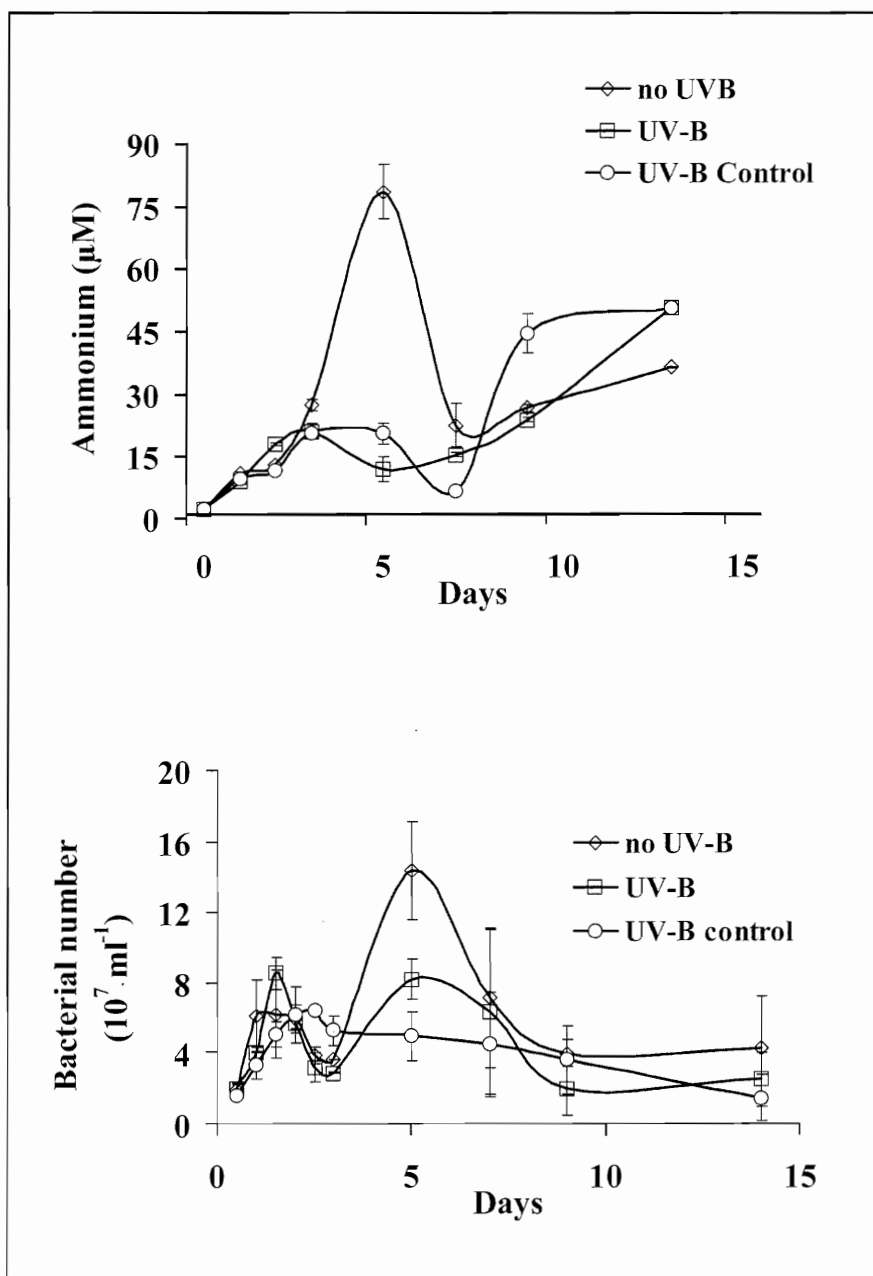


Figure 2. Ammonium concentration ( $\mu\text{M}$ ) and bacterial number ( $10^7 \text{ ml}^{-1}$ ) in UV experiment.

### “Top-Down” vs. “Bottom-Up” Control

The levels of nutrient availability (“bottom-up”) and predation pressure (“top-down”) define the dominance of ecological processes inside of the microbial food web. These two factors affect the diversity, size and abundance of bacteria, influencing the niche delimitation and the competition between them and phytoplankton (Chrzanowski & Simek, 1990; Psenner & Somaruga, 1992). It is well-known that morphology and size of bacteria can change in short time scales depending on the nutrient supply or grazing pressure. Availability of nutrients favors the occurrence of larger bacteria, while the predation pressure selects the smaller size (Jürgens & Güde, 1994). Modification of shape and size is one of the existing adaptation mechanisms of these microorganisms. Larger number of filamentous and attached bacteria have been observed in more eutrophic environments, while small bacteria dominate in oligotrophic regions. This suggests that larger forms are inferior competitors under conditions substrate limitation (Jürgens, 1994).

Studies on the influence of organic and inorganic nutrients on the bacterial dynamics of the Patos Lagoon estuary have demonstrated that carbon is important for bacterial growth; however, it is not a limiting factor (Cesar & Abreu, 1995). Inorganic nutrients (N and P), on the other hand, are likely to control their activity (Cesar *et al.*, 1996). The influence of inorganic nutrients (N and P) concentration on the bacterial dynamics were evaluated along a salinity and nutrient gradient in the estuarine region. Highest bacterial abundance ( $3.4 \times 10^6$  cells  $\times$  ml<sup>-1</sup>) and growth was observed at the station with highest N: P ratio (150:1), with low nutrient concentration. Enrichment experiments with different N:P ratios showed highest bacterial growth at low (5:1) and higher (22:1 and 70:1) N:P ratios, supporting the hypothesis that bacterial growth in the Patos Lagoon estuary depends not only on the amount of inorganic nutrients (N and P), but especially on their proportions in the water column (Cesar, 1997; Cesar & Abreu, 1998) (Fig. 3). Moreover, at the end of enrichment experiment bacteria with different morphological characteristics were observed. It seems that different nutrient conditions allow for the growth of distinct bacterial assemblages, most adapted to the amount and proportions of N and P (Cesar, 1997).

As described before, the predation pressure on the bacteria in the Patos Lagoon favors the occurrence of smaller cells. It seems that the reduction in size is a successful strategy to escape predators (Abreu *et al.*, 1992). Moreover, the results point out to different prey size preferences by flagellates and ciliates, i.e., flagellates consume small free bacteria, while ciliates ingest larger attached bacteria (Abreu *et al.*, 1992; Brepöhl *et al.*, 1996). Studies conducted with estuarine water with different levels of nutrients and predators show that bacteria and predator size distribution change throughout time. It was observed that the reduction in bacterial size may be followed by the decrease of flagellate dimensions, indicating that prey and predator populations suffer continuous adaptations in order to maximize the

matter and energy transfer along the microbial food web (Reynaldi *et al.*, 1999). Finally, it must be considered that the trophic interactions between protozooplankton and bacteria have great importance to the nutrient cycling of the Patos Lagoon estuary. Since smaller bacteria are not efficiently grazed either by flagellates or by ciliates, and most of them are in a dormant state, it is possible that most of nutrient cycling is conducted by protozooplankton, which efficiently consume bacteria and excrete nutrients back to the water (Cesar & Abreu, 1998).

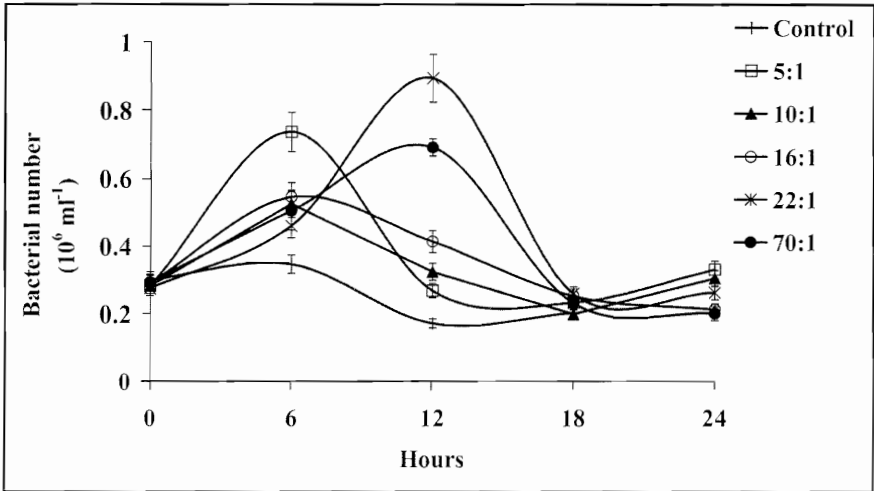


Figure 3. Bacterial number ( $10^6 \text{ ml}^{-1}$ ) with different N:P ratios (Adapted from Cesar, 1997).

### Technological Applicability and Future Perspectives

New concepts related to the ecology of aquatic microorganisms were applied to the aquaculture of the shrimp *Farfantepenaeus paulensis* in a joint project with the Aquaculture Laboratory from the Fundação Universidade Federal do Rio Grande. We studied the potential of microorganisms as food source, and their role in the control of water quality (Abreu *et al.*, 1998; Thompson *et al.*, 1998; Thompson *et al.*, 1999).

Microorganisms have probably been playing an important role in rearing systems, nourishing cultivated organisms. The increase of bacterial in tanks may sustain higher growth of protozooplankton or benthic meiofauna which, in turn, increment the growth of hearing fish, crabs or mollusks. The addition of live food is also a potential source of microorganisms. In our studies, we observed that phytoplankton added to tanks with *F. paulensis* larvae brings together large number of bacteria and flagellates. Similarly, the supply of *Artemia* nauplii works as a vector, introducing huge number of bacteria to the tanks (Thompson *et al.*, 1998).

Although bacteria represent an important food source, their small size (0.5-1.5  $\mu\text{m}$  length) represents a problem to their consumption by cultivated organisms. The results of our study showed that the *F. paulensis* larvae grew better when they consumed flagellates and ciliates. All larvae that received supplementary food in the form of flagellates and ciliates showed larger cephalothorax length than those in the control treatments. (Thompson *et al.*, 1999).

Microorganisms are also key components in the control of water quality. Their presence influence the dissolved oxygen and nutrients levels, as well as pH. In a set of four experiments, we tested the usefulness of biofilm, a consortium of microbial communities associated to a matrix of extracellular polymeric substances attached to a submersed surface, in reducing the levels of Ammonium and Phosphate in rearing systems with shrimp juveniles of *Farfantepenaeus paulensis*. Tanks with biofilm always had low levels of Ammonium and Phosphate. Pennate diatoms and cyanobacteria present in biofilm were responsible for taking up the largest amount of Ammonium from the water, even under intensive culture conditions (1,000 g shrimp/g/m<sup>3</sup>), though nitrifying bacteria also play an important role. Moreover, the presence of biofilm guaranteed smaller exportation of Phosphorus (33% less) and Ammonium (25% less) and larger output of Nitrate+Nitrite, contributing to reducing the eutrophication of the water body which receives effluents from the rearing system (Abreu *et al.*, 1998; Thompson, 1999).

The major problem we have faced in our studies is the proper identification of bacterial species. No ecological study is complete if we do not know the species involved. Current methods of identification consider the morphological characteristics of the cells or colonies and are complemented by physiological and biochemical tests (Schlegel, 1995). This approach is time-consuming and not precise. Moreover, most bacteria present in the water are in dormant state, and do not grow in most media used, thus it is impossible to grow isolated cells in order to perform and identification. Thus, cultivation techniques are inadequate to describe the species and assemblages of *in situ* bacteria (Höfle & Brettar, 1995; Pollard, 1998).

The use of molecular biology techniques seems to be the most appropriate way to identify aquatic bacteria, flagellates and ciliates. Some studies have shown new aspects of aquatic microbial ecology (Lee & Fuhrman, 1990; 1991). Moreover, molecular biology methods are becoming simpler and less expensive. It seems that we are approaching a new turning point similar to that when the epifluorescence microscope became more popular and accessible. We now intend to implement some molecular biology techniques in our laboratory. Likewise, we will continue the studies on the microbial food web of the Patos Lagoon estuary and adjacent coastal water, especially the influence of dissolved inorganic nutrients on the bacterial dynamics, as well as the effect of UV radiation on macrophyte microbial decomposition.

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