

## **OECOLOGIA BRASILIENSIS**

Roland, F. & Vidal, L.O. 2001. Heterotrophic bacterial respiration: a relevant measurement for the understanding of plankton metabolism. pp. 97-116. In: Faria, B.M.; Farjalla, V.F. & Esteves, F.A. (eds). *Aquatic Microbial Ecology in Brazil*. Series Oecologia Brasiliensis, vol. IX. PPGE-UFRJ. Rio de Janeiro, Brazil.

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### **HETEROTROPHIC BACTERIAL RESPIRATION: A RELEVANT MEASUREMENT FOR THE UNDERSTANDING OF PLANKTON METABOLISM**

ROLAND, F. & VIDAL, L.O.

#### **Resumo**

Há muito tempo, a importância funcional de microorganismos autotróficos e heterotróficos no metabolismo de plâncton e em cadeias tróficas aquáticas tem sido o foco de muitos trabalhos em ecologia aquática. Bactérias planctônicas foram recentemente reconhecidas como um componente abundante e importante da biota aquática. Bactérias utilizam grande parte do carbono ciclado em ecossistemas aquáticos. Uma parte significativa do carbono produzido por bactérias é transferida a níveis tróficos superiores; assim, a estrutura da comunidade bacteriana é determinada por controle "top-down". A real ligação entre a energia, como o carbono orgânico dissolvido, entre os níveis tróficos mais baixos e os mais elevados é influenciada pela magnitude dos processos respiratórios. Na verdade, a intensidade das taxas respiratórias é uma expressão da perda de carbono no sistema. Em nosso ponto de vista, a lacuna ecológica é: *Por que e como as taxas bacterianas de respiração são, comparativamente, tão altas em termos do equilíbrio energético no nível do plâncton*. Todos os esforços de estimativas da respiração bacteriana devem levar em consideração este aspecto teórico. O objetivo deste capítulo é enfatizar a importância dos dados de respiração bacteriana e sugerir alguns enfoques produtivos para a ecologia aquática microbiana. Aspectos metodológicos também são discutidos; além disto, mostraremos alguns dados preliminares de respiração bacteriana em um lago de planície de inundação amazônico. Nossos dados sugerem que os ecossistemas aquáticos amazônicos são fontes em potencial de CO<sub>2</sub> para a atmosfera.

Palavras-chave: respiração bacteriana, metabolismo planctônico, métodos, e lagos de planície de inundação amazônicos.

#### **Abstract**

The functional significance of both microbial autotrophs and heterotrophs in overall plankton metabolism and aquatic food webs has been the focus of great deal of aquatic ecology for a long time. Planktonic bacteria have been, more recently, recognized as an abundant and important component of the aquatic biota. Bacteria utilize a large fraction of the carbon that flows within aquatic ecosystems. A significant amount of the carbon produced by bacteria moves up to higher trophic levels; thus, bacterial community structure is driven by a top-down control. The realistic linking between energy as dissolved organic carbon from lower to higher trophic levels is influenced by the magnitude of the respiratory processes. Indeed, the intensity of respiration rates is an expression of the sinking of carbon in the system. In our point of view, the ecological gap still is: *Why and how heterotrophic bacterial respiration rates are, comparatively, so high in terms of energetic balance at plankton level*. All efforts at approaching bacterial respiration may take into account this theoretical regard. The objective of the present chapter is to bring up the importance of bacterial respiration data and suggest some fruitful approaches to aquatic microbial ecology. Methodological aspects are discussed as well; moreover, we also will show some preliminary data of bacterial respiration in a floodplain Amazonian lake. Our data suggests that Amazonian aquatic ecosystems are potentially sources of CO<sub>2</sub> to the atmosphere.

Key-words: bacteria respiration, plankton metabolism, methods, Amazonian flood plain lakes.

## Introduction

The functional significance of both microbial autotrophs and heterotrophs in overall plankton metabolism and aquatic food webs has received considerable attention over the past three decades (e.g. Pomeroy, 1974; Azam *et al.*, 1983; Pedrós-Alió & Brock, 1983; Cho & Azam, 1988; Pomeroy & Wiebe, 1988; Baines & Pace, 1991; Vaque & Pace, 1992; del Giorgio *et al.*, 1999). The microbial loop concept has been the theoretical scenario for researchers; thus, more recently, the importance of planktonic bacteria, for instance, has been frequently and broadly approached.

Nowadays, planktonic bacteria are recognized as an abundant and important component to the aquatic food web. Several studies have documented that bacteria utilize a large fraction of the carbon flowing within aquatic ecosystems (Cole *et al.*, 1988). Pelagic bacterial communities utilize DOM as an energy source, the latter being produced mainly by phytoplankton. It has been estimated that 5 to 50% of fixed carbon is released as dissolved organic matter (DOM) (Larsson & Hagström, 1982). Sequentially, the energy flows, as food, from bacteria to protozoans and to some metazoans (Pace & Cole, 1996). Hence, a significant fraction of the carbon produced by bacteria moves up to higher trophic levels (Wylie & Currie, 1991). In this sense, bacterial communities structure is driven by a top-down control. The realistic linking between energy as DOM from lower to higher trophic levels is influenced by the magnitude of the respiratory processes. The intensity of respiration rates is, in fact, an expression of the sinking of carbon in the system. In many systems, and under a quite different environmental and physiological conditions, bacterial carbon is largely respired even within a microbial food web (Ducklow *et al.*, 1986).

The ecological planktonic bacterial component also is driven by a bottom-up control. The community plays a variable role in nutrient recycling depending on resource availability. Bacteria may either regenerate or consume limiting nutrients such as nitrogen and phosphorus depending on the C:N and C:P ratios of available substrates (Caron, 1991). When bacteria is acting as nutrient sink, consumers of bacteria become a primary vector of nutrient recycling (Pace & Cole, 1994).

As a matter of fact, heterotrophic bacteria contribute to the cycles of nutrients and carbon in two major ways: by the production of new bacterial biomass (secondary production) and by the remineralization of organic matter. This dual character of planktonic bacteria in aquatic ecosystems is a central paradigm of contemporary microbial ecology (Billen, 1984; Cole, 1999; del Giorgio & Cole, 1998; Ducklow & Carlson, 1992). However, the balance between the bacterial ability to link and sink carbon has been accounted as another central idea.

The significance of respiration for microbial ecology has been reported for a long time (Pomeroy, 1966, 1968; Williams, 1970). Almost 30 years ago, Pomeroy (1974) established: "we know much less about respiration in the ocean than about photosynthesis". His statement is absolutely up-to-date. The aquatic

microbial scientists continue to invest great efforts improving their knowledge on production rather than on respiration. We believe this to be true for autotrophic metabolism, and which certainly occurs to heterotrophic processes as well, especially regarding bacterial metabolism. Many authors have reported that a large amount of phytoplankton production photosynthesis in marine and freshwater systems (30-60%) is processed by bacteria (Cole *et al.*, 1988; Ducklow & Carlson, 1992; Williams, 1981). However, part of this carbon is respired (Jahnke & Craven, 1995), and lost of the system as inorganic carbon (del Giorgio & Cole, 1998). It has been reported, for instance, that bacterial respiration is generally high and shows trends to exceed phytoplankton net production in oligotrophic systems (del Giorgio *et al.*, 1997).

Although a certain lack of plankton respiration data is mentioned, we must point out that the literature has improved research on aquatic microbial respiration processes in both marine (e.g. Biddanda *et al.*, 1994; Holligan *et al.*, 1984; Hopkinson *et al.*, 1989; Pomeroy *et al.*, 1995, Serret *et al.*, 1999; Williams, 1998; Williams, 1984; Williams & Purdie, 1991) and freshwater ecosystems (Benner *et al.*, 1995; Chin-Leo & Benner, 1992; Cole *et al.*, 1989; Jensen *et al.*, 1990; Markager *et al.*, 1992; Quay *et al.*, 1995; Roland & Cole 1999; Schwaerter *et al.*, 1988).

The strongest limitation on planktonic respiration studies is mainly due to methodology (Cole & Pace, 1995). There are currently several ways to estimate, mainly, the consumption of dissolved oxygen and/or production of inorganic carbon (see below a brief overview about methods). However, most of these methodology spends a relatively long time to provide a single data. Furthermore, the reproducibility is quite compromised. Another question is relative to fractionation of plankton communities, which implies in a some separation by membranes or nets according to size classes. That is the reason why the absolute majority of efforts has been focus on community respiration in order to understand respiration at ecosystem level (e.g. Biddanda *et al.*, 1994; Holligan *et al.*, 1984; Iriarte *et al.*, 1991; Jensen *et al.*, 1990; Markager *et al.*, 1992; Pomeroy *et al.*, 1995; Quay *et al.*, 1995; Williams, 1981; Williams & Purdie, 1991). Even though, at populational level and over some controlled conditions it is possible to find out a large number of studies involving different plankton levels (Robinson *et al.*, 1999). The review on respiration rates in heterotrophic and free-living protozoa made by Fenchel & Finlay (1983), for example, evaluate the relationship between size cells and respiration rates. The relevance of respiration to carbon metabolism in planktonic ciliates was focused by Stoecher & Michaels (1991). Evaluation of microalgal growth and respiration responses was made by Geider & Osborne (1989), Ploug & Grossart (2000) and Markager *et al.* (1992).

More recently, several studies have been reported a lot of bacterial respiration rates data and discussed them due to its importance in terms of magnitude to the total balance (Williams, 1984; Schwaerter *et al.*, 1988; Iriarte *et al.*, 1991; Benner *et al.*, 1995; del Giorgio *et al.*, 1997; Sondergaard *et al.*, 1997; Williams, 1998;

Roland & Cole, 1999; del Giorgio *et al.*, 1999; del Giorgio & Cole, 1999; Ploug & Grossart, 1999). In order to amplify the understanding of carbon flow in aquatic ecosystems, some authors have been produced qualitative and predictive models based on respiration rates (Touratier *et al.*, 1999; Roland & Cole, in press).

**Objective** – The central goal of the present chapter is to bring up the importance of bacterial respiration data and to suggest some fruitful approaches to aquatic microbial ecology, looking forward to the studies focusing ecological bioenergetics of plankton. Our purpose is, also, to show some preliminary data of bacterial respiration in a floodplain Amazonian lake.

### Measurements of Respiration Rate

An extensive discussion about measurements of respiration rate can be found in Williams (1984). Here, we just provide some simple ideas to encourage researchers to invest their efforts to produce respiration rate data, especially concerning pelagic and heterotrophic bacterial community.

Although we recognize that there are several modern and sophisticated methods, for example, electron transport systems (ETS; see Packard, 1985 and Iriarti *et al.*, 1991) or stable isotopes intracellular ratios ( $^{18}\text{O}$ : $^{16}\text{O}$ ; see Quay *et al.*, 1995), to estimate respiration rates, we will show, in this chapter, techniques concerning the consumption of dissolved oxygen and production of inorganic carbon as  $\text{CO}_2$ .

#### *Plankton Sampling and Size Fractionation*

In an ecosystemic approach, one of the strongest barriers in microbial ecology is actually to obtain the different planktonic fractions separately. The most common procedure is to use nets and/or membranes. The combination among specific supplies may result in an adequately-expected sample; in fact, a structural analysis of the plankton should be made in advance. Besides, we strongly recommend an accurate microscopic overview of the original sample. Roland & Cole (1999), for example, have filtered natural water in a double GF/D filter (Whatman) and got only bacterial populations. The filtered samples were regularly examined in terms of other heterotrophic (protozoans and flagellates), using epifluorescence techniques. Another way to have samples for incubation is from one-specific or population cultures. This kind of sample must be obtained through microbiological procedures associated with chemical selective factors.

An important decision is where to incubate the sample in order to obtain changes in respiratory products – dissolved oxygen or  $\text{CO}_2$ . Glass bottles or plastic bags are the most commonly-used enclosures. There has, however, been a longstanding concern that keeping a sample in a small bottle induces artifacts (Williams, 1984). The advantages using these kinds of enclosures are actually greater than the problems involved. Following changes directly in the environment could be an alternative way. This assumption, however, implies in some interference on the rates, promoted by the exchange of carbon dioxide and oxygen with the atmosphere.

Incubation time is, maybe, the greatest concern related to the methodological background. Most of the studies show bacterial respiration rates obtained from long time incubations (24 hours, for example). This period is, at least in theory, too extensive; physiological states may change dramatically in a few hours. The challenge, in this sense, is in fact how to achieve understanding of the daily trends in respiration data. This curve should provide the best time of incubation.

### *Respiration Studies*

We currently have two major ways to estimate respiration rates at plankton level. First, by evaluating changes in dissolved oxygen. There are different procedures to obtain dissolved oxygen concentration. Probes and microelectrodes are broadly used, although lack of accuracy sometimes compromises the precision of the rates. Conventional oxygen electrodes display a level of accuracy between 1-5% and, thus, can only be used in very favorable circumstances (Williams, 1984). The classic Winkler method is, undoubtedly, the best way to approach oxygen metabolism. This technique, with some modification, can yield a precision of 0.1%-0.3% (Bryan *et al.*, 1976). Automatic and potentiometric detection are the most sophisticated improvements in terms of refinement for end-point detection (e.g. Granelli & Granelli, 1991). However, the titration course still remains an objection even after accuracy is improved. The time to secure a single piece of data varies from 3 to 5 minutes. In a recent paper, Roland *et al.* (1999) reported a very precise and accurate improvement to the Winkler technique. The iodine-product of Winkler chemicals added—is read in a spectrophotometer in 430 wavelength. In this way, it is possible to have as many samples as planned in order to obtain very strong data and to read hundreds of samples in a few hours.

Measuring the carbon dioxide production is another way to estimate regular plankton metabolism rates. Changes in CO<sub>2</sub> may be obtained by alkalinity titration or by published CO<sub>2</sub>-stripping techniques. The partial pressure of CO<sub>2</sub> may also be quantified using normal chromatograph. However, depending on the biological activity, in both cases methodological precision is not reasonable. Salonen & Kononen (1984), for example, determined respiration rates assuming changes in CO<sub>2</sub>, but they comment that the method should be used with extreme caution for estimating respiration in different size classes of plankton. An alternative, but quite expensive method, is via radiochemical procedures, by measuring the release of <sup>14</sup>CO<sub>2</sub> from added <sup>14</sup>C-labelled organic compounds. So, many studies have been using this procedure (e.g. Rai, 1979; Stoecker & Michaels, 1991).

### *Respiratory Quotient*

To convert dissolved oxygen consumed to carbon respired, many authors have assumed a respiratory quotient (RQ) of 1.0, based on the respiration of organic matter, through Redfield's stoichiometry (Redfield *et al.*, 1963): one molecule of O<sub>2</sub> consumed to one of carbon respired (Chin-Leo & Benner, 1992; Biddanda *et al.*, 1994; del Giorgio *et al.*, 1997; Williams, 1998; Roland & Cole, 1999). Respiratory quotients vary between 0.7 and 1.1, depending on the composition of the substrates

(Kleiber, 1961; Parsons *et al.*, 1984 in Biddanda *et al.*, 1994). This RQ is unconstrained by observations for open sea bacteria, but it is likely to be an overestimate of the CO<sub>2</sub> evolution from the respiration of organic matter, including proteins and lipids in addition to carbohydrates (Geider, 1997). Oceanographers typically use a photosynthetic quotient of 1.25 O<sub>2</sub> evolved per CO<sub>2</sub> assimilated to convert measurement of primary production from carbon to oxygen equivalents (Grande *et al.*, 1989) for comparison with respiration rates measured as O<sub>2</sub> consumption.

### *Biomass*

One line of thought is: respiration is not restricted to a single group of organisms but common to all. The trend is toward confirming that bacterial populations tend to respire comparatively more than other components in the plankton (Simon *et al.*, 1992). The central goal is to approach somehow, a specific respiration rate. Bacterial biomass should be evaluated knowing how much carbon is respired by a certain biomass. It might lead us to understand the real role of heterotrophic bacteria to energetic balance. A very high level of community respiration could be either in fact high or surprisingly not so high depending on the magnitude of the biomass.

We have three steps in order to get bacterial biomass as carbon incorporated. (1) Bacterial abundance – samples may be filtered at 0.2 µm membrane and stained using either acridine-orange (Hobbie *et al.*, 1977) or DAPI (4,6-diamidino-2-phenylindole; Porter & Feig, 1980). Bacterial abundance may be estimated by using an epifluorescence microscope at 1,200x or higher; (2) Bacterial size – there are several ways to measure the size of bacterial particles: (a) measuring directly under the microscope; (b) taking photographs of the counted fields; (c) processing images in an image analyzer and (d) using flow cytometry; (3) Carbon factor – a conversion factor is need to convert cell number to carbon. The factor of 20 fg C cell<sup>-1</sup> (Lee & Fuhrman 1987) is one of the most commonly value used.

### **Some Insights on Bacterial Respiration and Perspective of Studies**

Fundamentally, in terms of energy flow as carbon pathway, fundamentally, bacteria can exhibit two major functions: (a) link energetic resources from an unavailable organic pool to a higher trophic level through effective production; or (b) sink metabolic products synthesized by the aquatic food web to inorganic pool and so to export carbon via respiration processes. It is very known how chemical and biological forces such as DOC, nutrients, grazing can drive heterotrophic bacterial production. However, investigative effects of physical attributes (e.g. light, temperature and turbidity) on bacterial production still are open doors in microbial metabolism studies. Hence, we still need improvements in order to understand bacteria as linkers of carbon. On the other hand, the effect of all these ecological forces over bacterial respiration rates is rarely investigated.

Figure 1 illustrates the functions of plankton communities in an ecosystemic view. The defined system – plankton – is a web of relationships among communities having both input and output of energy/matter. Both phytoplankton and bacteria biomass, in terms of energy, are severely controlled by one or a combination of abiotic ecological drivers (light, temperature, turbidity etc). Energetically, in fact, phytoplankton and bacteria take up a similar level (input of  $E$ ). A similar situation is observed in relation to the available pool of inorganic nutrients ( $N_i$ ). The difference is that bacteria can move up organic carbon ( $N_o$ ), but bacteria can also return organic carbon ( $DOC$ ) to the inorganic pool ( $CO_2$ ). Planktonic grazers such as ciliates, flagellates and micro-zooplankton act by linking energy from the plankton level to higher trophic levels ( $G1$ ). Respiration rates may be influenced by this carbon flux, especially mediated by bacteria. Bacterial growth efficiency [ $BGE=BP(\text{bacterial production})/BP+BR$  (bacterial respiration)] is one way to evaluate the power of respiration for carbon balance. The plankton level acts as a link to higher trophic levels ( $G2$ ) and/or as sink to other compartments of the system or even to other systems ( $Ex$ ).

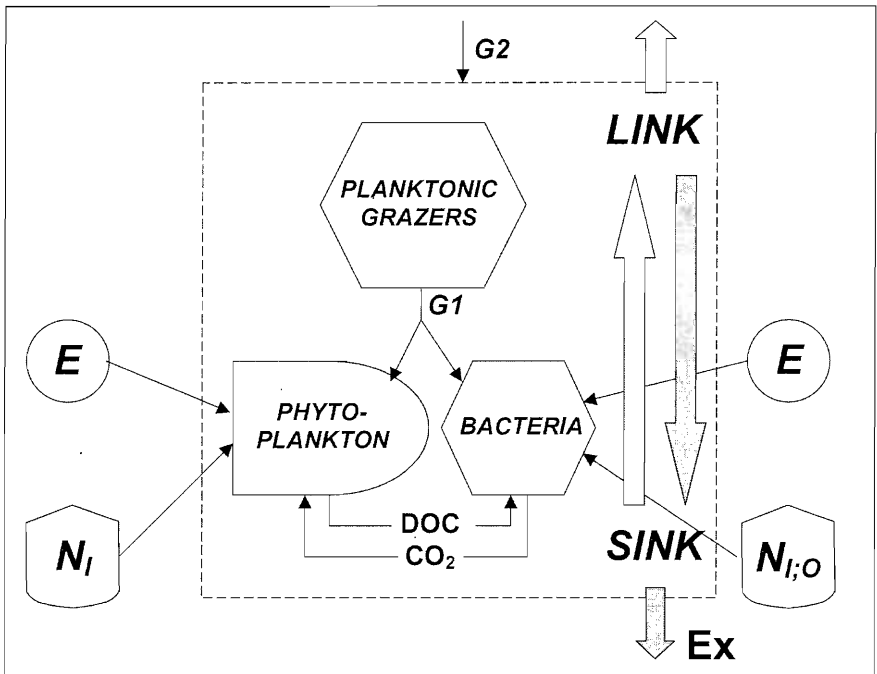


Figure 1. Schematic diagram of plankton level compartment. The symbols are designed according to the energetic approach proposed by H. Odum:  $E$  = energy;  $N$  = nutrients as matter;  $I$  = inorganic and  $O$  = organic;  $G$  = grazing pressure;  $Ex$  = exportation. The dotted line limits the ecosystem as defined.

In order to support the required improvement on bacterial respiration studies we following suggest some lines of investigation:

- Methods – improve the methodological ability to get instantaneous respiration measurements coupled to bacterial production, and so measure value of realistic changes in BGE;
- Drivers – simulate ecological gradients and sample in a broad cross temporal and spatial scale, in order to evaluate the range of bacterial respiration. Tropical aquatic ecosystems, particularly shallow and flood plain lakes must be approached due to, mainly, because the fast metabolism in daily scales;
- Function – estimate the bacterial contribution to the plankton respiration, measuring the entire planktonic communities respiration under ecological forces – temperature, light, nutrients, physical substrates etc;
- Biological view – consider grazing pressure on bacterial dynamics, population studies (biomass, growth/respiration rates and physiological states) estimating bacterial biomass and so specific respiration rates. Further, invest efforts applying cell biology methods in order to understand the biochemical respiratory process;
- System – quantify the production of CO<sub>2</sub> in terms of carbon flux to the atmosphere and understand the contribution of the energy source– autochthonous or allochthonous – to carbon balance mediated by bacteria;
- Management – design experiments looking at management activities such as aquaculture, eutrophication etc, and define the limits of UV light on respiratory process and, fundamentally, look at the ecological role of aquatic ecosystem to the green-house effect.

In our point of view, the ecological gap still is: *Why and how heterotrophic bacterial respiration rates are comparatively so high in terms of energetic balance at plankton level.* All suggested approaches listed before may take into account this theoretical regard.

### **A Study Case of Bacterial Respiration**

In order to illustrate the contribution of bacterial respiration to the total planktonic respiration, some data obtained from an Amazonian flood-plain lake - Lake Batata - will be shown.

#### *Study Site*

Lake Batata is located on the right bank of the Trombetas river (56°14'W and 1°28'S). Its area ranges from 29.5 km<sup>2</sup> during the high water phase to 18 km<sup>2</sup> during the low water phase (Roland & Esteves, 1998). Both morphometry and depth change considerably during the year, as a function of the river's water level (Panosso *et al.*, 1995). Lake depth usually oscillates between 0.5 and 9.0 meters



during each hydrological cycle. For over 10 years (1979-1989), the lake has received a large amount of bauxite tailings ( $50,000 \text{ m}^3 \text{ day}^{-1}$ , Lapa & Cardoso, 1988). The tailings were originally spread into 30% of the lake area; however, it has been widely redistributed due to intensive resuspension (Roland & Esteves, 1993). According to OECD criteria (Vollenweider & Kerekes, 1982), lake Batata is, on average, an oligo-mesotrophic system (Huszar *et al.*, 1998), with  $< 20 \mu\text{g L}^{-1}$  of total phosphorus concentrations. The water-column is generally quite well mixed and rarely anoxic (Esteves *et al.*, 1994); pH ranges between 6 and 7 and conductivity is usually  $< 10 \mu\text{S cm}^{-2}$ .

### *Material and methods*

Every three months (March 1998 – March 2000), according to the water level change, we collected samples to estimate phytoplankton biomass as chlorophyll *a* by fluorometry and bacterial abundance by epifluorescence microscopy. Bacterial abundance was not converted to biomass. Hence, we did not calculate specific respiration rates.

During the low water period (December-1999) three different experiments were run: (1) simulating a gradient of mineral turbidity; (2) combining turbidity and a gradient of DOC and (3) selecting UV radiation from PAR (Photosynthetically Active Radiation). In all experiments, treated samples (filtered in double GF/D Whatman filters) were incubated during 24 hours in glass bottles. In the UV radiation experiment, quartz-glass bottles were used. Dissolved oxygen concentration was obtained using Winkler technique and the iodine was read in a spectrophotometer (Roland *et al.*, 1999). The change in dissolved oxygen concentration was converted to carbon assuming  $\text{RQ}=1$ .

### *Data*

A very strong relationship ( $r^2=0.69$ ) was observed between phytoplankton biomass and bacterial abundance (Fig. 2). The values for phytoplankton biomass changed from 2 to  $12 \mu\text{g Chl } a \text{ L}^{-1}$ . The change in bacterial abundance was from  $1.3$  to  $2 \cdot 10^9 \text{ cell L}^{-1}$  for most of the values. Two of them were higher than  $2 \cdot 10^9 \text{ cell L}^{-1}$ , which were obtained during the low water period. In this hydrological phase, primary productivity is high (Roland *et al.*, 1997), and so the potential energy from phytoplankton to bacteria increases substantially. The coupling between phytoplankton and bacteria drive bacteria abundance (Anesio *et al.*, 1997). However when phytoplankton biomass is diluted during the increase in water level (Huszar, 2000) bacterial abundance is maintained by the increment of organic allochthonous from the flooded area. Thus, bacterial metabolism is alternatively supported by very labile DOC from algae during the low water phases and by POC (particulate organic carbon) and DOC from high plants as detritus during the flood. In this hydrological period, mineral particles make easier to bacteria the access to energy by generating aggregates. During the low water phase, bacterial abundance is in a maximum level due to the combination between carbon sources and aggregate formation.

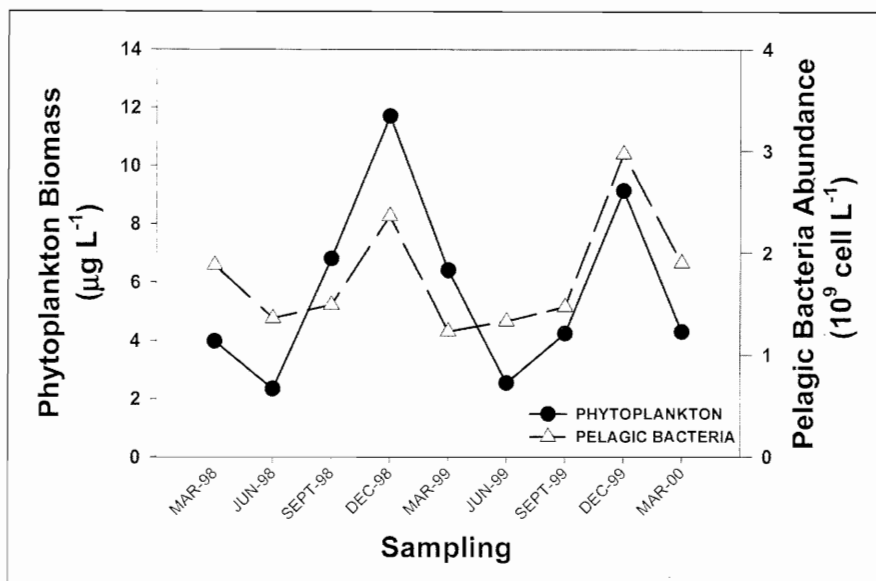


Figure 2. Temporal variation on phytoplankton biomass (left, full circles) and bacterial abundance (right, open triangles).

By measuring bacterial respiration rates in the presence and absence of mineral turbidity in the experiment, it was possible to observe that turbidity slightly inhibits respiration (Fig. 3A). All values in the absence of turbidity were higher than in the presence of turbidity. Applying this pattern to the field data (Fig. 2) we could suggest that bacteria, in the presence of turbidity, are more efficient than in its absence. On the other hand, turbidity optimizes bacterial production at the same time that it inhibits bacterial respiration. Actually, the main issue is that respiration is less changeable than production, because bacteria have some ability to control more respiration than production when under pressure by environmental stress (del Giorgio & Cole, 1998; Roland & Cole, 1999). A similar idea can be discussed in relation to the effect of UV radiation. In the presence of UV radiation, respiration rates are lower than with only PAR radiation (Fig. 3B). In this case, all values were over the 1:1 line. In natural conditions, this effect should be even higher. Notably, the UV radiation limits primary production and so DOC is released. Hence, this kind of energy may reduce secondary production as well. Bacterial metabolism, in this sense, is affected in both attributes – production and respiration. However, it seems that the effect of UV radiation, in terms of rates, depresses more production than respiration. On the other hand, UV radiation has a strong capacity to bleaching the organic matter and so promoting some increment in the labile-dissolved organic pool (Reche *et al.*, 1998) This is the main starting point to bacterial metabolism. The compensation between both effects – cellular damage and increment of energy as a source – should be more investigated.

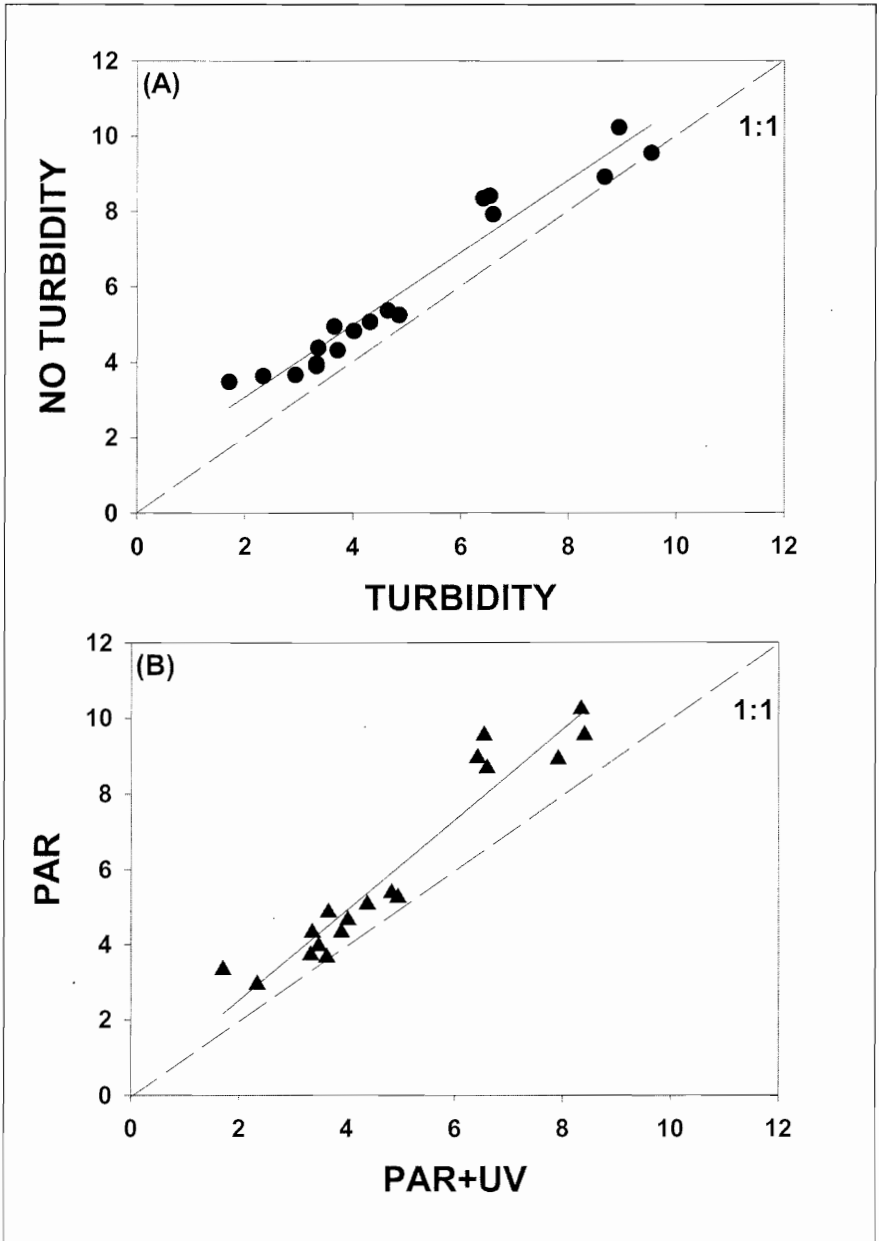


Figure 3. Bacterial respiration rates ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ) in experiments with turbidity (A) and light radiation (B). Dotted lines means 1:1 unit.

Data from an experiment combining organic carbon (DOC), as source of energy, and absence/presence of turbidity have shown increase of respiration rates according to increment in DOC in the presence or in the absence of turbidity (Fig. 4). The central point extracted from this data is that, when physical (tailings) and biological (organic matter) substrates are available at the same time, respiration rates increase substantially. This combination is, exactly the scenario observed during low water periods, when bacterial abundance is maximum. If this occurs, it is because the increment of production was higher than the increment in respiration. As a consequence, bacterial growth efficiency is supposed to be higher in these conditions.

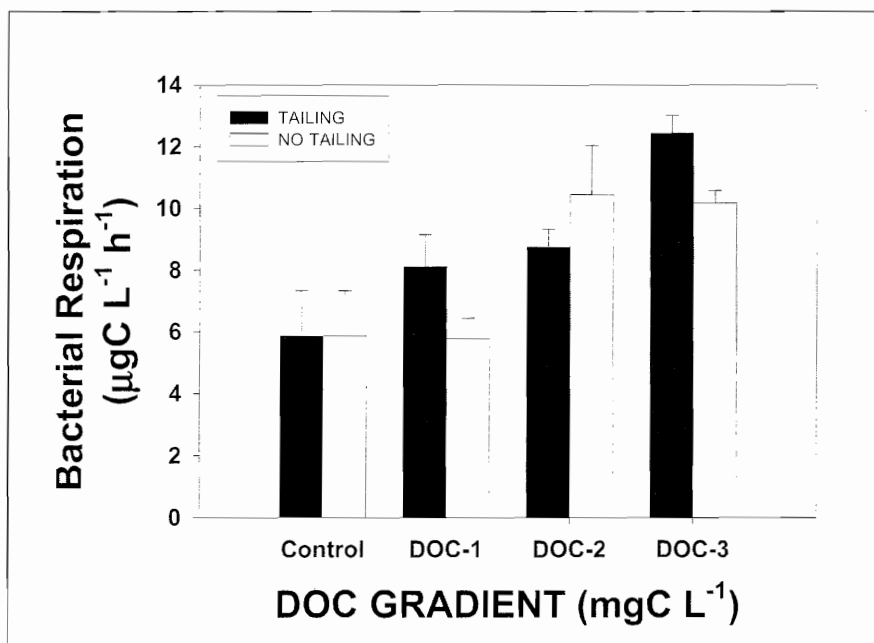


Figure 4. Changes in bacterial respiration rates under a gradient of dissolved organic carbon (DOC) in two different conditions (presence and absence of tailing) in terms of turbidity.

The proportion of bacterial respiration in relation to total bacterial and algal respiration, in different experimental manipulation levels (DOC and tailings) is shown in Figure 5. Most of data in this proportion is higher than 0.5. This means that, over this point, bacteria is the main community converting organic carbon to inorganic pool. Considering that bacteria exhibits very fast metabolism, it is possible to assume its importance to carbon balance. If we have an increment in DOC and turbidity, according to the flooding (Fig. 6), the role of bacteria in terms of respiration is remarkable.

It is quite known that Amazonian waters are very biologically productive and certainly bacteria support this production in some way. However, our data brings up the fact that those aquatic ecosystems are also potential to export carbon to the atmosphere.

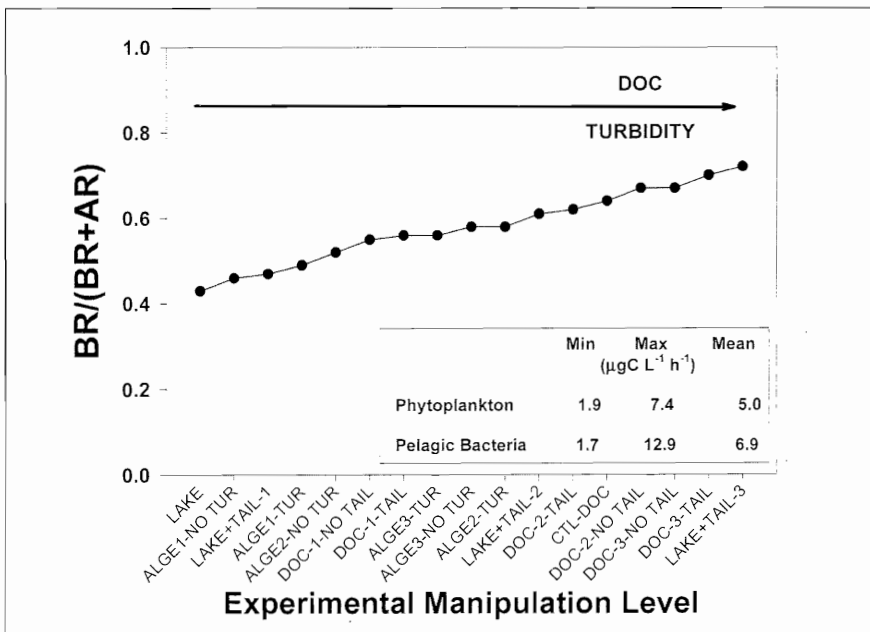


Figure 5. Relationship between ratio bacterial respiration (BR) to the sum bacterial respiration plus algal respiration (BR+AR) and experimental treatment. The data were arranged in rank order from lowest to highest ratio. Experimental manipulation: LAKE = natural sample; ALGAE = adding phytoplankton biomass; NO TUR = without natural turbidity; TUR = with natural turbidity; DOC = adding dissolved organic carbon; TAIL = adding tailing. Numbers represent the level of the treatment.

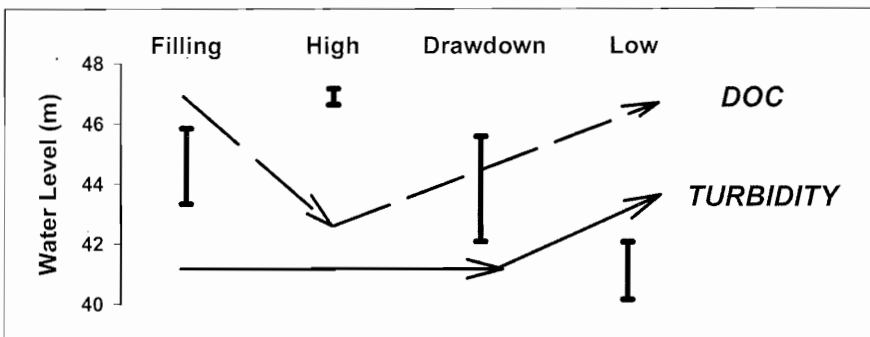


Figure 6. The tendencies of dissolved organic carbon (DOC) and turbidity in the lake Batata throughout the hydrological cycle. The bars show the range in the water level for each phase.

## References

- ANESIO, A.M.; P.C. ABREU & F.A. ESTEVES 1997. Influence of the hydrological cycle on the bacterioplankton of an impacted clear water Amazonian Lake. *Microb. Ecol.*, **34**: 66-73.
- AZAM, F.; T. FENCHEL; J.G. FIELD; J.S. GRAY; L.A. MEYER-REIL & F. THINGSTAD 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**: 257-263.
- BAINES, S.B. & M.L. PACE 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnol. Oceanogr.*, **36**(6): 1078-1090.
- BENNER, R.; S. OPSAHL; J. CHIN-LEO; J.E. RICHEY & B. FORSBERG 1995. Bacterial carbon metabolism in the Amazon River system. *Limnol. Oceanogr.*, **40**(7): 1262-1270.
- BIDDANDA, B.; S. OPSAHL & R. BENNER 1994. Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. *Limnol. Oceanogr.*, **39**(6): 1259-1275.
- BILLEN, G. 1984. Heterotrophic utilization and regeneration of nitrogen. pp.: 313-355. In: HOBBIIE, J.E.; P.J.leB. WILLIAMS (eds.) *Heterotrophic Activity in the Sea*, New York. Plenum.
- BRYAN, J.R.; J.P. RILEY & P.J.leB. WILLIAMS 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. *J. Exp. Mar. Biol. Ecol.*, **21**: 191-197.
- CARON, D.A. 1991. Envolving role of protozoa in aquatic nutrient cycles. pp.: 387-415 .In: REID, P.C. (ed). *Protozoa and their role in marine processes*. Springer-Verlag, Berlin.
- CHIN-LEO, G. & R. BENNER 1992. Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. *Mar. Ecol. Prog. Ser.*, **87**: 87-103.
- CHO, B.C. & F. AZAM 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature*, **332**: 441-443.
- 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.

- COLE, J.J.; N.F. CARACO & D.L. STRAYER 1989. A detailed organic carbon budget as an ecosystem-level calibration of bacterial respiration in an oligotrophic lake during midsummer. *Limnol. Oceanogr.*, **34**(2): 286-296.
- COLE, J.J. & M.L. PACE 1995. Why measure bacterial production? A reply to the comment by Janke and Craven. *Limnol. Oceanogr.*, **40**(2): 441-444.
- COLE, J.J. 1999. Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. *Ecosystems*, **2**: 215-225.
- del GIORGIO, P.A.; J.J. COLE & A. CIMBLERIS 1997. Respiration rates in bacteria exceed phytoplankton in unproductive aquatic systems. *Nature*, **385**: 148-151.
- del GIORGIO, P.A. & J.J. COLE 1998. Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.*, **29**: 503-541.
- del GIORGIO, P.A.; J.J. COLE; N.F. CARACO & R.H. PETERS 1999. Linking planktonic biomass structure to plankton metabolism and net gas flux in northern temperate lakes. *Ecology*, **80**: 1422-1431.
- DUCKLOW, H.W. & C.A. CARLSON 1992. Oceanic bacterial production. *Adv. Microb. Ecol.*, **12**: 113-181.
- DUCKLOW, H.W.; D.A. PURDIE; P.J. LeB. WILLIAMS & J.M. DAVIES 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science*, **232**: 865-867.
- ESTEVEZ, F.A.; S.M. THOMAZ & F. ROLAND 1994. Comparison of the metabolism of two floodplain lakes of the Trombetas River (Pará, Brazil) based on a study of diel variation. *Amazoniana*, **13**: 33-46.
- FENCHEL, T. & B.J. FINLAY 1983. Respiration rates in heterotrophic, free-living protozoa. *Microb. Ecol.*, **9**(2): 99-122.
- GEIDER, R.J. & B.A. OSBORNE 1989. Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. *New Phytol.*, **112**: 327-341.
- GEIDER, R.J. 1997. Photosynthesis or planktonic respiration? *Nature*, **388**: 132-132.
- GRANDE, K.D.; P.J.L. WILLIAMS; J. MARRA; D.A. PURDIE; K. HEINMANN; R.W. EPPLEY & M.L. BENDER 1989. Primary production in the North Pacific gyre: a comparison of rates determined by the  $^{14}\text{C}$ ,  $\text{O}_2$  concentration, and  $^{18}\text{O}$  methods. *Deep. Sea. Res.*, **36**: 1621-1634.

- GRANELLI, W. & E. GRANELLI 1991. Automatic potentiometric determination of dissolved oxygen. *Mar. Biol.*, **108**: 341-348.
- HOBBIE, J.E.; R.J. DALEY & S. JASPER 1977. The use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, **22**: 1225-1228.
- HOLLIGAN, P.M.; P.J. LeB. WILLIAMS; D. PURDIE & R.P. HARRIS 1984. Photosynthesis, respiration and nitrogen supply of plankton populations in stratified, frontal and tidally mixed shelf waters. *Mar. Ecol.-Prog. Ser.*, **17**(2): 201-213.
- HOPKINSON, C.S.Jr; B. SHERR & W.J. WIEBE 1989. Size-fractionated metabolism of coastal microbial plankton. *Mar. Ecol. Prog. Ser.*, **51**: 155-166.
- HUSZAR, V.L.M. 2000. A comunidade fitoplânctônica e sua relação com o pulso de hidrológico e o rejeito de bauxita. pp.: 91-104. In.: BOZELLI, R.; F.A. ESTEVES & F. ROLAND (eds.) *Lago Batata: Impacto e Recuperação de um Ecossistema Amazônico*, Rio de Janeiro, Inst.Biologia-UFRJ/Soc. Bras. Limnologia.
- HUSZAR, V.L.M.; L.H.S. SILVA; P. DOMINGOS; M. MARINHO & S. MELO 1998. Phytoplankton species composition is more sensitive than OECD criteria to the trophic status of three Brazilian tropical lakes. *Hydrobiologia*, **369/370**: 59-72.
- JAHNKE, R.A. & D.B. CRAVEN 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration rate measurements. *Limnol. Oceanogr.*, **40**(2): 436-441.
- JENSEN, L.M.; K. SAND-JENSEN; S. MARCHER & M. HANSEN 1990. Plankton community respiration and along a nutrient gradient in a shallow Danish estuary. *Mar. Ecol. Prog. Ser.*, **61**: 75-85.
- LAPA, R.P. & W. CARDOSO 1988. Tailings disposal at the Trombetas bauxite mine. pp.: 65-76. In: BOXAL, L.G. (ed.). *117<sup>th</sup> Annual Meeting TMS Light Metals Committee*. Proc. Phoenix
- LARSSON, U. & A. HAGSTROM 1982. Fractionated phytoplanktonic primary production, exudates release and bacterial production in a Baltic eutrophication gradient. *Mar. Biol.*, **67**: 57-70.
- LEE, S. & J.A. FUHRMAN 1987. Relationships between biovolume and biomass of naturally-derived marine bacterioplankton. *Appl. Environ. Microbiol.* **52**: 1298-1303.



- MARKAGER, S.; A.M. JESPERSEN; E. MADSEN & R. WEISBURD 1992. Diel changes in dark respiration in a plankton community. *Hydrobiologia*, **238**: 119-130.
- PACE, M.L. & J.J. COLE 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. *Microb. Ecol.*, **28**: 181-193.
- PACE, M. L. & J.J. COLE 1996. Regulation of bacteria by resources and predation tested in whole-lake experiments. *Limnol. Oceanogr.*, **41**(7): 1448-1460.
- PACKARD, T.T. 1985. Measurements of electron transport activity of microplankton. pp.: 207-261. In: JANNASH, M. & P.J.leB. WILLIAMS (eds). *Advances in aquatic microbiology*; Academic Press, London, vol. 3.
- PANOSSO, R.; D. MUEHE & F.A. ESTEVES 1995. Morphological characteristics of an Amazon floodplain lake (lake Batata, Para State, Brazil). *Amazoniana*, **13**: 245-258.
- PEDRÓS-ALIÓ, C. & T.D. BROCK 1983. The impact of zooplankton feeding on the epilimnetic bacteria of a eutrophic lake. *Fresh. Biol.*, **13**: 227-239.
- PLOUG, H. & H.P. GROSSART 1999. Bacterial production and respiration in suspended aggregates - a matter of the incubation method. *Aquat. Microb. Ecol.*, **20**(1): 21-29.
- PLOUG, H. & H.P. GROSSART 2000. Bacterial growth and grazing on diatom aggregates: Respiratory carbon turnover as a function of aggregate size and sinking velocity. *Limnol. Oceanogr.*, **45**(7): 1467-1475.
- POMEROY, L. R. & R.E. JOHANNES 1966. Total plankton respiration. *Deep-Sea Res.*, **13**: 971-973.
- POMEROY, L.R. 1968. Respiration of ultraplankton in the upper 500 meters of the ocean. *Deep-Sea Res.*, **15**: 381-391.
- POMEROY, L.R. 1974. The ocean's food web, a changing paradigm. *BioScience*, **24**: 409-504
- POMEROY, L. R. & W.J. WIEBE 1988. Energetics of microbial food webs. *Hydrobiologia*, **159**: 7-18.
- POMEROY, L.R.; J.E. SHELDON; W.M.Jr. SHELDON & F. PETERS 1995. Limits to growth and respiration in the Gulf of Mexico. *Mar. Ecol. Prog. Ser.*, **117**: 259-268.

- PORTER, K.G. & Y.S. FEIG 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**: 943-948.
- QUAY, P.D.; D.O. WILBUR; J.L. RICHEY & A.H. DEVOL 1995. The  $^{18}\text{O}$ :  $^{16}\text{O}$  of dissolved oxygen in rivers and lakes in the Amazon Basin: determining the ratio of respiration to photosynthesis rates in freshwaters. *Limnol. Oceanogr.*, **40**(4): 719-729.
- RAI, H. 1979. Microbiology of central Amazon lakes. *Amazoniana*, **6**: 583-599.
- RECHE, I.; M.L. PACE & J.J. COLE 1998. Interactions of photobleaching and inorganic nutrients in determining bacterial growth on colored dissolved organic carbon. *Microb. Ecol.*, **36**(3): 270-280.
- REDFIELD, A.C.; B.H. KETCHUM & F.A. RICHARDS 1963. Influence of organisms on the composition of seawater. pp.: 26-77. In: M.N. HILL (ed.) *The sea*, V.2. Wiley.
- ROBINSON, C.; S.D. ARCHER & P.J. leB. WILLIAMS 1999. Microbial dynamics in coastal waters of East Antarctica: plankton production and respiration. *Mar. Ecol. Prog. Ser.*, **180**: 23-36.
- ROLAND, F. & F.A. ESTEVES 1993. Dynamics of phosphorus, carbon and nitrogen in amazonian lake impacted by bauxite tailing (Batata lake, Pará, Brasil). *Verh. Internat. Verein. Limnol.*, **25**: 925-930.
- ROLAND, F.; F.A. ESTEVES & F.A.R. BARBOSA 1997. The influence of bauxite tailings on the light regime and its consequence on phytoplankton primary production in an Amazonian floodplain lake. *Verh. Internat. Verein. Limnol.*, **26**: 765-767.
- ROLAND, F. & F.A. ESTEVES 1998: Effects of bauxite tailing on PAR attenuation in an Amazonian crystalline water lake. *Hydrobiologia*, **377**: 1-7.
- ROLAND, F. & J.J. COLE 1999. Regulation of bacterial growth efficiency in a large turbid estuary. *Aquat. Microb. Ecol.*, **20**: 31-38.
- ROLAND, F.; N.F. CARACO; J.J. COLE & P. del GIOGIO 1999. Rapid and precise determination of dissolved oxygen by spectrophotometry: Evaluation of interference from color and turbidity. *Limnol. Oceanogr.* **44**(4): 1148-1154.
- ROLAND, F. & J.J. COLE 1999. Prediction of bacterial growth efficiency in a riverine ecosystem. *Verh. Int. Verein. Limnol.*, In press
- SALONEN, K. & L.K. KONONEN 1984. Applicability of size fractionation to assess respiration in different size classes of plankton. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **19**: 223-227.

- SCHWAERTER, F.; M. SONDERGAARD; B. RIEMANN & L.M. JENSES 1988. Respiration in eutrophic lakes: the contribution of bacterioplankton and bacterial growth yield. *J. Plankton Res.*, **10**(3): 515-531.
- SERRET, P.; E. FERNANDEZ; J.A. SOSTRES & R. ANADON 1999. Seasonal compensation of microbial production and respiration in a temperate sea. *Mar. Ecol. Prog. Ser.*, **187**: 43-57.
- SIMON, M.; B.C. CHO & F. AZAM 1992. Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. *Mar. Ecol. Prog. Ser.*, **86**: 103-110.
- SONDERGGARD, M. & J. THEIL-NIELSEN 1997. Bacterial growth efficiency in lakewater cultures. *Aquat. Microb. Ecol.*, **12**: 115-122.
- STOECKER, D.K. & A.E. MICHAELS 1991. Respiration, photosynthesis and carbon metabolism in planktonic ciliates. *Mar. Biol.*, **108**(3): 441-447.
- TOURATIER, F.; L. LEGENDRE & A. VEZINA 1999. Model of bacterial growth influenced by substrate C : N ratio and concentration. *Aquat. Microb. Ecol.*, **19**(2): 105-118.
- VAQUE, D. & M. PACE 1992. Grazing on bacteria by flagellates and cladocerans in lakes contrasting food-web structure. *J. Plank. Res.*, **14**: 307-321.
- VOLLENWEIDER, R.A. & J. KEREKES 1982. The loading concept as basis for controlling eutrophication philosophy and preliminary results of OECD programme on eutrophication. *Prog Wat. Technol.*, **12**: 5-38.
- WILLIAMS, P.J.leB. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. I. Size distribution of population and relationship between respiration and incorporation of growth substrates. *J. Mar. Biol. Assoc. U. K.*, **50**: 859-870.
- WILLIAMS, P.J.leB. 1981. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. *Oceanol. Acta*, **4**: 359-364.
- WILLIAMS, P.J.leB. 1984. A review of measurements of respiration rates of marine plankton populations. pp.: 357-389. In: HOBBIIE, J.E. & P.J.leB. WILLIAMS (eds.) *Heterotrophic Activity in the Sea*, New York. Plenum.
- WILLIAMS, P.J.leB. & D.A. PURDIE 1991. *In vitro* and *in situ* derived rates of gross production, net community production and respiration of oxygen in the oligotrophic subtropical gyre of the North Pacific Ocean. *Deep Sea Res.*, **138**: 891-910.

WILLIAMS, P.J.leB. 1998. The balance of plankton respiration and photosynthesis in the open ocean. *Nature*, **394**: 55-57.

WYLIE, J.L. & D.J. CURRIE 1991. The relative importance of bacteria and algae as food sources for crustacean zooplankton. *Limnol. Oceanogr.*, **36**: 708-728.

**Address:**

ROLAND, F. & VIDAL, L.O.

Departamento de Biologia/UFJF

Campus Universitário - Juiz de Fora - MG - Brazil - 36036-330

e-mail: roland@icb.ufjf.br