

Role of allochthonous and autochthonous dissolved organic matter (DOM) as a carbon source for bacterioplankton in boreal humic lakes

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## CONTENTS

<b>List of publications</b>	4
<b>The author's contribution</b>	4
<b>Abbreviations</b>	5
<b>1. Abstract</b>	6
<b>2. Introduction</b>	7
2.1 Dissolved organic matter in aquatic ecosystems	7
2.2 Degradation of DOM	9
2.3 Special features of humic lakes	11
<b>3. Objectives and hypotheses</b>	12
<b>4. Material and methods</b>	14
4.1 Study sites	14
4.2 Experimental design	15
4.2.1 Laboratory experiments	15
4.2.2 Field studies	15
4.2.3 Methodological aspects	16
<b>5. Results and discussion</b>	17
5.1 Bioavailability of DOM	17
5.1.1 Bacterial production vs. DOC concentration	17
5.1.2 Labile fraction of DOM	17
5.1.3 Bacterial growth efficiency	18
5.1.4 Significance of inorganic nutrients	20
5.2 Interactions between bacteria and phytoplankton	21
5.3 Temporal and spatial variation in DOM	23
<b>6. Conclusions</b>	25
<b>7. Acknowledgements</b>	26
<b>8. References</b>	27

## LIST OF PUBLICATIONS

- I. Tulonen, T., Salonen K. & Arvola L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia* 229: 239-252.
- II. Arvola, L. & Tulonen, T. 1998. Effects of allochthonous dissolved organic matter and inorganic nutrients on the growth of bacteria and algae from a highly humic lake. *Environmental International* 24: 509-520.
- III. Tulonen, T. 1993. Bacterial production in a mesohumic lake estimated from [<sup>14</sup>C]leucine incorporation rate. *Microb. Ecol.* 26: 201-217.
- IV. Tulonen, T., Kankaala, P., Ojala A. & Arvola L. 1994. Factors controlling production of phytoplankton and bacteria under ice in a humic boreal lake. *J. Plankton Res.* 16: 1411-1432.
- V. Arvola, L., Kankaala, P., Tulonen, T. & Ojala, A. 1996. Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi). *Can. J. Fish. Aquat. Sci.* 53: 1646-1662.
- VI. Tulonen, T., Kankaala, P., Arvola, L. & Ojala, A. 2000. Growth and production of bacterioplankton in a deep mesohumic boreal lake. *Arch. Hydrobiol.* 147(3): 311-325.

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## THE AUTHOR'S CONTRIBUTION

- I. K.Salonen (KS) and L.Arvola (LA) planned the experiments and supervised the work. T.Tulonen (TT) conducted most of the laboratory work, analysed the data and wrote the article together with KS and LA.
- II. LA and TT designed the experiments, analysed the data and wrote the article. TT performed the experiments with the bacteria and LA with the algae. LA supervised the work.
- III. TT designed and performed the methodological experiments. The field work was conducted with P.Kankaala (PK) and LA, who also supervised the work. TT analysed the data and wrote the article.
- IV. LL, PK, A.Ojala (AO) and TT designed the experiments and conducted the field work. Laboratory experiments were performed by TT (bacteria) and PK (phytoplankton). TT analysed the data and wrote the article, with contributions from PK, LA and AO.
- V. LA, PK and TT designed the experiments and conducted the field work. TT was responsible for the bacterioplankton studies. TT contributed to the writing of the article.
- VI. TT and PK designed the experiments and conducted the field work. TT performed the bacterial experiments and analysed the data. She wrote the article together with PK, with contributions from LA and AO.

**ABBREVIATIONS**

BGE	bacterial growth efficiency
BP	bacterial production
BR	bacterial respiration
DOC	dissolved organic carbon
DOM	dissolved organic matter
EOC	extracellular organic carbon
LDOC (LDOM)	labile dissolved organic carbon (matter)
MW	molecular weight
PP	primary production
POC (POM)	particulate organic carbon (matter)
RDOC (RDOM)	refractory dissolved organic carbon (matter)
TOC	total organic carbon

## 1. ABSTRACT

The continuous leakage of dissolved organic matter (DOM) from terrestrial ecosystems in the Boreal Zone results in the high concentrations of organic substrates in lakes and the brown colour of the water. Bacterioplankton play an important role in the carbon flow of aquatic ecosystems, since bacterial mineralization is one of the main processes affecting the DOM pool. On the other hand, bacteria form the base of the microbial food web and are the main nutritional source for protozoa. In humic lakes, bacterioplankton may utilize the allochthonous carbon originating from drainage basins and the autochthonous carbon fixed by primary producers. This thesis is focused on the factors affecting the bacterial degradation of DOM originating from both sources. The role of DOM as a carbon and energy source for bacteria was investigated using experimental and field data collected from 2 lakes, the highly humic Lake Mekkojärvi and mesohumic Lake Pääjärvi, with mean dissolved organic carbon (DOC) concentrations of 21 and 10 mg C l<sup>-1</sup>, respectively.

The laboratory experiments, performed with epilimnetic lake water or inflow water from Lake Mekkojärvi, showed that bacterioplankton could readily utilize about 5% of the allochthonous carbon pool, which indicated the refractory nature of the allochthonous DOM. The allochthonous substrates resulted in bacterial growth efficiencies (BGEs) of between 18% and 24%, determined as the amount of new bacterial biomass produced per unit of organic carbon substrate used by bacteria. Experiments with different molecular-weight fractions indicated that the high-molecular-weight compounds, which comprised the bulk of allochthonous DOM, were utilized more efficiently (BGE 26%) than the low-molecular-weight compounds (BGE 3%). Addition of nutrients enhanced the BGE to values of over 40%.

The bioavailability of DOC varied seasonally in the epilimnion of Lake Pääjärvi. The ratio between daily bacterial production (BP) and DOC concentration was low in winter (mean 0.07) but clearly increased in spring and summer, ranging between 0.4 and 1.1. The high summer values suggested a pronounced role for autochthonous carbon and for labile substrates excreted by phytoplankton at that time of the year. This was supported by the positive relationship between BP and primary production (PP) in the epilimnion ( $r^2 = 0.43$ ,  $p < 0.001$ ,  $n = 37$ ). In summer the ratio between BP and PP in the euphotic zone was a mean of 0.25, and (assuming a BGE of 26%) the autochthonous carbon fixed by phytoplankton was usually able to satisfy the carbon demand of bacterioplankton. Net heterotrophy prevailed and the bacterioplankton utilized mainly allochthonous DOM as a carbon source in autumn and winter, when the production of autochthonous carbon could not support the bacterial production. Considering the BP in the entire water column of Lake Pääjärvi, it was evident that allochthonous carbon was the most important energy source for bacterioplankton on an annual basis. However, seasonal and spatial variation occurred in the quality of the DOM supply and in the availability of inorganic nutrients. Experimentally, it was shown that the addition of recently produced 'fresh' allochthonous DOM enhanced microbial activity in the euphotic zone, especially in autumn and winter. Simultaneous enrichment with DOM and inorganic phosphorus frequently resulted in the highest rates of BP. In the aphotic hypolimnion microbial activity could be stimulated by higher temperature, addition of inorganic phosphorus and 'fresh' DOM.

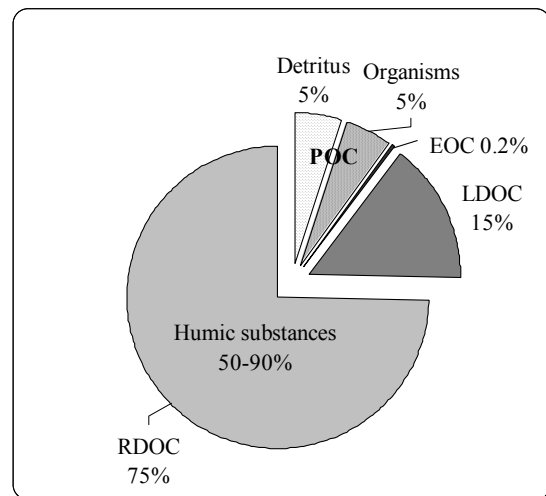
## 2. INTRODUCTION

### 2.1 Dissolved organic matter in aquatic ecosystems

The aquatic environment is the major global reservoir of organic carbon (Benner 1998). In all aquatic systems from seas and groundwater to marshes, the bulk of the total organic carbon (TOC) pool consists of dissolved organic carbon (DOC; Kortelainen 1999). TOC can be classified according to its solubility. The DOC fraction is determined as the C concentration of the water passing through 0.2- or 0.45- $\mu\text{m}$  pore size filters or through GF/F fibre glass filters. The C retained on the filter constitutes that fraction of particulate organic carbon (POC) that is roughly 10% of the TOC in most aquatic systems (Wetzel 1984, Fig. 1). Total organic matter (TOM) also includes other elements such as oxygen, hydrogen, nitrogen and phosphorus in addition to C and is, together with dissolved organic matter (DOM) and particulate organic matter (POM), analogous to TOC, DOC and POC.

From the biological point of view, the DOM pool can be classified as biologically labile (LDOM) and refractory dissolved organic matter (RDOM). In a cross-system analysis performed by Søndergaard & Middelboe (1995) of data collected from lakes, rivers and seawater, an average of 17% of the DOC pool (15% of TOC) was found to be labile, i.e. easily available for bacterial consumption. However, clearly lower values were measured in other studies (Tranvik 1988, Søndergaard et al. 1995, Raymond & Bauer 2000). The LDOM pool is comprised mostly of low-molecular-weight (low-MW) compounds such as

dissolved free amino acids, carbohydrates and fatty acids, vitamins, nucleotides, pigments and steroids (Münster et al. 1999b). Part of the LDOM fraction is produced photosynthetically and excreted as dissolved extracellular organic carbon (EOC), mainly by phytoplankton in pelagic areas and by macrophytes and epiphytic algae in the littoral zone. The proportion of EOC in the TOC pool is low in humic lakes; e.g. in summer EOC comprises only 0.2% of TOC in mesohumic Lake Pääjärvi (Kankaala et al. 1996).



**Figure 1.** An approximation of the different fractions of the total organic carbon pool in aquatic systems. The DOC pool is comprised of excreted organic carbon (EOC), labile organic carbon (LDOC) and refractory organic carbon (RDOC).

Since POC usually constitutes about 10% and LDOM 15% of TOC in aquatic environments, at least 75% of TOC can be termed refractory DOC, implying that it is not significantly affected by microbial utilization (Fig. 1). Usually

this fraction comprises hundreds of macromolecules with complex chemical compositions and structures (Münster & Chróst 1990). Humic substances also belong to this category; these are biogenic, heterogenous organic substances that can be characterized as being yellow to black in colour, of high-MW and refractory (Aiken et al. 1985, McKnight & Aiken 1998). Humic substances account for about 50% of the DOC pool in most surface waters, but in highly coloured waters they can account for as much as 90% of the DOC (Kronberg 1999).

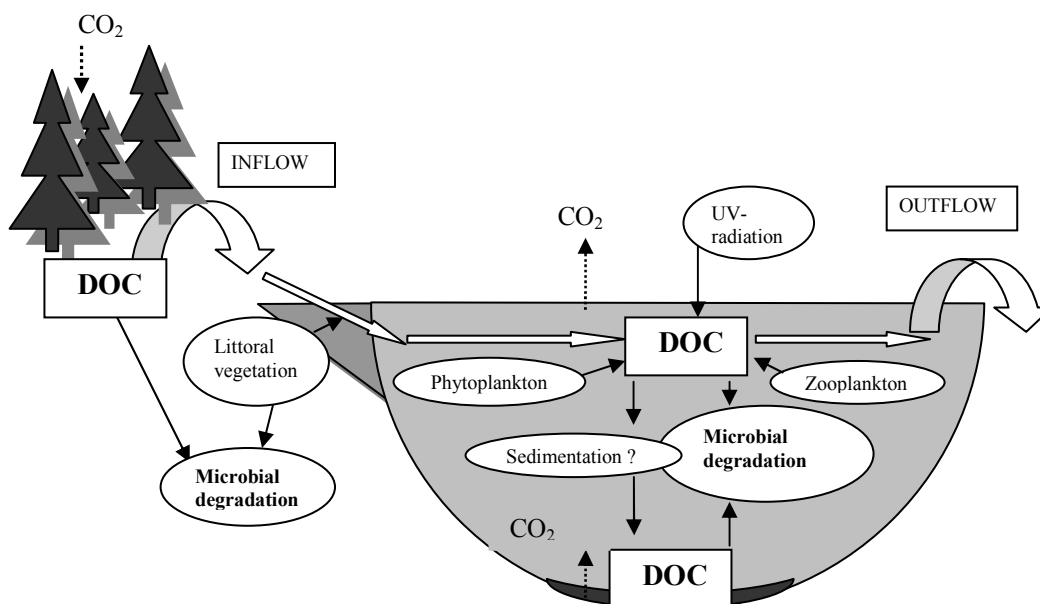
Transformation of POM to DOM is one of the essential factors determining the rates of microbial processes in aquatic systems. POM includes both living organisms (bacteria, phytoplankton, protozoa and metazoa) and particulate detritus (dead organic material). The ratio between living biota and detritus can vary seasonally, as well as in different environments. The proportion of detritus in Lake Pääjärvi was estimated to be roughly 10-30% of POC in spring and summer and 50% in autumn (Kankaala et al. 1996).

The POM and DOM fractions in aquatic systems can originate from 2 different sources (Fig. 2). Firstly, autochthonous organic matter is produced in lakes by phytoplankton and other photosynthetic organisms. The autochthonous C pool also comprises detritus and DOC excreted by photosynthetic organisms (EOC) or by heterotrophic organisms such as micro- and mesozooplankton. Secondly, aquatic systems receive POM and DOM from terrestrial sources, since allochthonous organic matter leaches from the catchment area into rivers and lakes. Allochthonous C also originates

primarily from the photosynthetically produced C, mainly from vascular plants. However, allochthonous DOM usually passes through several degradation stages on its way to the lake and thus is comprised mostly of refractory, high-MW compounds.

The input of allochthonous C always exceeds the input of photosynthetic, autochthonous C in humic lakes. In a large humic lake, the proportion of autochthonous C was found to be about 4% of the TOC input of the lake during the summer stratification period (Jonsson et al. 2001) and in a small humic lake < 15% on an annual basis (Arvola et al. 1999). Cole et al. (2002), who studied the contribution of autochthonous and allochthonous C in a humic lake with the <sup>13</sup>C technique, showed that allochthonous DOC accounted for more than 90% of the TOC in the water column. To what extent allochthonous carbon is actually transported further in the food web in aquatic environments, has been one of the critical questions in aquatic ecology in recent decades.





**Figure 2.** Schematic presentation of DOC flow from terrestrial ecosystems through the lake ecosystem.

## 2.2 Degradation of DOM

Prokaryotic, heterotrophic bacteria are the main biological organisms capable of significantly utilizing the DOM pool to support their energy and C demands. Assimilated organic substrates are needed to generate energy in the form of ATP (adenosine triphosphate). In addition, organic substrates are transformed through a series of intermediate metabolites and used in the biosynthesis of essential organic molecules, to be finally produced as new bacterial biomass or respired as inorganic C (Madigan et al. 1997). In aquatic environments, the many biochemical transformations of organic and inorganic constituents that occur are mediated by heterotrophic bacteria, which have enzyme systems required for these reactions (Chróst 1990). The

degradation of organic matter is largely dependent on the metabolic capability of bacteria to synthesize the required enzyme and transport systems (Chróst 1991, Münster 1993, Münster & De Haan 1998). Microbial extracellular enzymes can cleave the DOM, especially polymeric substances, which are too large to be readily assimilated and must first be transformed into smaller molecules through enzymatic depolymerization. Production of enzymes facilitates the survival of bacteria when easily available energy and nutrient sources become limiting. Such situations prevail e.g. in the aphotic water layers during stratification and under the ice in winter. Enzyme-producing bacteria can also be superior competitors of organic and inorganic nutrients (Chróst 1990).

In many aquatic ecosystems, especially in those with large pelagic areas, bacteria receive DOM mostly from compounds leaking from photosynthetically active phytoplankton (EOC), plankton detritus or from the grazing food chain. Most of this DOM is thought to be easily available for bacteria and plays an important role as a C source for bacterioplankton (Anderson & Ducklow 2001, Moran et al. 2002). Cole et al. (1988) suggested that in general about 40% of the primary production (PP) is channeled via bacteria in pelagic habitats.

Both in the water column and in the sediments, bacteria are the main biological component involved in the degradation and mineralization of the DOC pool and can significantly affect the fate of DOC in aquatic systems (Chróst 1990). In addition, the efficiency of bacteria in utilizing DOC and producing bacterial biomass influences the flow of C and energy through the microbial food web that further leads to higher trophic levels (Azam et al. 1983). Finally, the mineralization processes occurring in aquatic ecosystems may significantly affect the release of CO<sub>2</sub> to the atmosphere (Cole et al. 1994, del Giorgio & Duarte 2002).

However, microbial mineralization is only one of the loss processes affecting the standing stock of DOM in freshwater. The bulk of DOM is dependent not only on the degradation processes, but also on the rates of import, washout and PP occurring in the lake (Fig. 2). Abiotic mineralization, such as photochemical degradation, and flocculation or aggregation of DOM followed by sedimentation, can also decrease the amount of DOM in the

water column. So far, DOC loss by sedimentation has not been studied extensively (Curtis 1998, Tranvik 1998). In addition, some algae were found to be capable of utilizing dissolved organic substrates as nutrient sources and zooplankton of feeding partially on detritus (Sepers 1977, Salonen & Hammar 1986, Jones 1992, Van Wambeke 1994, Ojala et al. 1995), but the impact of these processes on the total DOM flux has not been sufficiently examined.

Recently, photolytic degradation of TOC and especially of DOM has been examined (Salonen & Vähätalo 1994, Vähätalo 2000 and references therein, Engelhaupt et al. 2003). Photochemical reactions degrade substrates to low-MW compounds, mostly to CO<sub>2</sub>. In humic lakes this abiotic process is restricted to the epilimnetic layers of the lake and is often similar to the vertical attenuation of ultraviolet-alpha (UV- $\alpha$ ) radiation. Although photochemical mineralization was estimated to cover only about 10–17% of total mineralization (Vähätalo 2000), it promotes the cleavage of polymers to labile substrates and may stimulate bacterial degradation of DOM (Lindell et al. 1995, Bushaw et al. 1996). Seasonally, photochemical degradation can play an important role in transforming DOM in aquatic systems. Photolytic mineralization in the Boreal Zone is mainly restricted to the ice-free period and the rates are clearly lower during the long ice-covered period in winter.

### 2.3 Special features of humic lakes

DOC concentrations in aquatic environments can range from values  $< 0.5 \text{ mg l}^{-1}$  in seas to  $30 \text{ mg l}^{-1}$  in coloured lakes and up to  $60 \text{ mg l}^{-1}$  in swamps, marshes and bogs (Kortelainen 1999). Quantitatively, the highest DOC concentrations are found in humic, brown-coloured lakes that are commonly found in temperate and cold regions of the Boreal Zone. Since lakes receive their high load of DOM from terrestrial sources, climate and hydrological conditions are important factors contributing to the leaching of organic matter from catchments (Fig. 2). In Finland, where peatlands cover about one-third of the land area, the cold climate and flat topography provide favourable conditions for organic matter accumulation in terrestrial and aquatic ecosystems (Kortelainen 1999). About 60% of the lakes in southern Finland have DOC concentrations  $> 10 \text{ mg l}^{-1}$  (Kortelainen 1993). Climate-warming scenarios indicate that future loads of DOM from catchment areas may increase in Scandinavia (Forsberg 1992, McCarthy et al. 2001, Holmberg 2003). Climate fluctuations or land-use changes that increase allochthonous DOM input to lakes can greatly affect the structure and function of ecosystems and can also cause practical problems in lake management (Carpenter & Pace 1997).

Partly as a consequence of high DOC concentration, there are several features that are common in humic lakes of the Boreal Zone: brown water colour, low penetration of light and predominance of the red part of the spectrum, acidity, low alkalinity, conductivity and low concentrations of free inorganic nutrients

(Keskitalo & Eloranta 1999). In summer the layer of epilimnetic water is thinner in humic lakes than in clear-water lakes and hypolimnetic oxygen depletion commonly occurs especially in small humic lakes. All these features have ecological consequences that can modify the structure and function of the food web in humic lakes (Arvola et al. 1999).

There are 2 basic factors affecting phytoplankton production in humic lakes. Firstly the penetration of solar radiation is depressed due to humic substances, and secondly the large DOM pool affects the chemical environment by altering the bioavailability of inorganic nutrients and potentially toxic chemicals (Jones 1998). Reduced species richness of the phytoplankton community can occur in humic lakes (Eloranta 1986) and phytoplankton production is evidently lower in humic lakes compared with clear-water lakes (Arvola et al. 1999). However, the high proportion of mixotrophic algae and migratory ability of flagellated algae, especially in small humic lakes, may compensate for the poor light environment (Ilmavirta 1983, Salonen & Jokinen 1988, Jones 1993, Jansson et al. 1996). Jones (1998) pointed out that mixotrophy or mobility of algae may not be a phenomenon of humic lakes alone, but can rather be adaptations occurring in all small lakes sheltered from wind and characterized by reduced turbulence and shallow epilimnia. In larger humic lakes, where the euphotic layer can be thinner than the epilimnion, water movements can frequently transport algae below the euphotic zone, causing a reduction in PP. The total nutrient concentrations in humic lakes can be high, but the interaction of phosphate with humic substances and iron

depresses the bioavailability of phosphorus (Ohle 1935, Jones et al. 1988). Recently, N was also suggested to be a limiting factor for phytoplankton production in humic lakes (Jansson et al. 1996, Järvinen & Salonen 1998). In comparison to PP, the characteristics of humic lakes are not as clearly influential at higher levels in the food web. For example, no clear correlation between zooplankton abundance and water colour or TOC concentration was found (Sarvala et al. 1999). Still, fish populations in humic lakes are known to be affected by poor light conditions, acidity and stratification of temperature and oxygen (Rask et al. 1999).

The key role played by bacteria in humic lakes is emphasized by the fact that the rate of total respiration usually exceeds that of PP (Salonen et al. 1992, del Giorgio & Peters 1994, Kankaala et al. 1996). This suggests that the production of phytoplankton in humic lakes cannot support the C demand of the heterotrophic food chain and that the C cycle is partly based on the allochthonous DOC supply. Although a significant proportion of the bacterial C can be channelled through the microbial loop to higher trophic levels (Salonen & Hammar 1986, Hessen et al. 1990, Salonen et al. 1994), there is clear evidence that most of the C can also be lost via respiration within the complex food web of the humic lake (Kankaala et al. 1996, Cole et al. 2002). In recent years it has been shown that most lakes appear to be net sources of CO<sub>2</sub> to the atmosphere (Cole et al. 1994, Kortelainen et al. 2000, Sobek et al. 2003). The degradation processes acting on allochthonous DOM in humic lakes may play an especially important role in

overall C balance between terrestrial and aquatic ecosystems and the atmosphere.

Although the high DOC pool in humic lakes ensures a high potential growth capacity for bacteria (Tranvik & Höfle 1987, Tranvik 1988), the measured rates of BP are usually rather low when compared with those in more eutrophic lakes or estuaries. However, BP measurements are seldom accompanied by bacterial respiration (BR) measurements, which is essential in determining the gross production of bacteria and the total C flow in aquatic ecosystems (Jahnke & Craven 1995, del Giorgio & Cole 1998, del Giorgio & Duarte 2002). Generally, DOC concentration correlates positively with bacterial production rate (Tranvik 1988, Jansson et al. 2000) but with bacterial abundance or biomass the correlation is not always so clear (Münster et al. 1999b, Nürnberg & Shaw 1999, Jansson et al. 2003).

### 3. OBJECTIVES AND HYPOTHESES

In all lakes there are 2 primary energy sources, namely light exploited by photosynthetic organisms such as phytoplankton and macrophytes, and organic C exploited mainly by bacterioplankton. BP is based on both autochthonous C fixed by primary producers and on allochthonous C produced in the drainage basin. Allochthonous C typically predominates over autochthonous C production in humic lake ecosystems, which offers a special environment for the growth of bacterioplankton and affects the overall C flow from the base to the top of the food chain.

This thesis focuses on the role played by DOC, originating from both autochthonous and allochthonous sources, in the growth processes of bacterioplankton in boreal humic lakes and aims:

\* to investigate the degradation rate of DOM by bacterioplankton

\* to investigate factors affecting the bioavailability and degradation of DOM in humic lake water

\* to evaluate the importance of autochthonous DOM for bacterial

growth and to consider the interactions between bacterioplankton and phytoplankton

\* to study the spatial and temporal variation in the bioavailability of autochthonous and allochthonous DOM

The main objectives and key hypotheses of the publications included in the thesis are shown in Table 1.

**Table 1.** Main objectives and hypotheses of the publications included in the thesis.

Publ.	Main objectives	Key hypothesis
I	Degradation of allochthonous DOM Importance of inorganic nutrients	Molecular weight of organic compounds and availability of nutrients affects the bioavailability of DOM
II	Degradation of allochthonous DOM Importance of inorganic nutrients	Availability of inorganic nutrients affects the degradation of DOM
III	Estimation of <sup>14</sup> C-leucine method in humic freshwater Factors regulating bacterial growth at different times of the year	Allochthonous DOM is an important C source for bacteria in humic lakes on an annual basis
IV	Factors regulating bacterial growth in winter	Low temperature and quality of substrate supply determine the growth of bacterioplankton under the ice
V	Effects of allochthonous DOM and P additions on plankton processes and bacterial growth Interaction between bacterioplankton and phytoplankton	Additional P benefits more bacteria than phytoplankton and enhances bacterial utilization of DOM
VI	Vertical distribution of bacterial biomass and production Factors regulating bacterial growth both in the epi- and hypolimnia	Phytoplankton production is insufficient to sustain the total bacterial production in the lake

## 4. MATERIAL AND METHODS

### 4.1 Study sites

This study is based on experimental and field data collected from 2 boreal humic lakes, Lake Mekkojärvi and Lake Pääjärvi, situated in the upper course of the Kokemäenjoki River water system in the municipality of Lammi (25°3'E, 61°13'N), southern Finland.

#### *Lake Mekkojärvi*

Lake Mekkojärvi is a small (area 0.35 ha), shallow (maximum depth 4.3 m) and acidic (pH 5.0-6.5) headwater lake in the Evo State Forest Area. The lake is surrounded by a spruce and Scots pine forest and a small inlet brings water and high allochthonous inputs into the lake from the catchment area. The shoreline is comprised of a solid floating *Sphagnum* mat and narrow *Warnstorfia* moss belt. The lake is highly humic, having a water colour of 300-600 mg Pt l<sup>-1</sup>. The mean DOC concentration in the epilimnion is 21 mg l<sup>-1</sup>, ranging between 18 and 24 mg l<sup>-1</sup> (Münster et al. 1999a). Dissolved free amino acids and carbohydrates comprise 0.3% of the DOC pool. In the epilimnion, total P concentration averages 17 µg P l<sup>-1</sup> and total N concentration 1000 µg N l<sup>-1</sup>, respectively. The total N:P ratio varies between 50 and 60 in the surface water. The lake exhibits steep thermal and chemical stratification in summer; in consequence, inorganic nutrients are rapidly depleted in the euphotic zone. Bacterial densities vary over a wide range between 2.2 and 7.3 x10<sup>6</sup> cells ml<sup>-1</sup> (Arvola et al. 1992, Münster et al. 1992). Small cells predominate in the epilimnion and larger cells and higher biomasses are found in the anaerobic hypolimnion (Kuuppo-Leinikki &

Salonen 1992). Due to the thin euphotic layer (0-1 m), phytoplankton production is low and the algal species present are mostly flagellates, capable of vertical migration. There are no planktivorous fish in the lake, and the crustacean zooplankton community is dominated almost exclusively by *Daphnia longispina* (Arvola et al. 1992, Salonen & Lehtovaara 1992, Ojala & Salonen 2001).

#### *Lake Pääjärvi*

Pääjärvi is a large (area 1340 ha), deep (max. depth 87 m) oligomesotrophic lake situated near the Lammi Biological Station, University of Helsinki. Its drainage area is 244 km<sup>2</sup>, of which 15% is comprised of cultivated land and 8.2% of lakes (Hakala et al. 2002). The mean residence time of the water is 3.3 yr. Dimixis prevails in the lake and in summer the thermocline lies between 5-10 m. The entire water column is well oxygenated throughout the year. The water is brown-coloured (80-90 mg Pt l<sup>-1</sup>) with an mean DOC concentration of 10.3 mg l<sup>-1</sup> (range 9-14 mg l<sup>-1</sup>). Allochthonous inputs and high N loads from cultivated land flow into the lake via 5 main rivulets. The total P concentrations (mean 9.5 µg P l<sup>-1</sup>) in the lake are low compared with the high concentrations of N (mean 1403 µg N l<sup>-1</sup>). Phytoplankton production is restricted to the shallow euphotic (about 3.5 m) zone. The bacterial densities measured varied between 0.8 and 5.9 x10<sup>6</sup> cells ml<sup>-1</sup> in the epilimnion and between 0.8 and 2.7 x10<sup>6</sup> cells ml<sup>-1</sup> in the hypolimnion (III, IV, VI, Salonen 1981). Bacteria in Lake Pääjärvi are mainly grazed by protozoans and phytoplankton by the numerous species of ciliates and metazooplankton. Among phytoplankton, cryptophytes are usually the most abundant algal group (V,

Ilmavirta et al. 1977, Sarvala et al. 1981, Kankaala et al. 1996).

## 4.2 Experimental design

### 4.2.1 Laboratory experiments

Bacterioplankton growth was studied in batch cultures (Table 2). Water was collected either from the inflow or from the epilimnion (depth 0-0.5 m; I, II) of Lake Mekkojärvi or from the epilimnion (0-3 m, III; 0-2 m, IV) and from the hypolimnion (10 m, VI) of Lake Pääjärvi. Sample water was sterilized by autoclaving or filtration (< 0.1 µm). The cultures were inoculated with bacterioplankton by filtering lake water through 0.6- or 1.0-µm membranes using dilutions between 1:2 and 1:50 (I-III); the winter samples were incubated without dilution (IV). In the experiments with hypolimnetic water (VI), the culture media consisted of 80% of lake water filtered through 1.0-µm Nuclepore membranes (bacterioplankton inoculum) and 20% sterilized lake water (< 0.1 µm). The cultures were incubated at temperatures corresponding to the lake temperatures (3.5-20.0°C).

Different treatments were used in the experiments. Humic inflow water (allochthonous DOM) was diluted (II) with deionized water (MQ-water, Millipore) and nutrients and vitamins were added to the cultures by using Jaworski's medium (JM; Culture Collection of Algae and Protozoa, CCAP 1988). Sterilized humic water (I) was fractionated by ultrafiltration into different MW fractions and culturing performed with and without nutrient additions. Culturing was also performed

with different combinations of hypolimnetic lake water (VI), using allochthonous (sampled from a brook running from peatland) or autochthonous (sampled from the surface water during the phytoplankton maximum) DOM and P additions. The effects of allochthonous DOM and P additions on bacterial growth in winter at low light intensity and low water temperature were investigated in Lake Pääjärvi (IV). The lake water was collected from the epilimnion under the ice sheet. The unfiltered sample water was enriched with inflow water and/or P.

### 4.2.2 Field studies

The field studies were focused on Lake Pääjärvi (Table 2). The role of autochthonous DOM and the interaction between bacteria and phytoplankton were investigated in the epilimnion during May-October (III, V, VI) and during December-May (IV). In summer 1991, samples were taken from the euphotic water layer every third day during 3 different study periods of 16-17 days from the western end of the lake (depth 7 m) and in summer 1992 monthly from the same bay and from the middle of the lake (depth 54 m). Vertical samples at the midlake station were taken from the surface to a depth of 45 m. Winter samples were taken in the western bay from 2 depths (about 0-1 m and 1-2 m; III, IV). Bacterial abundance and biomass in the lake samples were determined with an epifluorescence microscope. BP was measured using the <sup>14</sup>C-leucine method (Chin-Leo & Kirchman 1988) and PP using the <sup>14</sup>C method (Schindler et al. 1972).

**Table 2.** Experimental design in publications I-VI.

Publ.	Lake	Sampling	Laboratory experiments				Field studies	
			Batch cultures	Nutrient/DOC enrichment	Molecular fractionation	Conversion factors	Experiments with P/DOC	Measurements in lake
I	Mekkojärvi	Inflow water Epilimnion	X	X	X			
II	Mekkojärvi	Inflow water	X	X				
III	Pääjärvi	Epilimnion	X			X	X	
IV	Pääjärvi	Epilimnion	X	X		X	X	
V	Pääjärvi	Epilimnion				X	X	
VI	Pääjärvi	Epi- and hypolimnia	X	X			X	

The effect of allochthonous DOM and P on bacterial growth was determined in an experiment performed in the field both in winter (IV) and during the open water period (V). In winter, experimental tubes were filled with the epilimnetic lake water and kept under the ice for 3.5 mo in Lake Pääjärvi. Some of the tubes were enriched with inflowing brook water representing allochthonous DOM and additional P input. During the ice-free season, the enclosure experiments *in situ* were performed 3 times (in spring, summer and autumn) to evaluate the effects of allochthonous humic matter and P on the metabolic processes throughout the plankton community and further on the growth of bacterioplankton. One control and 3 enriched enclosures (volume 8.5

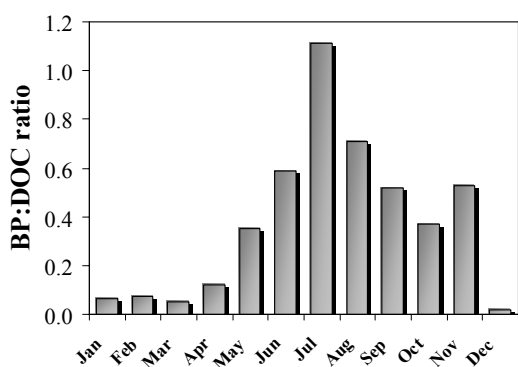
m<sup>3</sup>, depth 3 m) were situated at the western end of Lake Pääjärvi and were sampled every third day for about 2 wk from depths of 0-1, 1-2 and 2-3 m.

#### 4.2.3 Methodological aspects

In recent decades new methods for assessing BP have been developed, which are mainly based on radiolabelling techniques (Fuhrman & Azam 1980, Kirchman et al. 1985, Riemann & Bell 1990). All the methods used for either production or biomass measurements are dependent on certain conversion factors. In this thesis, BP was determined with the <sup>14</sup>C-leucine technique, which measures leucine incorporation into proteins and is



therefore closely related to bacterial biomass production. The level of maximum incorporation of  $^{14}\text{C}$ -leucine and the conversion factors from leucine incorporation to biomass were determined empirically for the pelagic zone of Lake Pääjärvi (III). In addition, the conversion factor from bacterial biomass to C production was determined with a laboratory experiment (III) and was used in all studies concerning bacterioplankton of Lake Pääjärvi (IV-VI).



**Figure 3.** Ratio between bacterial production (BP,  $\mu\text{g C l}^{-1} \text{d}^{-1}$ ) and concentration of DOC ( $\text{mg l}^{-1}$ ) in the euphotic zone (0-3 m) of Lake Pääjärvi in 1991-1992 (III, IV, VI). Ratios are mean monthly values.

## 5. RESULTS AND DISCUSSION

### 5.1 Bioavailability of DOM

#### 5.1.1 Bacterial production vs. DOC concentration

Both the concentration and bioavailability of DOM are critical factors that determine the growth of bacterioplankton in aquatic systems. The ratio between BP and DOC

concentration (BP:DOC) was used as an indicator of the bioavailability of DOC (Bergström & Jansson 2000). The results of this thesis (III, IV, VI) showed that the ratio between daily production rates and DOC concentration in the euphotic zone of Lake Pääjärvi varied from low winter values (mean 0.07) to values between 0.59 and 1.11 in summer, from June to August (Fig. 3). The ratio in autumn was an average of 0.47, which was clearly higher than that in May (0.35). In the hypolimnion the ratio was usually low (mean 0.15 in summer). In summer, the role of extracellular excretion of DOC by phytoplankton may explain the higher ratios observed in the epilimnion compared with those occurring in winter or in the aphotic layer. The increasing discharge in autumn brings new organic carbon and nutrients to the lake and in consequence the bioavailability of DOM for bacteria can be enhanced. For example, the high BP:DOC ratio in November 1991 coincided with a high precipitation of about 90 mm in that month (data not shown). According to Bergström and Jansson (2000), the BP:DOC ratios of a humic lake in Sweden correlated well with the high inflow episodes. The authors suggested that this was connected with the better bioavailability of fresh, allochthonous DOM imported to the lake from the drainage area.

#### 5.1.2 Labile fraction of DOM

Both field data and laboratory experiments have shown that bacterial growth is strongly correlated with the DOC concentration of lake water (Tranvik 1988, Jansson et al. 2000); however, only a small fraction of the total DOC pool is easily bioavailable for bacterioplankton (I, II). The inflow

water of Lake Mekkojärvi contained 27 and 20 mg l<sup>-1</sup> of allochthonous DOC, of which bacterioplankton consumed in bioassays 4.7-5.9% in 3-4 d. Most of the allochthonous DOC (about 95%) was thus refractory to immediate bacterial utilization. Bacteria utilized 6-14% of DOC from the epilimnetic water of humic lakes and 7-11% of DOC in less humic lakes in bioassays performed by Tranvik (1988). In his study, the bulk of readily available DOC was measured from water taken from the pelagic zone, where differentiation between autochthonous and allochthonous DOC is impossible. Therefore the autochthonous LDOC may explain the higher values observed by Tranvik (1988) compared with the approximately 5% consumption of allochthonous C observed in this thesis (I and II). The LDOC varied from 5 to 9% in eutrophic lakes from Denmark, where DOC was mostly of autochthonous origin (Søndergaard & Borch 1992, Søndergaard et al. 1995), i.e. the values were in fact lower than in the oligotrophic or humic lakes (Tranvik 1988). According to Raymond & Bauer (2000) bacteria could mineralize only about 3% of DOC in 5 d in estuaries receiving high allochthonous load. Similarly, bacteria were able to consume 2.8-8.0% of DOC in tropical brown-water lagoons, where most of the DOM originated from decomposition of macrophytes and surrounding terrestrial vegetation (Farjalla et al. 2002). In consequence, the generally referred value of 17% DOC consumption, based on measurements in lake, river and seawater (Søndergaard & Middelboe 1995), seems rather high (see Fig. 1). Raymond and Bauer (2000) suggested that methodological differences can partially explain the variation, because

Søndergaard and Middelboe (1995) used indirect methods to determine LDOC. Generally, laboratory experiments are performed by filtering water to remove POC and grazers, but at the same time bacteria are removed from their natural environment. In consequence, interactions between bacteria and phytoplankton, grazers, viruses and further circulation of nutrients can be altered (Hygum et al. 1997, Raymond & Bauer 2000). Münster and Chróst (1990) emphasized that under natural conditions the turnover time of labile compounds is very short due to rapid assimilation by bacteria. Continuous exudation rates of labile compounds and their accessibility determine the rate of bacterial utilization in natural environments.

### 5.1.3 Bacterial growth efficiency

The critical issue is how efficiently bacteria can simultaneously utilize both DOM fractions, i.e. the LDOM and RDOM, under different environmental conditions. One way to assess the bioavailability of this DOM pool is to measure bacterial growth efficiency (BGE). Measurements of BP rates are not often accompanied by measurements of BR rates, which are essential when considering the degradation of DOM in different environments (del Giorgio & Cole 1998). However, determination of the BGE also gives information about the respiratory activity of bacteria, in addition to the degradation ability. BGE is determined as the amount of new bacterial biomass produced per unit of organic C substrate used by bacteria and is a way to relate BP and BR:  $BGE = (BP)/(BP+BR)$ . The detailed review by del Giorgio and Cole (1998) suggests that the BGE in natural aquatic systems may vary with BP and trophic status of

the ecosystem. The BGE can be as low as 1% in highly oligotrophic systems and around 50% in most eutrophic systems. Experimental evidence suggests that the quality of DOC, rather than rate of supply, regulates the BGE in most aquatic systems. The results of this thesis showed that the BGE of ambient bacteria varied between 18% and 24%, using allochthonous DOM from the inflow of Lake Mekkojärvi (I, II). The BGEs in the surface water of Lake Pääjärvi or Lake Mekkojärvi were not determined, but in humic lakes from Sweden Tranvik (1988) measured BGEs between 13% and 35%. Values in his study did not differ remarkably from those in clear-water lakes (22-36%), which may emphasize the oligotrophic nature of both humic and clear-water lakes. Higher BGEs (up to 60%) have been measured in eutrophic aquatic systems, especially in estuaries (Kroer 1993, Middelboe & Søndergaard 1993, del Giorgio & Cole 1998 and references therein). When del Giorgio and Cole (1998) compared different sources of organic matter and measured BGEs, the collected data implied that organic matter excreted by phytoplankton (EOC) regularly showed higher growth efficiencies than organic detritus derived from phytoplankton, seaweeds, vascular vegetation or animal faeces.

It is worth noting that the conversion factors, which are needed to convert bacterial biomasses or production rates to C, can vary greatly between different sources (Lee & Fuhrman 1987, Bell 1990, Jørgensen 1992), which often makes comparison of the data from different studies and environments difficult. Usually the conversion factors are taken from the literature, although it is generally recommended that they be

determined under ambient conditions, as was done in this thesis for both biomass and production measurements (III).

The key factor in the determination of BGE appears to be the role played by the maintenance energy cost, which reflects the respiration rates of bacteria. The BGE can be low, although the fraction occupied by low-MW compounds in the water is high. This was emphasized in our study, in which bacteria utilized a high proportion of allochthonous DOC (about 30%) from the low-MW fraction (< 1000 MW) but showed a low BGE of 3% (I). When high-MW compounds (1000-100 000 MW) were also present, bacteria consumed only 4% of the DOC but showed a clearly higher BGE (26%) than in low-MW fraction, which was also the case when nutrients were added in excess. This clearly implies that the nutritional value of allochthonous high-MW compounds was better than that of low-MW compounds.

Why then are the BGE values rather low (18-24%) in humic waters, which are rich in DOC with high nutritional value? Most of the DOM is comprised of polymeric compounds that cannot be incorporated directly into bacterial cells, but must first be degraded by enzymes (Chróst 1990, Wetzel 1991, Münster & De Haan 1998, Münster et al. 1999b). In Lake Mekkojärvi, high-MW compounds (> 10 000 MW) comprised about 60% of the total DOM pool in the inflow water, indicating that most of the DOM pool must be processed by exoenzymes before it can be assimilated by bacteria. This suggests that the biosynthesis of these substrates exerts high energy requirements on the cell with a consequent decline in BGE. The autochthonous DOC pool can be

mineralized with lower energy costs, as was shown in a study of 2 eutrophic lakes where the mean BGEs of 32% and 42% were obtained during the summer months (Kristiansen et al. 1992). Microbial enzyme activities can vary seasonally and are basically regulated at the cellular and organismal levels, but so far little is known of their genetic or species specific aspects (Münster & De Haan 1998). Due to the variability of methods, the microbial enzyme activities are not often comparable between different aquatic ecosystems. However, there is some evidence that the enzymes in humic waters can be reversibly immobilized by the humic compounds (Wetzel 1991).

#### 5.1.4 Significance of inorganic nutrients

The BGE of the humic water batches was 24%, which was clearly lower than the BGE of 42% determined for glucose with nutrients in distilled water (I); however, after nutrient enrichment the BGE in humic water also increased to 46%. This implies the importance of inorganic nutrients in the degradation processes of bacterioplankton. In general, our results showed clearly that addition of inorganic nutrients can stimulate microbial activity and bioavailability of DOM for bacterioplankton (I, II, V, VI, Smith & Prairie 2004).

The concentrations of N and P in humic lakes are generally low compared with the C concentrations. The total N and P pools consist mostly of organic compounds and concentrations of inorganic nutrients are low especially during the steepest stratification in the epilimnion, which suggests that N and P may limit the growth of both

heterotrophic and autotrophic organisms (Salonen et al. 1992, Jansson 1998, Järvinen & Salonen 1998, Arvola et al. 1999). In our study, where JM was added without N to the humic water (I), it dramatically decreased both the bacterial yield and BGE. Although the C:N ratio of DOM has been found to dictate the availability of organic C to bacteria in many laboratory investigations (Goldman et al. 1987, Touratier et al. 1999, Hunt et al. 2000), under ambient conditions bacteria are exposed to multiple sources of N and there is conflicting evidence for the role of N in regulating BGE in natural aquatic ecosystems (del Giorgio & Cole 1998). The large DOM pool may also satisfy the N demand of bacterioplankton in humic lakes which is supported by our results suggesting that the bacteria are capable of using the N bound in the DOM pool (II). The C:N ratios in N-rich Lake Pääjärvi ranged between 7 and 8, which emphasizes the minor role played by N in limiting the degradation processes of DOM in this lake (VI).

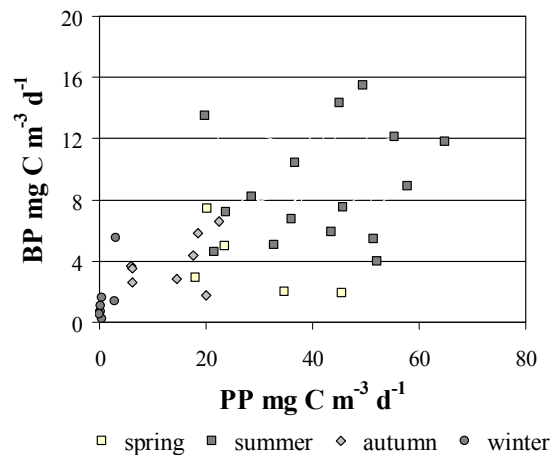
P limits the growth of phytoplankton in many oligotrophic lakes (Schindler 1977). Recently, P limitation of bacterial growth as well has been found in many lakes (Toolan et al. 1991, Le et al. 1994, Jansson et al. 1996, Carlsson & Caron 2001). In humic lakes, P is associated with humic colloids in Fe-P-humus complexes, and the bioavailability of this P is evidently low for both phytoplankton and bacteria. In consequence, the turnover of humus-bound P to the biota is often slow (Jansson 1998). Our results showed that P enrichment promotes bacterial growth both under ice-free and winter conditions (IV-VI). However,

stimulation of bacteria after P addition in the enclosure experiments could also have been caused by enhanced algal growth and its excretion of DOC (V), since clear evidence that bacteria could more efficiently utilize added P than algae was not found. Competition for P between bacteria and phytoplankton has been discussed in many investigations (Thingstad et al. 1993, Joint et al. 2002). Due to their small size, bacteria have been found to be superior competitors, especially in low concentrations of P. Due to their high affinity, it has even been suggested that bacteria may indirectly limit PP by depriving phytoplankton of nutrients (Joint et al. 2002). The capability of bacteria for utilizing essential nutrients from organic compounds can lead to outcompetition of phytoplankton in humic lakes (Drakare et al. 2002, Jansson et al. 2003). Grazing and excretion of dissolved nutrients from heterotrophic flagellates and zooplankton may, however, increase the turnover and supply of bioavailable nutrient fractions both for bacteria and phytoplankton (Goldman & Dennet 1992, Nagata & Kirchman 1992).

## 5.2 Interactions between bacteria and phytoplankton

Humic lakes are often described to be oligotrophic in nature, due to the rapid attenuation of light, low pH and low concentrations of inorganic nutrients present. Although PP is restricted to a shallow zone, production in the water column does not necessarily deviate greatly from that in oligotrophic clear-water lakes (Arvola et al. 1999, Nürnberg & Shaw 1999). The low

penetration of harmful UV irradiation, additional C and nutrients originating from allochthonous matter (processed by bacterioplankton) and vertical migration of phytoplankton are all factors that can compensate for the adverse effects of humic substances. The positive relationship between bacterioplankton and phytoplankton is more or less clear in most oligotrophic and eutrophic lakes and marine environments, with BP averaging 20% of PP (Cole et al. 1988). The interaction between bacteria and algae is more complex in humic lakes and the ratio of BP:PP is often high (del Giorgio & Peters 1994, Jansson et al. 2000). However, labile organic compounds excreted by phytoplankton may play important roles as C sources for bacteria at certain periods of the year in the euphotic zone of humic lakes (IV-VI).

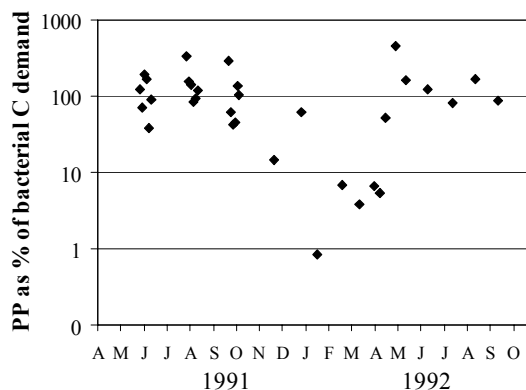


**Figure 4.** Relationship between primary production (PP) and bacterial production (BP) in the epilimnion (depth 0-3 m) of Lake Pääjärvi (linear regression,  $r^2 = 0.43$ ,  $p < 0.001$ ,  $n = 37$ ). Data collected from years 1991-1992 (III, IV, VI).

EOC produced by phytoplankton is comprised mostly of low-MW compounds that are easily available for bacterioplankton. Many studies have emphasized that bacteria can utilize C compounds excreted by phytoplankton with high efficiency, with BGEs often being near 50% (Coveney & Wetzel 1989, Sundh & Bell 1992). In Lake Pääjärvi, the daily excretion of phytoplankton in summer was about 0.2% of the total DOC pool (Kankaala et al. 1996). Although the contribution of EOC to the DOC pool is low in humic lakes, it does not necessarily imply that the significance of EOC for BP is low. For example, the addition of autochthonous C together with P (VI) resulted in similar growth rates for bacteria ( $0.64 \mu \text{d}^{-1}$ ) as enrichment of fresh allochthonous DOM + P ( $0.60 \mu \text{d}^{-1}$ ). BP in the early spring was enhanced immediately after the slight increase in phytoplankton production under the ice cover, and a peak in BP followed the spring phytoplankton maximum (III). Furthermore, the addition of P stimulated PP, especially during the warmest season following enhanced BP rates (V). In July and August, 55-56% of BP but only 17-18% of biomass occurred in the euphotic zone of Lake Pääjärvi. Regression analyses for annual datasets (III, IV, VI) showed a significant relationship between PP and BP (Fig. 4). The ratio BP:PP ratio was lowest during the growing season from May to September (mean 0.25). However, in addition to PP and excretion or leakage of labile compounds by phytoplankton, there may also be other reasons for the better growth of bacteria observed in the euphotic zone. In the summer months, UV radiation can cleave RDOM into

smaller molecules (Vähätalo 2000), active grazing can accelerate nutrient and DOC turnover (Goldmann & Denner 1992, Hygum et al. 1997, Anderson & Ducklow 2001) and higher temperatures can increase metabolism of bacteria (Autio 1992, Felip et al. 1996).

Since there is clear evidence for a positive relationship between BP and PP, it is reasonable to ask if phytoplankton C fixation covers the C demand of bacterioplankton in the upper water column of Lake Pääjärvi. This can be determined by using a BGE of 26%, which is close to the mean determined for oligotrophic and humic waters (Tranvik 1988, I). Conditions where the bacterial C assimilated plus BR exceeds total phytoplankton C fixation, prevailed in Lake Pääjärvi during the cold season from November to April (Fig. 5). PP appeared to cover the C demand of bacteria in the upper water layers in most cases during the summer stratification period (May-September). If the bacteria are assumed to utilize merely the supply of EOC compounds, measured as 30% of the PP (V) and utilized with a BGE of 50% (Sundh & Bell 1992), this would only occasionally cover the C requirements of bacterioplankton. On an annual basis, BP clearly exceeded PP in the epilimnion, the mean ratio being 1.8 (III, IV, VI). Furthermore, when the BP of the entire water column is considered, it is even more obvious that net heterotrophy prevails in Lake Pääjärvi throughout the year (VI). Phytoplankton C fixation covered only 38-54% of the total bacterial C demand between May and August and in September even less (16%) in the deep pelagic area of the lake.



**Figure 5.** Primary production (PP) as percentage of bacterial C demand in the euphotic zone (0-3 m) at the western end of Lake Pääjärvi in 1991-1992 (III, IV, VI). Values are log-transformed. Assumed BGE was 26%.

### 5.3 Temporal and spatial variation in DOM

Discharge largely determines the temporal variation in allochthonous DOM input into lakes in Boreal Zone, which may in turn reflect the quality and also the quantity of DOM in the lake water during the year (Curtis 1998). Bergström and Jansson (2000) demonstrated a close correlation between discharge events and BP in a humic lake in Sweden. Similarly, enhanced bacterial growth was measured in late autumn just before the ice formation in Lake Pääjärvi (Fig. 4A in III), which may indicate that the high autumnal inflow has brought fresh organic C and nutrients into the epilimnion. Positive responses following the addition of humic water were also found in autumn in the enclosure experiments (V). BP clearly exceeds the primary production in autumn (VI) which emphasizes the importance of

allochthonous DOM as a C source for bacteria at that time of the year.

The ice cover in Lake Pääjärvi lasts about 5 mo, usually from December to late April (VI), which effectively decreases light penetration and phytoplankton production. However, DOC concentrations ( $10.4 \text{ mg C l}^{-1}$ , IV) during the winter months did not differ from those in summer or autumn ( $10.5 \text{ mg C l}^{-1}$ , VI). Earlier, I showed that heterotrophy prevailed under the ice cover in Lake Pääjärvi (Fig. 5) and that bacterioplankton were highly dependent on the allochthonous DOM supply. Considering the low discharge in winter and the long retention time of the lake (about 3 yr), bacteria must rely mainly on refractory compounds, which are mineralized at low growth efficiency. Low temperature is one of the essential factors in determining the degree of microbial activity, which was also shown in the data from Lake Pääjärvi with a high correlation between BP and water temperatures  $< 7^{\circ}\text{C}$  (III). However, the refractory nature of DOM under the ice are emphasized, because experiments performed during the winter months indicated that microbial activity could be stimulated by newly exported allochthonous DOM added together with P (IV). Similar results, in which the addition of nutrients and organic compounds enhanced bacterial growth at low temperatures, were shown by Pomeroy et al. (1991) and Wiebe et al. (1992). Interestingly, our results also indicated that in winter bacterial communities can be controlled by small flagellates and that grazers may significantly affect the circulation of nutrients and organic substrates under the ice (VI).

Discharge in spring consists mostly of water from melted snow, and organic substances are more refractory than during the high inflow periods in autumn. In May, the bioavailability of DOM in Lake Pääjärvi was lower than in September (Fig. 3). The spring maximum of phytoplankton production, however, can temporarily stimulate BP (III, V). The maximum BP rates and biomasses in the epilimnion were always measured during the summer months, which can be explained not only by the higher phytoplankton and EOC production present but also partly by the higher temperature, more rapid turnover of nutrients and organic substrates and by more intensive photodegradation of DOM.

Due to the steep thermal and/or chemical stratification of the water column, there can also be clear vertical differences in the bioavailability of DOM in the lake. Firstly, photolytic degradation of refractory compounds and release of labile substances in the surface layers are evidently maximal in midsummer, whereas in the aphotic water layer the quality of DOM is different due to the lack of photolysis. Evidence for the positive effect of light on bacterial growth was achieved in study I, in which the BGE clearly decreased when cultured in dim light ( $2 \mu\text{E m}^{-2} \text{s}^{-1}$ ), compared with cultures incubated under higher light intensities ( $200 \mu\text{E m}^{-2} \text{s}^{-1}$ ). Secondly, organic matter sinking through the water column has passed through many degradation phases and the LDOM may already have been effectively utilized in the upper water layers. The poor bioavailability of DOM in the aphotic water was shown in experiments where the addition of 'fresh' allochthonous DOM increased

the hypolimnetic BP more than did temperature increase or addition of P(VI). Although bacterial activity was low in the hypolimnion, most of the bacterial biomass (74-90%) was found under the euphotic water layer in the central part of Lake Pääjärvi. The results support the earlier observations of Cole and Pace (1995) that although the growth rates of bacteria are low in deeper waters, the greater water volume of the hypolimnion implies a higher total bacterial biomass and higher BP compared with the epilimnion. This suggests that in many humic lakes the quantitative role played by microbial mineralization in the hypolimnion can be vital to determining the fate of DOM (Gurung et al. 2002). In the anoxic hypolimnion - a typical phenomenon in many poorly mixed humic lakes - BP and substrate availability are generally believed to be reduced (Bastviken et al. 2001). However, high BP rates and degradation rates of polymeric organic compounds similar to those observed in the epilimnion have also been reported in anoxic environments (Cole & Pace 1995, Ochs et al. 1995, Münster et al. 1999a). The quality of organic substrates and the availability of inorganic nutrients also appear to regulate microbial growth in anoxic water layers (Bastviken et al. 2001), thus corresponding to the situation observed in oxic layers.



## 6. CONCLUSIONS

1. The allochthonous DOM pool, originating from the catchment area of the highly humic Lake Mekkojärvi, consisted mostly of high-MW compounds. The bacterioplankton were readily able to utilize about 5% of the allochthonous dissolved organic substrates, but utilized high-MW compounds more efficiently than low-MW compounds.
2. The growth efficiency of bacterioplankton in humic water varied between 18% and 24%, which implies that most of the assimilated C was respired. The availability of inorganic nutrients enhanced the BGE and degradation activity of allochthonous DOM.
3. The quality of DOM and its bioavailability for bacterioplankton varied seasonally in the epilimnion of the mesohumic Lake Pääjärvi. This resulted probably from changes occurring in the DOM input from the catchment area.
4. The role played by autochthonous organic matter as a C source for bacterioplankton in Lake Pääjärvi was significant in spring and summer: BP in the epilimnion constituted a mean of 25 % of PP and photosynthesis could frequently cover the C demand of bacteria.
5. Considering both the vertical distribution and the annual production of bacterioplankton, the allochthonous DOM was clearly the main C source for bacteria, because the PP could not support the total BP

in the lake. The degradation processes acting on allochthonous DOM in humic lakes of the Boreal Zone may contribute greatly to the transfer of terrestrial C, through processes in the aquatic ecosystem, to the atmosphere.

6. Although environmental conditions in humic lakes guarantee a potentially large DOC supply for bacteria, BP and degradation of organic substrates are often regulated by the quality of DOM and availability of inorganic nutrients.

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