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Attached Bacterial Communities in Lakes – Habitat-Specific Differences

BY

ANN-LOUISE HAGLUND

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Abstract
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For many years, the importance of microorganisms attached to surfaces in littoral zones and wetlands has been disregarded when describing aquatic ecosystem dynamics. Supporting evidence is scarce but convincing that these microbial communities are not only very productive but can often serve as major regulators of nutrient and carbon dynamics in many freshwaters. In order to determine the quantitative importance of epiphytic bacteria for the overall carbon turnover, I compared the relative contribution of epiphytic bacteria on the submerged macrophyte *Ranunculus circinatus*, sediment and free-living bacteria to the total bacterial production. Sediment bacteria generally dominated total bacterial biomass in the littoral zone. Although the epiphytic biomass on *R. circinatus* was ten times lower than the biomass of sediment bacteria, it often contributed at least equally to the total bacterial production. Thus, the results presented in this thesis confirm that most bacterial biomass and production in shallow lakes is associated with surfaces, and that in littoral zones with dense macrophyte stands, epiphytic bacteria can contribute significantly to the overall carbon turnover.

There is increasing evidence that not all cells in natural bacterial communities are metabolically active. In Lake Erken, there were large differences in the fraction of active bacteria between different habitats, while the within-habitat differences were small. The sediments had the largest bacterial fraction, followed by epiphytic bacteria, while in the water column only a few percent of the bacteria were active. In this thesis the fraction of active bacteria is connected to environmental fluctuations. I hypothesize that smaller fluctuations in chemical, biological or physical factors result in large active bacterial fractions. Thus, small environmental fluctuations within a habitat allow large active bacterial fractions, while the active fraction is constrained when the environmental fluctuations are large.

Keywords: bacteria, freshwater, sediment, epiphyton, bacterioplankton, metabolically active

Ann-Louise Haglund, Department of Ecology and Evolution, Limnology, Norbyv 20, Uppsala University, SE-752 36 Uppsala, Sweden

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Till mina Föräldrar,
Birgit och Gunnar

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I Haglund, A., E. Törnblom & B. Boström. Quantitative importance of epiphytic versus sediment and planktonic bacteria for the overall carbon turnover in littoral zones. (*Submitted*)

II Haglund, A., E. Törnblom, B. Boström & L. Tranvik. (2002) Large differences in the fraction of active bacteria in plankton, sediments, and biofilm. *Microbial Ecology*. 43:232-241.

III Haglund, A., P. Lantz, E. Törnblom & L. Tranvik (2003) Depth distribution of active bacteria and bacterial activity in lake sediment. *FEMS Microbiology Ecology*. 46:31-38.

IV Haglund, A. & H. Hillebrand. The effect of grazing and nutrient supply on periphyton associated bacteria. (*Accepted by FEMS Microbiology Ecology*)

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Introduction

The majority of studies of aquatic microbial ecology focus on pelagic habitats while our knowledge of benthic habitats lags far behind. For many years, the importance of microorganisms attached to surfaces in littoral zones and wetlands have been disregarded when describing aquatic ecosystem dynamics. Supporting evidence is scarce but convincing that these microbial communities are not only very productive but can often serve as major regulators of nutrient and carbon dynamics in many freshwaters (Wetzel and Søndergaard, 1998). Most lakes in the world are shallow with mean depths much under 10 meters, thus the littoral zone dominates over the pelagic among most standing water ecosystems (Wetzel, 1990). According to Wetzel and Søndergaard (1998), most bacterial productivity in shallow lakes is associated with surfaces e.g. on macrophytes (epiphytic), in sediments (epipelic), and on rocks and stones (epilithic).

The emergent, free-floating, and submerged macrophytes in the littoral zone have high productivity and produce leachate supporting a large microbial biomass (e.g. Søndergaard, 1983; Moran and Hodson, 1989; Mann and Wetzel, 1996). The attached bacteria live in close proximity with algae, cyanobacteria, protozoans, fungi and other small heterotrophic biota surrounded by polysaccharides forming a biofilm. There is an intensive internal recycling of nutrients, including carbon, and gases within the attached microcommunities. The bacteria supply the algae and cyanobacteria with nutrients and the primary producers emit exudates that are readily taken up by the heterotrophic bacteria (Haack and McFeters, 1982; Neely and Wetzel, 1995).

Compared to bacteria in other types of biofilms, epiphytic bacteria have an advantage since they can profit from leachates from the host macrophyte as well as exudates from surrounding algae and cyanobacteria (e.g. Søndergaard, 1983; Moran and Hodson, 1989; Mann and Wetzel, 1996). There are several records of high activities and growth rates in these communities (e.g. Kirchman *et al.*, 1984; Neely and Wetzel, 1997) but reports on the quantitative importance of epiphytic bacteria as compared to other littoral bacterial communities in the overall carbon turnover on a lake ecosystem scale are scarce.

Active versus non-active bacteria

There is increasing evidence that not all cells in natural bacterial communities are metabolically active (e.g. Winding *et al.*, 1994; del Giorgio and Scarborough, 1995). However, when calculating cell-specific properties of bacteria, e.g. specific growth rate, all bacteria are typically considered equally active. Consequently, variations in cell-specific properties may be masked when it is ignored that a considerable fraction of the bacteria is inactive, or even non-living. Most reports of the active fraction of aquatic bacteria focus on free-living bacteria (reviewed in del Giorgio and Scarborough, 1995; Sherr *et al.*, 1999). Active bacterial fractions in planktonic habitats range between a few percent and almost 100%. Studies on the active fraction in attached communities are comparatively few. In sediments, the total bacterial activity is generally low, considering the high bacterial abundance, which implies long doubling times or a small active fraction. Some studies report rather large fractions of active bacteria in sediment, ranging from 4 to 67%, which favors the hypothesis of long doubling times (Quinn, 1984; Flindt and Nielsen, 1992; Fischer and Pusch, 1999; van Duyl *et al.*, 1999; Proctor and Souza, 2001; Luna *et al.*, 2002). In epiphytic habitats, on the other hand, the high bacterial activity and short doubling times indicates a large fraction of metabolically active bacteria. Previous studies report active fractions between 31 to 70% of the total bacterial community (Quinn, 1984; Al-Hadithi and Goulder, 1989; Kang and Goulder, 1996).

Control of active bacteria

Three processes determine the fraction of active bacteria. First, the growth rate of active bacteria determines the production of new cells; second, the rate of inactivation and death; third, the loss rates from the active and inactive pools (del Giorgio and Scarborough, 1995). These processes are regulated by several mechanisms including temperature, grazing, viral infection, nutrient and carbon supply, all of which vary over time and between habitats.

The importance of temperature for production of new cells and activation of dormant cells has been thoroughly documented (e.g. Quinn, 1984; Tabor and Neihof, 1984; White *et al.*, 1991). At least on a seasonal scale, temperature may explain up to 80% of the variation in the fraction of active pelagic bacteria (Sommaruga and Conde, 1997; Jugnia *et al.*, 2000). This dependence on temperature disappears if shorter time spans are considered during which temperature is more uniform (del Giorgio and Scarborough, 1995; Smith, 1998). Then other factors such as substrate availability and grazing become more important.

The fraction of active bacteria has been shown to increase with nutrient concentrations in both lake, marine and estuarine systems (del Giorgio and Scarborough, 1995). Accordingly, there is a positive relationship between

system productivity, measured as chlorophyll *a*, and the fraction or abundance of active bacteria, supporting the importance of substrate supply (e.g. Søndergaard and Danielsen, 2001; Yager *et al.*, 2001). Interestingly, as del Giorgio and Scarborough (1995) point out, the fraction of active cells is generally larger in estuaries than in lakes with comparable nutrient, chlorophyll and DOC concentrations. In addition, lakes seem to have larger proportions of active bacteria than marine systems at comparable chlorophyll concentrations and total bacterial abundances. Hence, other factors besides substrate limitation seem to be important regulators of the proportion of active bacteria.

Selective grazing on the most active or dividing bacterial cells may have an impact on the active fraction. Flagellates and other micrograzers have been found to preferentially graze metabolically active cells (Sherr *et al.*, 1992; Gonzalez *et al.*, 1993; del Giorgio *et al.*, 1996b) with smaller active bacterial fractions as a consequence. Other studies show increasing active fractions in the presence of micrograzers due to changes in the bacterial species composition towards more grazing resistant forms (Sherr *et al.*, 1999; Berman *et al.*, 2001).

Viruses are very numerous in aquatic systems and are generally thought to be responsible for about 10-50% of the total bacterial mortality in surface waters (Fuhrman, 1999). Thus, viral infection may have a significant influence on bacterial production (Mathias *et al.*, 1995; Weinbauer and Höfle, 1998) and active fraction (Yager *et al.*, 2001).

Almost all studies on the control of the active bacterial fraction are pelagic studies. There are a few reports on attached bacteria. Starvation has been found to decrease the fraction of active bacteria in biofilms (Schaule *et al.*, 1993) and the active proportion increased with enrichment in both sediments (Flindt and Nielsen, 1992; Luna *et al.*, 2002) and on macrophytes (Kang and Goulder, 1996). The abundance or active fraction of bacteria is most likely regulated by the same processes in attached and pelagic communities, but the individual importance of the regulating processes may differ between habitats within a lake.

Other factors affecting attached communities

In contrast to pelagic microbial communities, attached communities may be subject to external factors influencing the structure of the biofilms and thus the biomass and activity of associated microbes. Water currents may reduce the thickness of the biofilm through sloughing and thereby change community structure and diffusion rates (Fischer, 2003).

Macro-invertebrate grazers, e.g. snails and some insect larvae, may have an effect on the whole attached community since the large size difference between the herbivore and the biofilm-associated microbes makes it difficult for the herbivore to graze on specific species. Thus, macro-invertebrate

grazers can have a direct effect on attached communities by reducing biofilm biomass through grazing (e.g. Lamberti, 1996). Macro-invertebrate grazers may also have an indirect effect disrupting the structure of the biofilm, which may alter the diffusion of nutrients and gases through the biofilm (Riber and Wetzel, 1987; Bothwell, 1989; Burkholder *et al.*, 1990) and/or change the species composition within the biofilm. In addition, fecal pellets or excretion of waste products by the macro-invertebrate grazers may change the nutrient availability for the biofilm-associated microbes (Sterner *et al.*, 1992; Elser and Hassett, 1994; MacKay and Elser, 1998; Kahlert and Baunsgaard, 1999). There are only few studies on the effect of macro-invertebrate grazers on attached bacteria. Bacteria are obviously consumed by macro-invertebrates (Lamberti and Resh, 1983; Mulholland *et al.*, 1991) but they may be assimilated less effectively than algae (Morales and Ward, 2000).

Questions addressed in this thesis

The role of attached bacteria in freshwater systems is the main focus of this thesis, especially the control of the active bacterial fraction and cell-specific activity of these bacteria compared to bacteria in other communities. The following questions are addressed:

- Are epiphytic bacteria important for the overall carbon turnover in littoral zones?
- Are there habitat-specific differences in bacterial traits?
- Do environmental fluctuations control the active bacterial fraction?
- Can macro-invertebrate grazers control benthic bacterial communities?

Methods

Most studies were conducted in the littoral zone of Lake Erken, a relatively large mesotrophic lake situated 60 km north of Stockholm, Sweden. The enclosure experiments in paper IV were performed in Lake Erken and in a shallow embayment at the Baltic Sea. The two sites were of similar productivity but had different macrograzer communities due to the salinity (~5 g kg^{-1}) of the coastal waters.

Bacterial abundance and biomass were determined using epifluorescence microscopy. The fluorogenic tetrazolium salt 5-cyano-2,3-ditolyltetrazolium chloride (CTC) was used in paper I, II and IV to detect bacterial cells with an active electron transport system (Rodriguez *et al.*, 1992). The samples were counterstained with SYTO 13 to determine total bacterial abundance (del Giorgio *et al.*, 1996a). In paper III, the Live/Dead *Bac*Light Bacterial Viability Kit was used to distinguish live cells, with intact cell membranes, from dead cells, with damaged cell membranes. In addition, 4´,6´-diamidino-2-phenylindole hydrochloride (DAPI) was used for total bacterial counts and a washing step was added, removing unspecific DAPI staining, to detect cells containing nucleoids (NuCC-method) (Zweifel and Hagström, 1995). The two latter methods are not proof of metabolic activity but rather indicate potential viability of a cell.

Artificial electron acceptors, tetrazolium salts, are common indicators of electron transport system (ETS) activity. Zimmerman *et al.* (1978) introduced the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) and Rodriguez *et al.* (1992) developed a method using CTC, 5-cyano-2,3-ditolyl tetrazolium chloride. In bacteria with an active ETS, the tetrazolium salt CTC is reduced to an insoluble red fluorescent product, which can be visualized with microscopy. Thus, bacteria obtained as active are separated from inactive by an arbitrary threshold that is constituted by the microscopically visible amount of reduced CTC in the cell. CTC is more directly coupled to respiratory activity than INT (Smith and McFeters, 1997). The CTC method often yields smaller fractions of active bacteria than e.g. microautoradiography or universal 16S rRNA probes (e.g. Karner and Fuhrman, 1997). Over the years there has been some criticism of the CTC-method e.g. that all bacterial strains are not capable of reducing CTC (Smith and McFeters, 1997), and that CTC can be poisonous to the bacteria (Ullrich *et al.*, 1996; Servais *et al.*, 2001). Sherr *et al.* (1999) showed that a number of taxonomically diverse bacterial strains are capable

of reducing CTC. They suggested that cells that are able to reduce detectable amounts of CTC constitute the most active cells in the bacterial community, and thus are responsible for the bulk of bacterial metabolism. Despite uncertainties on how widely applicable the CTC method is, it has been used to detect respiring bacteria in several different habitats over the last decade.

Both the Live/Dead *Bac*Light Bacterial Viability Kit and the NuCC-method have previously been used on planktonic bacteria. Apart from Paper III there is only one report on using these methods in sediment. Luna et al. (2002) used a method similar to the Live/Dead Kit as well as the NuCC-method on a coastal marine sediment profile.

Heterotrophic bacterial production was measured by incorporation of [^{3}H]thymidine into DNA (Bell, 1993) or [^{14}C]leucine (Kirchman, 1993) into protein, with some modifications described in detail in papers I-IV. In paper III, total sediment metabolism was estimated using a heat conduction multichannel microcalorimeter (Boström and Törnblom, 1990). Both bacterial production methods and heat calorimetry are compared and discussed in detail in paper III.

In addition to calculating bacterial specific growth rate using the total bacterial biomass (SGR_{total}), the specific growth rate of the active (SGR_{active}) or viable bacteria are reported in paper I and III. SGR_{active} represents the mean growth rate of the active bacteria, while SGR_{total} represents the mean growth rate of the total bacterial community including dormant and dead bacteria. Hence, calculating SGR_{active} provides a more specific way to describe bacteria dynamics.

An enclosure experiment was performed in order to study the effect of nutrient additions and grazing by macro-invertebrates on biofilm-associated bacteria and algae (Paper IV). Grazer presence was manipulated using metal-frame cages mounted on concrete plates and covered by a 1 mm screen. Nutrients were supplied with a granulose slow-release NPK-fertilizer adding nitrogen and phosphorus to the water column. Unglazed ceramic tiles were used as substrate and pre-colonized for 3 to 12 months to establish natural biofilm communities.

Results and discussion

Quantitative importance of epiphytic versus sediment and planktonic bacteria for the overall carbon turnover

In order to determine the quantitative importance of epiphytic bacteria for the overall carbon turnover, I compared the relative contribution of epiphytic bacteria on the submerged macrophyte *Ranunculus circinatus*, sediment and free-living bacteria to the total bacterial production.

Sediment bacteria generally dominated total bacterial biomass in the littoral zone. Although the epiphytic biomass on *R. circinatus* was ten times lower than the biomass of sediment bacteria, it often contributed at least equally to the total bacterial production (Figure 1, Paper I). Bacterioplankton generally contributed only a few percent to the overall carbon production. On a couple of occasions the contribution of the bacterioplankton was significantly higher and reached up to 35% of the total bacterial production. These results support the findings of Theil-Nielsen and Søndergaard (1999) that epiphytic bacteria can have an important role in the overall carbon turnover in littoral zones of lakes. Other studies report a dominance of sediment bacteria in the total bacterial production (Moriarty *et al.*, 1985; Fischer and Pusch, 2001; Stanley *et al.*, 2003), but these studies were performed in areas with lower macrophyte densities and thus fewer surfaces for bacterial colonization. In dense macrophyte stands (98% areal coverage) epiphytic bacterial production can be 2 to 7 times higher than bacterioplankton, while in lakes with lower macrophyte areal cover (20%) epiphytic and bacterioplankton production were equally high (Theil-Nielsen and Søndergaard, 1999).

Thus, in littoral zones with dense macrophyte stands epiphytic bacteria can contribute as much as or more than sediment bacteria to the total bacterial production and be quantitatively important in the overall carbon turnover in lakes.

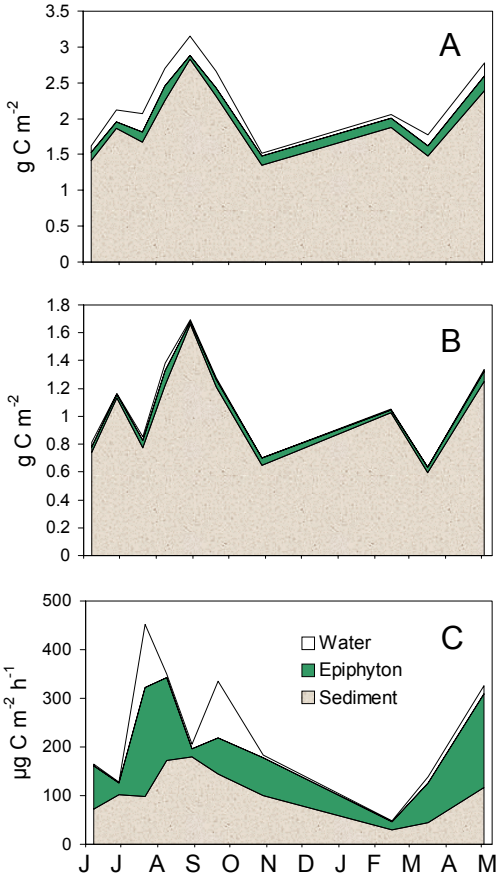

Figure 1. Contribution of different habitats (sediment, leaves on R. circinatus and water column) to the total (A) and active (B) bacterial biomass and bacterial production (C) per m^2 lake surface in the littoral zone of Lake Erken.

Active bacteria in different habitats

Between-habitat differences in active bacteria

In order to compare bacterial communities with variable bacterial densities in different habitats in Lake Erken, the fraction of bacteria reducing detectable amounts of CTC and bacterial cell-specific growth rates were estimated. There were considerable differences in the fraction of active bacteria between habitats in the littoral zone (Paper II). The sediments had the largest active fraction, followed by the *R. circinatus* leaves, and the water column with only a few percent active bacteria (Table 1, Paper II). There was a consistent trend of an increasing active proportion with increasing total abundance of the bacteria across all three habitats, as indicated by the log slope of

the regression of active bacteria as a function of total bacteria (Figure 2, Paper II). A larger active fraction of the bacteria at higher total bacterial abundance has been reported previously in studies of pelagic bacteria of lakes (del Giorgio and Scarborough, 1995; del Giorgio *et al.*, 1997) and coastal waters (Lovejoy *et al.*, 1996; Smith, 1998). The present results demonstrate that this trend extends across habitats. There is only one study analogous to this one, comparing the active fraction of bacteria in different habitats. Quinn (1984) studied the active fraction of bacteria in the sediment, on macrophyte leaves, and in the water column of a eutrophic lake. The INT and nalidixic acid techniques yielded active fractions from 10 to 32% in the sediments, 31 to 38% on the submerged macrophyte leaves, and 17 to 31% in the water column. Hence, the habitats did not differ as markedly as in Lake Erken. This may be due to more eutrophic conditions, since the proportion of metabolically active bacteria increases with lake productivity (del Giorgio and Scarborough, 1995). Possibly, the advantage of attached bacteria over free-living ones decreases with increasing overall nutrient availability, which would result in smaller between-habitat differences in

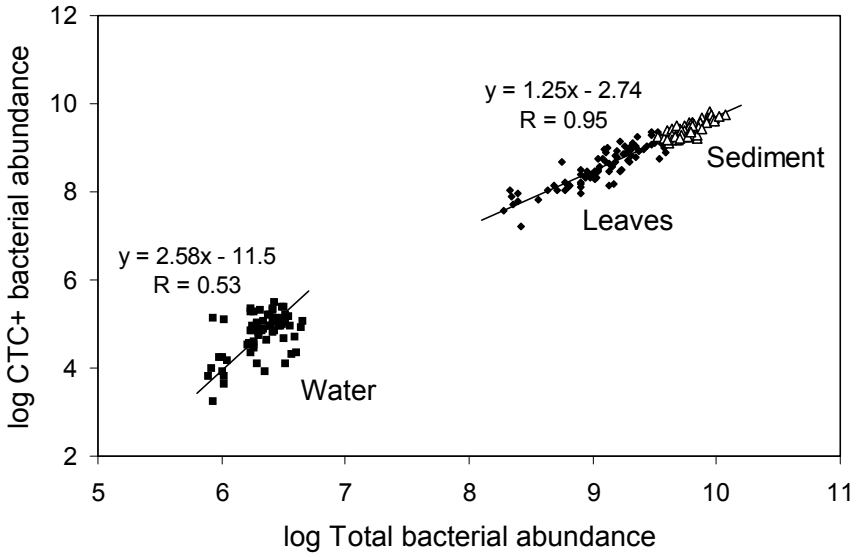

Figure 2. The abundance of active (CTC+) bacteria as a function of total bacterial abundance. Pooled data (n = 156) from the sediments and leaves of R. circinatus, and water column data (n = 66) expressed in comparable units, cells/gWW for leaves and sediment and cells/mL for water, in the littoral zone of Lake Erken.

Table 1. Seasonal mean ± sd (June 1999 to May 2000) in total and active (CTC+) bacterial abundance, active (CTC+) bacterial fraction, total and active (CTC+) bacterial biomass, bacterial production, and specific growth rate for the total and active (CTC+) bacteria in sediments, on *R. circinatus* leaves and in the water column in the littoral zone of Lake Erken. Within brackets is the factor of variation calculated as the max value/min value. The data for the sediments are given in gDW, for *R. circinatus* leaves in g leaf WW, and for the water column in L (n = 30. 27, and 29 for young, "middle-aged", and old leaves, respectively, n = 30 for the water column).

	Sediment	*R. circinatus* leaves			Water column
		young	"middle-aged"	old	
Total bacterial abundance[a] x 10^9	69±28 (5)	0.83±0.52 (12)	1.8±0.96 (9)	2.6±1.1 (4)	2.3±0.94 (5)
Active bacterial abundance[a] x 10^8	360±182 (6)	2.8±3.3 (111)	7.4±5.6 (25)	11±6.1 (9)	0.94±0.96 (178)
Active bacterial fraction (%)	50±8 (2)	32±17 (5)	39±20 (8)	40±14 (4)	4.6±4.2 (78)
Total bacterial biomass[b]	425±139 (4)	6.2±3.6 (8)	12±6.5 (7)	19±9.8 (6)	11±5.9 (13)
Active bacterial biomass[b]	223±83 (6)	2.0±2.0 (59)	4.8±3.3 (25)	7.0±4.3 (11)	1.0±1.2 (221)
Bacterial production[c]	0.24±0.13 (22)	0.026±0.026 (65)	0.077±0.073 (63)	0.16±0.15 (178)	0.020±0.033 (108)
Specific growth rate (total)[d]	0.013±0.007 (9)	0.11±0.09 (70)	0.14±0.11 (31)	0.23±0.26 (95)	0.035±0.045 (65)
Specific growth rate (active)[d]	0.027±0.014 (11)	0.42±0.37 (58)	0.42±0.38 (26)	0.63±0.94 (117)	1.5±1.8 (178)

[a] Units are cells (gDW or gWW or L)$^{-1}$ for sediment, leaves, and water, respectively.

[b] Units are µg C (gDW or gWW or L)$^{-1}$ for sediment, leaves, and water, respectively.

[c] Units are µg C h^{-1} (gDW or gWW or L)$^{-1}$ for sediment, leaves, and water, respectively.

[d] Units are day^{-1}.

more productive systems. Hence, in addition to between-habitat differences within single aquatic systems, these differences may change along gradients of productivity.

Specific growth rates for active bacteria (SGR_{active}) also differed between habitats (Table 1, Paper I). The sediment had the lowest SGR_{active} corresponding to a doubling time of 37 days (annual mean), the doubling time of the active bacteria on *R. circinatus* leaves was 1–2 days, while for active planktonic bacteria the doubling time was 16 hours. The high specific growth rate of planktonic bacteria would not have been detected using the total bacterial biomass in the calculations, which would generate specific growth rates in the same size range as for sediment bacteria (Table 1, Paper I). Thus, estimates of productivity and specific growth rate may be highly biased if the non-viable fraction of the bacterial community is not considered. In addition, the extent of the underestimation of specific growth rates will vary between bacterial communities with different fractions of active bacteria.

Within-habitat differences in active bacteria

To be able to study the effect of leaf age on bacterial traits, I collected leaves of different age from *R. circinatus*. There was no significant effect of leaf age on the fraction of active bacteria on *R. circinatus*. The mean fraction of active bacteria tended to be smaller on young leaves compared to "middle-aged" and old leaves (Table 1, Paper I) which may result from shorter colonization time of bacteria for the young leaf substrate. Hence, the bacterial community on the young leaves may be in another successional stage than the bacterial community on the older leaves. In addition, there was no difference in specific growth rate of active bacteria between leaves of different age (Table 1, Paper I). This may reflect similar conditions for active bacteria on all leaves.

Sediment samples from different depths below the sediment surface were collected from the profundal zone of Lake Erken to examine the changes in bacterial activity and distribution of viable bacteria with sediment depth. The sediments had a large (59 ± 2%, mean ± SD) fraction of viable bacteria (scored with Live/Dead Bacterial Viability Kit) (Paper III). Interestingly, the viable fraction did not change with sediment depth, thus there was no accumulation of dead cells in deeper sediments, a fact also reported by Miskin *et al.* (1998). In deep sediments of the Pacific Ocean there was a constant percentage of viable cells in a dividing stage down to 518 m (Parkes *et al.*, 1994). The specific growth rate for viable bacteria (scored with Live/Dead Bacterial Viability Kit) decreased with sediment depth (Paper III), which may be caused by a more refractory carbon source or lower nutrient availability (Paper III). The lack of within-habitat differences in the viable bacte-

rial fraction implies that there is something else apart from resource supply and/or quality regulating the active bacterial fraction.

Environmental fluctuations versus active bacterial fraction

In figure 3, I connect the large differences in the active bacterial fraction between habitats to the environmental fluctuations in these habitats. Environmental fluctuations include both chemical and biological changes i.e. carbon and nutrient supply and/or quality, changes in grazing pressure or viral lysis, and also physical factors like wave action or tidal water. The pelagic habitat is a comparatively dilute environment for bacteria, with large fluctuations in carbon and nutrient supply. This results in large variations in bacterial production and specific growth rates over the time period of one year (Table 1). Grazing and viral lysis are generally important regulators of bacterial mortality in the pelagic zone (e.g. Berninger *et al.*, 1991; Sanders *et al.*, 1992; Fuhrman, 1999). Selective grazing on active bacterial cells may be an additional reason for the large fluctuations in active bacterial abundance and biomass and the relatively small active bacterial fraction often found in planktonic habitats.

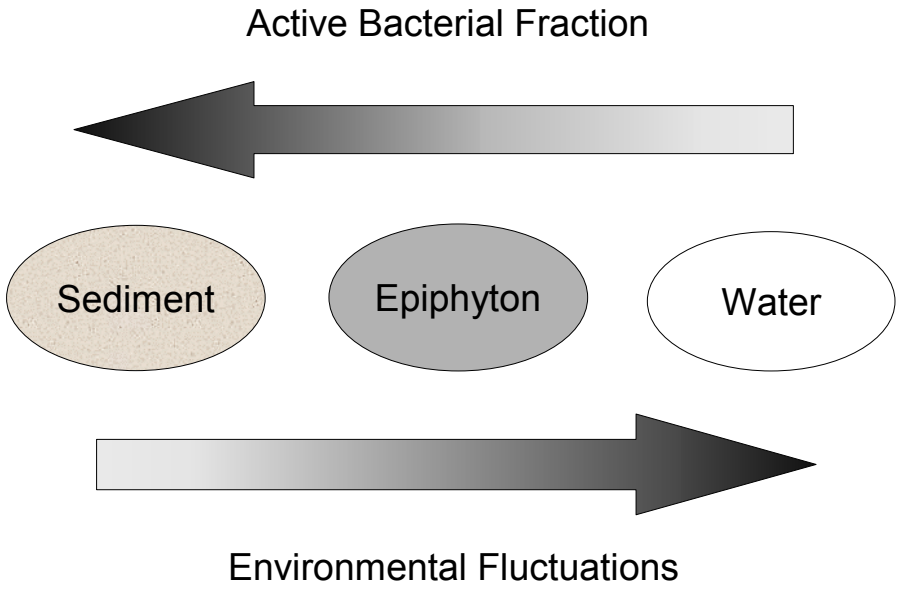

Figure 3. Active bacterial fraction versus environmental fluctuations (including chemical, biological and physical factors) in different habitats.

The epiphytic habitat provides an easily available carbon source supplied by the host macrophyte and biofilm-associated algae in close proximity to the bacteria, resulting in relatively high specific growth rates for the bacteria in these habitats. As in the pelagic habitat, there are rather large annual variations in bacterial activity but epiphytic abundance and biomass are relatively stable (Table 1). Since the host macrophyte probably generates a considerable amount of carbon and nutrients, which is readily assimilated by epiphytic bacteria, the epiphytic production is dependent on the annual fluctuations in primary production, i.e. exudate production of the macrophyte and biofilm-associated algae. Thus, the relatively high factor of variation in bacterial epiphytic activity may reflect fluctuations in exudate production.

The sediments offer a rather stable environment to the bacteria with a continuous but relatively recalcitrant carbon source. This results in low specific growth rates in bacterial sediment communities compared to pelagic and epiphytic habitats. The annual factor of variation was low for all bacterial traits in the sediments (Table 1).

The relatively large fraction of active bacteria in both littoral and profundal sediment, and the fact that there was no difference in the viable fraction of bacteria with sediment depth, indicates that in these habitats resource quality and supply are not the main/only regulator of the active fraction. There are some indications that grazing and viral lysis may not be important regulators of bacterial mortality in attached communities. High densities of protozoa have been recorded in both sediments (e.g. Hondeveld *et al.*, 1994) and epiphytic biofilms (Neckles *et al.*, 1994). However, most grazing studies show a minor impact of flagellate grazing on bacterial sediment communities (Hamels *et al.*, 2001). Viruses are numerous in freshwater sediments but the virus-to-bacteria ratio has generally been close to one or lower, i.e. an order of magnitude lower than what is typical in pelagic environments (Maranger and Bird, 1996; Ricciardi-Rigault *et al.*, 2000). Accordingly, the two mortality factors considered to be responsible for most of the mortality of bacteria in the water column may not be as effective in attached bacterial communities. This may contribute to the persistence of intact cells throughout the studied sediment profile and the general large fraction of active or viable bacteria in sediments and epiphytic biofilms. If grazing and viral lysis were important for bacterial mortality in sediments, there would most likely be an accumulation of dead bacteria-like particles with sediment depth. Thus, the small annual fluctuations in bacterial abundance and biomass in attached communities (Table 1) may be due to low or moderate grazing pressure.

In Lake Erken, the water column with relatively large environmental fluctuations, has a small active fraction while the habitats with attached bacterial communities, epiphytic and sediment, have large active fractions and most likely less environmental fluctuations. Hence, the active bacterial fraction increases when environmental fluctuations are small, as illustrated in the

conceptual model in figure 3. In a diatom-inhabited intertidal mudflat the effect of tidal water, i.e. fluctuations in water content of the sediment, on bacterial activity was studied. The surface sediment, which was emerged for several hours every day, had smaller active bacterial fraction than the subsurface sediment further down, which had no exposure to illumination or air (van Duyl *et al.*, 1999). Although the surface sediment profited from diatom exudates not available to the subsurface bacterial community, the fraction of active bacteria was larger in deeper unexposed sediment, possibly due to a less fluctuating environment. Accordingly, Proctor and Souza (2001) found larger active bacterial fractions in anoxic sediments possibly due to the absence of benthic invertebrates and hence absence of grazing and bioturbation. However, Mermillod-Blondin *et al.* (2004) report a positive effect of bioturbation on the fraction of active bacteria.

The effect of macro-invertebrate grazers on attached bacterial communities

As stated in the introduction, most aquatic studies focus on pelagic systems. This also applies to aquatic food webs where the contributions of benthic biota are seldom included in descriptions of lake dynamics (Vadeboncoeur *et al.*, 2002). Benthic food web studies often describe the effect of enrichment (bottom-up) or the effect of herbivory (top-down) on periphytic algae. Although the algae live in close association with bacteria, protozoa, fungi, and small meiofauna these heterotrophic organisms are seldom included in reports on the control of benthic food webs.

The effects of nutrient additions and grazing by macro-invertebrates on biofilm-associated algae and bacteria were studied by performing an enclosure experiment on three occasions from early spring to summer in mesotrophic Lake Erken and Väddö, at the Swedish Baltic coast (Paper IV). Grazing by macro-invertebrates did not control the bacterial biomass in Lake Erken or Väddö, although, the combination of nutrient additions and grazer presence could on some occasions reduce the bacterial biomass in Lake Erken. Instead, the presence of macro-grazers had a positive effect on specific bacterial activity in Lake Erken (Paper IV). The exact nature of this effect could not be clarified, but most likely the grazers changed the nutrient availability for the bacterial community. The macro-invertebrate grazers changed the algal community by selectively grazing on large canopy algae and thus changed the structure and species composition of the biofilm (Hillebrand and Kahlert, 2001). They also decreased C:P and N:P ratios of the biofilm thereby changing the nutrient conditions for the bacteria (Hillebrand and Kahlert, 2001). Whether the improved conditions were due to physical disruption of the biofilm or caused by the release of fecal pellets remains unclear.

In addition, the presence of macro-invertebrates changed the relationship between biofilm-associated bacteria and algae. It is generally assumed that these algae and bacteria have a mutualistic relationship, readily exchanging nutrients and gases, and thus maintaining high productivity in the biofilm (Haack and McFeters, 1982; Wetzel, 1993; Neely and Wetzel, 1995). We found a correlation between algae and bacteria at both sites, but this correlation weakened in the presence of macro-invertebrate grazers (lower r-values in regression analysis, Paper IV). Hence, macro-invertebrate grazers may indirectly reduce the importance of internal nutrient regeneration within the biofilm by increasing nutrient availability through feeding activities and /or fecal pellets production.

General discussion

Overall, the results presented in this thesis demonstrate that most bacterial biomass and production in shallow lakes is associated with surfaces. They also show that in littoral zones with dense macrophyte stands epiphytic bacteria may contribute as much as or more than sediment bacteria to the overall carbon turnover in lakes (Paper I). The success of these bacteria results in high specific growth rates and that a large fraction of the bacteria on macrophytes are metabolically active. The high specific growth rates are most likely due to the continuous supply of exudates from the host macrophyte and biofilm-associated algae, which are readily assimilated by the epiphytic bacteria. Low resource availability may explain the comparatively low specific growth rates of the sediment bacteria but not the large active bacterial fraction in this habitat. Four main processes regulating the active fraction were initially mentioned in this thesis, i.e. temperature, grazing, viral lysis, and nutrient and carbon supply. If resource availability/quality was the most important regulator, the fraction of active bacteria would most likely be very small in the sediment habitat due to the relatively recalcitrant carbon source present. In addition, temperature fluctuations did not cause the between-habitat differences in active bacterial fraction in Lake Erken since these differences were present although the temperature was constant. Hence, I conclude that the differences in active bacterial fraction may be due to differences is grazing pressure and/or viral lysis between habitats in Lake Erken. The influence of selective grazing on active pelagic bacteria has been documented in several studies. Usually, grazing results in a decrease in the fraction of active bacteria (Sherr *et al.*, 1992; Gonzalez *et al.*, 1993; del Giorgio *et al.*, 1996b) but grazing can also trigger a change in bacterial composition towards more grazing resistant forms and an increase in the active bacterial fraction (Sherr *et al.*, 1999; Berman *et al.*, 2001). Some reports indicate that grazing and viral lysis may not be as important for bacterial mortality in attached bacterial communities (Hamels *et al.*, 2001), which may result in larger active bacterial fractions in these habitats.

The findings of this thesis and the work of other scientists are summarized in the conceptual model in figure 3 where environmental fluctuations (fluctuations in chemical, biological and physical factors) is related to the fraction of active bacteria. In conclusion, increasing environmental fluctuations result in smaller active bacterial fractions.

The high rates of bacterial production in sediments and on macrophytes, as well as on other surfaces, have large implications for the flux of carbon and nutrients between benthic and pelagic zones and for the use of bacterial production by consumers (reviewed in Vadeboncoeur *et al.*, 2002). In the enclosure experiment performed in Lake Erken and Väddö, the macro-invertebrate grazers did not control the bacterial biomass. Instead, the presence of macro-invertebrate grazers had a positive effect on bacterial carbon production and specific growth rate, possibly by changing nutrient availability in the biofilm through their feeding activities. There were also indications that the macro-invertebrate grazers changed the relationship between bacteria and algae. I show that macrograzers may affect the production of bacterial carbon in the benthic zone mainly by changing nutrient availability and interactions within the biofilm community.

I conclude that epiphytic bacteria can contribute significantly to the total bacterial production in the littoral zone. Thus, attached bacteria are indeed responsible for most of the carbon turnover in shallow lakes. I found large differences in the active fraction between habitats in Lake Erken, while the within-habitat variation was small. I suggest that the degree of environmental fluctuations, including chemical, biological and physical factors, within a habitat regulates the fraction of active bacteria. Thus, small environmental fluctuations allow a large active bacterial fraction, while the active fraction is constrained when the environmental fluctuations are large.

Svensk sammanfattning (Summary in Swedish)

Fastsittande bakterier är viktiga för energiomsättningen i sjöar

Under många år ansågs mikroorganismer fastsittande på olika ytor, som exempelvis vattenväxter, stenar och sediment, i strandzoner och våtmarker oviktiga för näringsomsättningen i akvatiska system. Nu tyder allt på att dessa mikrosamhällen ofta reglerar energi- och näringsflödena i sötvatten. Eftersom de flesta av världens sjöar är grunda utgör strandzonen en stor del av sjöarnas totala yta, och därför är den mesta bakterieproduktionen i grunda sjöar associerad till fastsittande bakterier.

Fastsittande bakterier lever nära alger, cyanobakterier, svampar och andra mikroorganismer som tillsammans bildar en biofilm. Dessa organismer kan dra nytta av varandra och utbyta näringsämnen och energi. Bakterierna förser genom sina nedbrytningsprocesser algerna och cyanobakterierna med näring. Algerna och cyanobakterierna avger kolföreningar, som utgör restprodukter från deras fotosyntes. Bakterierna tar upp dessa och använder dem som energikälla.

Bakterier associerade till vattenväxter (epifyter) har en fördel gentemot bakterier i andra biofilmer eftersom de förutom ett internt samarbete med alger även kan dra nytta av kolföreningar som läcker från vattenväxten. Dessa bakteriesamhällen har uppvisat höga aktiviteter och höga tillväxthastigheter. Trots det så finns det väldigt få studier om hur viktiga epifytbakterier är för den totala energiomsättningen i sjöar. Mina resultat visar att epifytbakterier bidrar minst lika mycket till den totala bakteriella produktionen som sedimentbakterierna. Det bekräftar att i grunda sjöar med hög andel vattenväxter kan epifytbakterier spela en viktig roll för omsättningen av energi- och näringsämnen.

Alla bakterier är inte aktiva

Alla celler i ett bakteriesamhälle är inte metaboliskt aktiva. Några celler befinner sig i ett vilostadium och andra kan till och med vara döda. Det är naturligtvis bara de bakterier som är aktiva som kan delta i bakteriella processer. Om inte hänsyn tas till att alla bakterier i ett samhälle inte är aktiva eller levande får man en sned bild av exempelvis tillväxthastigheten hos bakterierna.

Tidigare studier av olika bakteriesamhällen rapporterar stora variationer i andelen aktiva bakterier. I vattnet varierar andelen aktiva från endast ett par procent till nästan 100%. Fastsittande bakteriesamhällen tenderar att ha större andel aktiva. I sedimenten rapporteras från 4 till 67% och epifytsamhällen har visat på andelar från 31 till 70%.

I sjön Erken fann jag stor skillnad i andelen aktiva bakterier mellan olika livsmiljöer för bakterier. I sedimenten var 50% av bakterierna aktiva, 37% var aktiva på vattenväxten hjulmöja, *Ranunculus circinatus*, och i vattnet endast 5%. Andelen aktiva ökade med det totala antalet bakterier i samhället, något som även rapporterats från tidigare studier av frilevande bakterier sk. bakterieplankton.

Faktorer som påverkar andelen aktiva bakterier

Andelen aktiva bakterier i ett bakteriesamhälle kan regleras av flera faktorer; temperatur, bakteriedödlighet p.g.a. att de äts upp (betas) eller infekteras av virus, samt tillgången av näring och energi. Både temperatur och tillgång av näring och energi har visat sig ha en positiv inverkan på andelen aktiva bakterier, men i vissa fall stämmer inte detta. Sjöar har exempelvis generellt högre andel aktiva bakterier än marina system trots liknande näringsförhållanden. I dessa fall regleras andelen aktiva bakterier uppenbarligen av något annat än tillgång till energi och näring som exempelvis betning eller virus infektioner i bakterieceller.

Vissa encelliga djur som äter bakterier t.ex. flagellater väljer att selektivt konsumera aktiva bakterier. Detta leder vanligtvis till en lägre andel aktiva bakterier i ett bakteriesamhälle. Ibland kan dock andelen aktiva bakterier öka eftersom betning kan leda till att bakteriearter som är resistenta mot betning dominerar i bakteriesamhället. Mellan 10 och 50% av bakteriedödligheten hos bakterieplankton beror på infektioner av virus. Förekomsten av virus kan därför också vara av stor betydelse för regleringen av andelen aktiva bakterier.

Nästan alla tidigare studier om hur den aktiva andelen bakterier regleras är gjorda på bakterieplankton. Andelen aktiva regleras sannolikt på liknande sätt vare sig bakteriesamhället är fastsittande eller frilevande, men vikten av de individuella processerna kan variera mellan olika livsmiljöer för bakterier i en sjö.

Miljöbetingade fluktuationer kontra andelen aktiva bakterier

De stora skillnaderna i andelen aktiva bakterier mellan olika livsmiljöer för bakterier i sjön Erken kan inte förklaras av skillnader i temperatur eller tillgång på näring eller energi. Prover från de olika bakteriesamhällena togs vid samma temperatur och visade ändå stora skillnader i andelen aktiva. Sedimentbakterierna hade alltid större andel aktiva än epifytbakterierna i Erken

trots att nedbrytningen av kolföreningar generellt sker långsammare i sediment. Den höga andelen aktiva bakterier i sediment beror alltså på någon annan faktor som t.ex. betning eller virusinfektioner. Vissa studier tyder på att betning och virusinfektioner inte är lika omfattande i fastsittande bakteriesamhällen som i frilevande. Lägre betningstryck på sedimentbakterier i jämförelse med bakterieplankton kan förklara varför andelen aktiva bakterier är högre i sediment.

Generellt utgör sediment en stabilare miljö för bakterier jämfört med den fria vattenmassan. I sedimenten har bakterierna tillgång till ett konstant flöde av näring och energi genom sina nedbrytningsprocesser. Bakterieplankton lever i en miljö med ständiga fluktuationer i näringstillgång och betningstryck. I figur 3 kopplas andelen aktiva bakterier samman med graden av miljöbetingade fluktuationer. I Erken har vattenmassan, som alltså är en ständigt fluktuerande miljö, en låg andel aktiva, medan sedimenten, som bidrar med en mer stabil miljö, har en hög andel aktiva bakterier. Miljöbetingade fluktuationer inkluderar kemiska, biologiska eller fysiska processer som exempelvis näringstillgång, betning eller tidvatten. Min hypotes är alltså att en ständigt fluktuerande miljö leder till en låg andel aktiva bakterier.

Större vattenlevande djur och insekter kan påverka fastsittande bakteriesamhällen

Biofilmer kan även betas av större djur och insekter (makrobetare) som exempelvis insektslarver, vattengråsuggor eller snäckor. Det finns endast få studier som rapporterar om effekten av makrobetare på bakterier som är associerade till biofilmer. Vissa resultat tyder på att även om bakterierna konsumeras av makrobetarna så kanske de inte assimileras lika effektivt som exempelvis alger.

Mina studier visar att makrobetarna inte har någon större effekt på den totala bakteriebiomassan i biofilmer. Däremot påverkas bakteriernas aktivitet positivt i närvaro av makrobetare. Detta kan ske genom att makrobetarna förbättrar näringstillförseln till bakterier och alger i biofilmen. Då betarna konsumerar biofilmen bildas kanaler som gör att näring i vattnet lättare kan nå bakterierna inbäddade i biofilmen. Dessutom ”gödslar” betarna biofilmen genom att avge näringsrika exkrementer.

Även samarbetet mellan bakterier och alger kan förändras i närvaro av makrobetare. Mina resultat tyder på att makrobetare, möjligen genom att förbättra näringstillförseln i biofilmen, gör att utbytet av energi och näring mellan alger och bakterier blir mindre viktigt för den bakteriella aktiviteten i biofilmer.

Sammanfattningsvis...

...visar mina resultat att det kan vara stor skillnad i andelen aktiva bakterier mellan olika livsmiljöer för bakterier i en sjö. Som nämndes inledningsvis, regleras andelen aktiva bakterier av faktorer som temperatur, bakteriedödlighet p.g.a. betning eller virusinfektion, samt tillgången av energi- och näringsämnen. Vikten av dessa faktorer för andelen aktiva bakterier varierar sannolikt mellan olika livsmiljöer för bakterier. Min hypotes är att det är graden av fluktuationer hos dessa faktorer, samt även fluktuationer hos fysiska processer, som leder till variationen i andelen aktiva bakterier mellan olika livsmiljöer. Livsmiljöer med endast små miljöbetingade fluktuationer leder till att bakteriesamhället har en stor andel aktiva bakterier, medan livsmiljöer med stora fluktuationer har en mindre andel aktiva bakterier.

Dessutom bekräftar mina studier att fastsittande bakteriesamhällen står för den mesta bakterieproduktionen i grunda sjöar och är sannolikt mycket viktiga för omsättningen av energi- och näringsämnen i sjöar.

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