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Factors influencing the biogeography of bacteria in fresh waters - a metacommunity approach

JÜRIG BRENDAN LOGUE



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Abstract

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One of ecology's primary goals is to comprehend biodiversity and its patterns of distribution over space and time. Since microorganisms play a pivotal role in key ecological processes, the diversity of microbial communities may have important implications for the stability and functioning of Earth's ecosystems. Thus, it is of utmost importance to develop a theoretical foundation but also a conceptual understanding for the mechanisms that generate and maintain microbial diversity.

The aim of this thesis is to investigate to what extent local freshwater bacterioplankton diversity, i.e. richness and community composition, is structured by local environmental interactions and/or regional processes. The key objective is to identify ecological linkages between lake bacterioplankton and bacterial communities in connected streams and the surrounding terrestrial landscape, thereby applying a metacommunity approach. To do so, I studied several natural lake bacterioplankton assemblages within different regions of Sweden and assessed both local environmental properties and regional parameters (e.g. dispersal, landscape position). The genetic composition of freshwater bacterioplankton diversity was determined by means of terminal-restriction fragment length polymorphism or 454 pyrosequencing.

From the review on the biogeography of bacterioplankton in inland waters it became clear that microbial diversity and its spatial distribution are governed by a complex interplay of both local and regional drivers. In one case, freshwater bacterioplankton communities were structured by local environmental conditions rather than by regional dispersal processes. These local environmental conditions seemed to be equally important in controlling both the total bacterioplankton community and its active fraction. In a study of bacterioplankton communities from five different regions, locally abundant aquatic bacteria were shown to be also regionally widespread, a pattern predicted by neutral theory. Yet, this degree of similarity decreased with increasing environmental heterogeneity. In another study, bacterioplankton richness was controlled mostly by nutrient content, indicating that productivity exerted influence on bacterioplankton richness. However, landscape position and productivity covaried, suggesting that the landscape dictates environmental properties, which then directly structure local bacterioplankton richness. Finally, a review synthesising results from empirical metacommunity approaches and comparing these to theory showed that yet a gap between empirics and theory exists. To conclude, local bacterioplankton diversity appeared to be mainly structured by local environmental properties. However, signatures of neutral processes driving local bacterioplankton community assembly were also recorded.

Keywords: bacteria, diversity, richness, community composition, dispersal, metacommunity, fresh water, biogeography

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"I make no apologies for putting microorganisms on a pedestal above all other living things. For, if the last blue whale choked to death on the last panda, it would be disastrous but not the end of the world. But if we accidentally poisoned the last two species of ammonia oxidizers, that would be another matter. It could be happening now and we wouldn't even know... ". (2006, Tom Curtis)

"A lake is the landscape's most beautiful and expressive feature. It is Earth's eye; looking into which the beholder measures the depth of his own nature". ("Walden", 1953, Henry David Thoreau)

To my family

List of Papers

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

- I Logue, J.B., Lindström, E.S. (2008) Biogeography of bacterioplankton in inland waters. *Freshwater Reviews*, 1(1): 99–114.
- II Logue, J.B., Mouquet, N., Peter, H., Declerck, P., Flohre, A., Gantner, S., Gültow, N., Hörtnagel, P., Meier, S., Pecceu, B., Hillebrand, H. Empirical approaches to metacommunities – a review and comparison to theory. Submitted to *Trends in Ecology and Evolution*.
- III Logue, J.B., Lindström, E.S. (2010) Species sorting affects bacterioplankton community composition as determined by 16S rDNA and 16S rRNA fingerprints. *The ISME Journal*, 4(6): 729–738.
- IV Östman, Ö., Drakare, S., Kritzberg, E.S., Langenheder, S., Logue, J.B., Lindström, E.S. (2010) Regional invariance among microbial communities. *Ecology Letters*, 13(1): 118–127.
- V Logue, J.B., Langenheder, S., Andersson, A.F., Bertilsson, S., Drakare, S., Lanzén, A., Lindström, E.S. Productivity influences freshwater bacterioplankton richness in oligotrophic lakes. Manuscript.

Reprints of papers I, III and IV were made with kind permission from the publishers Freshwater Biological Association, Nature Publishing Group and Wiley InterScience, respectively.

List of additional Papers

In addition to the papers that form the basis of this thesis, the author has made a substantial contribution to the following other papers.

- Logue, J.B., Bürgmann, H., Robinson, C.T. (2008) Progress in the Ecological Genetics and Biodiversity of Freshwater Bacteria. *BioScience*, 58(2): 103-113.
- Lymer, D., Logue, J.B., Brussaard, C.P., Baudoux, A.-C., Vrede, K., Lindström, E.S. (2008) Temporal variation in freshwater viral and bacterial community composition. *Freshwater Biology*, 53(6): 1163-1175.
- Berga, M., Logue, J.B., Lindström, E.S., Tranvik, L.J., Steger, K. Similar trends in bacterial community composition detected by phenotypic and genotypic fingerprinting in lake sediments. Submitted to *Aquatic Sciences*.
- Lindström, E.S., Logue, J.B., Andersson, E., Drakare, S., Johansson, J., Lymer, D., Östman, Ö. Multiple mechanisms shape microbial communities in lakes. Manuscript.
- Östman, Ö., Drakare, S., Kritzberg, E.S., Langenheder, S., Logue, J.B., Lindström, E.S. Spatial distances, environmental variation and the degree of community invariance among aquatic microbial communities. Manuscript.

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Abbreviations

16S rDNA	16S rRNA gene
BCC	Bacterioplankton Community Composition
BR	Bacterioplankton Richness
Chl-a	Chlorophyll-a
DNA	Deoxyribonucleic Acid
DDH	DNA-DNA Hybridisation
DOM	Dissolved Organic Matter
LCN	Lake Chain Number
OTU	Operational Taxonomic Unit
PC1	Principal Coordinate 1
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
TAR	Taxa-Area Relationship
TOC	Total Organic Carbon
Tot-N	Total Nitrogen
Tot-P	Total Phosphorus
t-RFLP	Terminal-Restriction Fragment Length Polymorphism
SSU rRNA	Small Subunit of Ribosomal Ribonucleic Acid
WRT	Water Residence Time

1. Introduction

1.1 General Background

Earth is teeming with microbial life-forms. Invisible to the naked eye, microorganisms occupy any and every imaginable habitat on Earth, in doing so defining the outer limits of life. Not only are microbes¹ ubiquitous, but they are also among the most abundant organisms on Earth. One millilitre of fresh water, for instance, can contain more than a million microbial cells (Whitman *et al.* 1998). It has been estimated that Earth is inhabited by more than $4\text{--}6 \times 10^{30}$ microbial cells (Whitman *et al.* 1998). That is, there are 10^9 times more microbial organisms on Earth than there are stars in the vastness of the universe. In addition, the microbial world is extremely diverse, in fact, microbial diversity is assumed to be unprecedented, to be unrivalled in the biological world. Estimates for the total number of microbial species on Earth range from 10^3 to 10^9 (Pedrós-Alió 2006). However, more important and, perhaps, even more remarkable than these mere numbers is the metabolic diversity of microorganisms. Microbes play a pivotal role in key ecological processes, sustaining almost every other form of life on Earth. They are of special importance to human life, providing services with regard to human health, biotechnology, agriculture and the food industry. Moreover, they act as reservoirs for the discovery of novel metabolic pathways and potentially useful genes for medicine and biotechnology.

1.2 Microbial Diversity

Microbial diversity defines the variety of microorganisms at the genetic, species, and ecosystem levels. It encompasses the ecological complexity in which microbial organisms occur and the ecological processes of which they are part. Thus, microbial diversity comprises aspects of richness, evenness, composition, and function.

The evolutionary history of microbial organisms mirrors the evolutionary history of Earth itself. These organisms have been thriving on Earth for over

¹ Microbe, microbial organism, microorganism. The term generally denotes members of the domains Bacteria and Archaea, as well as microscopic members of the domain Eukarya. At times, defined as having a mass of $<10^{-5}$ g and a length of $< 500\mu\text{m}$ (Madigan & Martinko 2006).

four fifth of its existence. Being a product of roughly 3.8 billion years of evolution, microbial diversity outpaces that of all other organisms on Earth (Forney *et al.* 2004). However, with several thousand microbial species currently known and described (Garrity 2005) (<http://www.bacterio.cict.fr/number.html>), the extent of microbial diversity is yet a matter of controversial dispute (Curtis & Sloan 2004; Curtis *et al.* 2006; Pedrós-Alió 2006). Basically, there are two diametrically opposing views: there are those who believe that microbial diversity is small and those who think it is large.

Those who believe that microbial diversity is small, i.e. in the order of 10^3 – 10^4 microbial taxa globally, base their argumentation on a fact and its corollary. The fact is that microorganisms are small and extremely abundant. They are, further, assumed to proliferate rapidly, disperse readily and unlikely to go extinct (Finlay *et al.* 1996; Finlay & Clarke 1999; Fenchel & Finlay 2004; Fenchel 2005a). Since microorganisms are believed to be unhindered by geographic boundaries, dispersal limitation is expected to be inexistent for microbial organisms, i.e. they can occur anywhere on Earth, presuming that specific habitat requirements are met: hence, the long-standing dictum among microbiologists that “everything is everywhere, but the environment selects” (Beijerinck 1913; Baas-Becking 1934). The corollary states that because allopatric speciation, i.e. speciation that takes place when a geographical barrier separates two populations of the same species, is unlikely, β -diversity² will be low and, as a consequence thereof, global diversity will only be marginally higher than local diversity (Finlay 2002). However, allopatry is not a must for microbial speciation (e.g. Rosenzweig *et al.* 1994). Given that the corollary is contradicted with the repeated appearance of new sequences from molecular surveys, Fenchel (2005b) put forth two arguments about why molecular surveys overestimate microbial diversity. The first is that molecular surveys always claim to have retrieved new sequences, which, in fact, might belong to already described microbial organisms that have not yet been sequenced. This indeed remains a possibility but with current sequencing power this question should be solved within no time. Secondly, according to Fenchel (2005b) the variation found in ribosomal ribonucleic acid (rRNA) gene sequences “...does not necessarily reflect phenotypic differentiation in terms of morphological or physiological properties”, implying that the diversity of sequences reported from molecular surveys does not really reflect the ‘true’ diversity. Though, whenever the similarity of small subunit (SSU) rRNA gene sequences from microorganisms has been compared with other taxonomic characters, SSU rRNA gene sequences from microbes have been shown to be extremely conservative (Rossello-Mora & Amann 2001). Hence, SSU rRNA gene sequences most probably underestimate microbial diversity and not the other way around (Box 1).

² β -Diversity is a measure of biodiversity, which compares species diversity between ecosystems or habitats. It is influenced by the turnover of species among habitats.

Box 1. The Bacterial Species Concept

Discussions about microbial diversity imply the existence of a theory-based species concept. However, microbial species demarcation is more arbitrary compared with delineating species for higher organisms. Genetic diversity within bacterial species, for instance, is not constrained by the cohesive force of genetic exchange as it is within sexually reproducing organisms, such as animals and plants.

A bacterial species or taxon is, at present, described as “a category that circumscribes a (preferably) genomically coherent group of individual isolates/strains sharing a high degree of similarity in (many) independent features, comparatively tested under highly standardised conditions” (Rossello-Mora & Amann 2001). In practice, a bacterial species is often defined simply as a group of strains exhibiting more than 70% DNA-DNA-hybridisation (DDH) similarity and more than 97% of 16S rRNA gene sequence identity (Rossello-Mora & Amann 2001; Stackebrandt *et al.* 2002). Strains that show >97% 16S rRNA gene sequence similarity yield DDH similarities ranging from <20% to 70%. By contrast, no two strains of >70% DDH similarity show a 16S rRNA gene sequence identity of <97%. Thus, taxa based on 97% 16S rRNA gene sequence identity obviously underestimate the number of microbial species.

Diversity can, however, be calculated with other units besides that of a species or taxon. Taxonomic operational units (OTUs) cluster individuals into non-overlapping classes according to a consistent classification criterion. Generally in microbiology, OTU clustering is carried out at a 16S rRNA gene sequence similarity of 97%.

Note that bacterial species delineation is not defined by a theory-based concept but rather as a more practical necessity, thereby entirely omitting ecological species properties (see Cohan 2001 for an approach integrating ecological species properties). Yet, a species concept is central to achieving a predictive understanding of the composition, structure, and function of microbial communities.

Those who believe that microbial diversity is large (10^6 – 10^9 microbial taxa worldwide) are greatly influenced by the cognisance that the presence of, and phylogenetic relationship between, microbial organisms in natural communities can be inferred from the analysis of sequences of conserved genes (typically 16S rRNA genes). Species-abundance or rarefaction curves (Figure 1) have often been used to estimate the extent of microbial diversity, just as mathematical models have been (e.g. Curtis *et al.* 2002). Another line of evidence, suggesting that microbial diversity is large, is the occurrence of endemism, which has, for instance, been reported for hot spring cyanobacteria (Papke *et al.* 2003), hyperthermophilic archaea (Whitaker *et al.* 2003), or purple non-sulphur bacteria (Oda *et al.* 2003). Hence, β -diversity may be higher than was previously expected and, therefore, global diversity should be higher than local diversity. Finally, most rRNA gene sequences as yet retrieved from Earth’s ecosystems bear no resemblance to the several thousand described microorganisms known from cultivation, and the microbes that could be cultured were hardly ever found in molecular surveys; a phenomenon, which indicates that microbial diversity is much larger than had been anticipated.

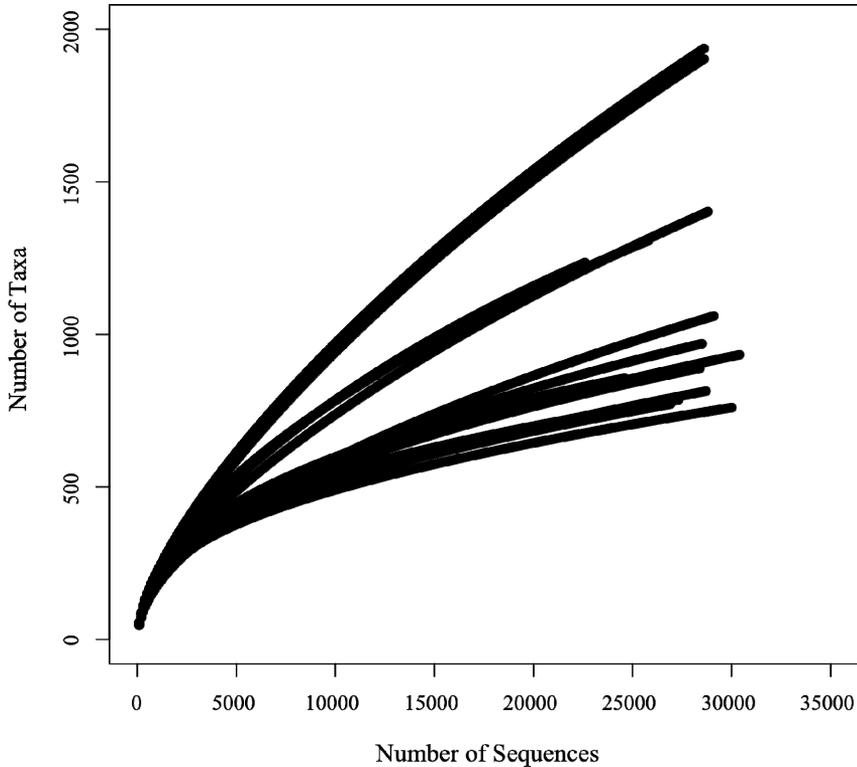


Figure 1. Rarefaction curves, showing the number of taxa versus the number of sequences retrieved from fourteen different libraries. A library of sequences is constructed with the ribosomal ribonucleic acid (rRNA) gene sequences from a particular sample. Each sequence represents the small subunit (SSU) rRNA gene (16S rDNA) of one member of the original community. As more sequences are being targeted, new taxa are detected. At the beginning of this process, almost every new sequence will belong to a new species but, as more sequences are examined, the curve of the number of taxa versus the number of sequences should approach an asymptote. This would indicate that most of the diversity in the library has been captured. However, most bacterial rarefaction curves generated so far have not reached an asymptote, indicating that many more sequences need to be retrieved to capture the real diversity of that community.

The phenomenon just described furthermore implies that some microbial organisms occur at such low abundance that molecular, cultivation-independent techniques cannot detect them and that microbes, which could be isolated using standard cultivation methods, are rarely numerically dominant in the communities from which they were obtained. This has, very recently, lead to the conception amongst microbial ecologists that there are “core” and “occasional” microbial species (Magurran & Henderson 2003). Core microbial species are the most abundant and dominant taxa and, since

they are well adapted, are maintained via active growth. Occasional microbial species constitute the long tail of rare taxa (or seed bank), termed the “rare biosphere” (Pedrós-Alió 2006; Sogin *et al.* 2006; Pedrós-Alió 2007). Occasional taxa may be rare because of inactivity or even dormancy, thereby escaping grazing and viral lysis. Or, they may be less abundant in spite of active growth yet being more susceptible to grazing and/or viral lysis (Jones & Lennon 2010). An important property of this “rare biosphere” is that a rare microbial species can become active and abundant should environmental conditions change and become favourable to sustain its growth (Pedrós-Alió 2006). Hence, rare microbial taxa may swap compartments that is from being dormant and in the seed bank to being active and abundant.

Recent methodological advances, i.e. the introduction of high-throughput sequencing technologies into the field of microbiology, now provide microbial ecologists with the tools necessary to dip into this “rare biosphere” and to explore microbial diversity to a greater acuity and depth. As these novel sequencing approaches are applied to diversity studies and the number of sequencing reactions increases to millions or hundreds of millions, we can expect the discovery of new sequences to increase microbial diversity by at least another order of magnitude. However, we might also discover that the “rare biosphere” or seed bank is indeed globally similar in composition and, hence, it is likely that ‘everything will be found everywhere’. The challenge, now, lies in gaining an understanding of the bigger picture behind these sequences.

1.3 Microbial Biogeography

A branch of study meeting the challenge of gaining an understanding of the bigger picture behind microbial diversity is that of microbial biogeography (see Box 2 for an explanation of biogeography). Since the study of biogeography provides an insight into the deeper and perhaps universally valid, ecological processes and mechanisms that underlie and maintain microbial diversity, resolving the question about the spatial scaling of microbial diversity will help to resolve the question about its extent. In addition, grasping the principles governing diversity and its distribution over space and time is needed in order to preserve diversity. Moreover, the study of biogeography is of great importance in understanding the specific role of an organism in its natural habitat, thus liaising diversity with ecosystem function (see Bell *et al.* 2005b for a microbial perspective and Reiss *et al.* 2009 for a general overview on the topic of diversity and ecosystem function). Its ultimate goal, though, is to develop a conceptual basis upon which the microbial world can be explored and, eventually, predicted.

Box 2. Definition of Biogeography

Biogeography is the science that attempts to document and understand spatial patterns of biological diversity. It aims to reveal where organisms live, at what abundance, and why (Martiny *et al.* 2006). Traditionally, it has been defined as the study of the distribution of organisms, both past and present. Modern biogeography, however, now includes studies of all patterns of geographic variation in nature – from genes to entire communities and ecosystems – elements of biological diversity that vary across geographic gradients including those of area, isolation, latitude, depth, and elevation. The fundamental question of biogeography is however: How does biological diversity vary over the surface of Earth?

It is important to differentiate between **historical biogeography**, i.e. the attempt to reconstruct the origin, dispersal, and extinction of taxa and biota, and **ecological biogeography**, i.e. the attempt to account for present distributions and geographic variation in diversity in terms of interactions between organisms and their physical and biotic environment (Lomolino *et al.* 2009).

The problems of distribution and variation on geographic scales were long since matters of primary interest to biologists, including Linnaeus, Darwin, and Wallace. However, unlike the studies of biogeographical patterns of animals and plants, the spatial (and temporal) scaling of microbial diversity is still poorly understood and has hitherto received attention only very recently (Horner-Devine *et al.* 2004a; Green & Bohannan 2006; Martiny *et al.* 2006; Ramette & Tiedje 2007). Interest in microbial diversity and the arrival of cultivation-independent molecular techniques have prompted a plethora of microbial biogeography studies. Microbial ecologists have now begun to examine whether microbial communities are distinct in different environments, whether microbial diversity changes with habitat heterogeneity and distance, and whether microbial diversity shows explainable patterns of distribution comparable to such of macroorganisms, for example the taxa-area relationship (TAR) (Rosenzweig 1995), the productivity-diversity relationship (Rosenzweig 1995) or patterns of co-occurrence (Horner-Devine *et al.* 2007).

1.3.1 Current Conceptual Perspectives on Freshwater Bacterioplankton Diversity

The Concept of Ubiquity/Cosmopolitanism

The traditional view amongst microbiologists is that of microbial species having a cosmopolitan distribution that is “everything is everywhere, but the environment selects” (Beijerinck 1913; Baas-Becking 1934). In brief, the most commonly claimed mechanism underlying a cosmopolitan distribution of microbes is that of large abundances, implying that dispersal is more likely, whereas extinction is less likely (Finlay & Clarke 1999; Fenchel & Finlay 2004). Body size and the fact that some microbes can form dormant

and resting-stages also enhance dispersal, in particular long-distance passive dispersal. As a consequence, the rate and geographic distance of dispersal are increased to levels where dispersal limitation is non-existent (thus, ‘everything is everywhere’). Moreover, the biogeography of microorganisms reflects the influence of contemporary environmental factors, meaning similar microbial communities are expected to be found in similar habitats and differentiated microbial assemblages along an environmental gradient (thus, ‘but the environment selects’). The concept, furthermore, implies that because of the absence of geographical barriers and local extinctions (dormancy, generation of resting-stages and large population sizes reduce the probability of extinction), every habitat will contain a majority of globally occurring microbial species in form of a seed bank (Fenchel *et al.* 1997; Finlay & Clarke 1999). Hence, microbial diversity is assumed to be high locally but low globally (see 1.2 Microbial Diversity).

The Metacommunity Framework

Understanding the mechanisms that underlie patterns of species distribution, abundance and interactions is central to community ecology. Traditionally, community ecology would have focused on either local deterministic processes (Clements 1916) or dynamics at the regional scale (Gleason 1926; MacArthur & Wilson 1967). The acknowledgement that community composition within a local habitat is affected by both local interactions and regional processes is one of the major achievements in community ecology within the last twenty years (Ricklefs 2004). It is, by now, generally accepted that diversity and its distribution over space and time are governed by a complex interplay of both local and regional drivers (Ricklefs 2004).

In taking a mechanistic approach towards the understanding of community dynamics, the metacommunity concept (Leibold *et al.* 2004; Holyoak *et al.* 2005) emphasises the interplay between local interactions and regional processes as a driver of community dynamics. The metacommunity concept has emerged only within the last decade and has readily been adopted in studies of microbial diversity. The term metacommunity describes a set of local communities that are linked by dispersal of multiple potentially interacting species (Wilson 1992), such that local interactions and regional processes compromise local community assembly and resultant ecosystem dynamics. Leibold *et al.* (2004) and Holyoak *et al.* (2005) have organised the various approaches to metacommunity theory into four different but partially coinciding paradigms: species sorting, mass effects, patch dynamics, and the neutral model. Each paradigm evokes different mechanisms of community assembly to explain local species coexistence within a metacommunity. In species-sorting metacommunities, habitat patches differ with regard to environmental conditions. Trade-offs in the ability to cope with these conditions allow species to regionally coexist by means of niche diversification and regional differences in resource exploitation. If applied to a global scale, this

first paradigm is essentially the same as the “everything is everywhere, but the environment selects”-hypothesis (see The Concept of Ubiquity/Cosmopolitanism and 1.2 Microbial Diversity). In mass-effects metacommunities, environmentally heterogeneous habitat patches are tightly interconnected via frequent dispersal in such a way that reproduction in a source habitat allows for persistence within a sink habitat (source-sink dynamics) as dispersal rates exceed rates of extinction. In patch-dynamics metacommunities, environmentally homogeneous patches are inhabited by species that exhibit a trade-off between dispersal and local dominance, such as a colonisation-competition trade-off in which successful competitors are poor colonisers and vice versa. Finally, in neutral metacommunities, species do not differ in their fitness and niche, thus, local interactions and species trade-offs play a negligible role. Community assembly, however, depends on frequency-dependent immigration events and speciation is assumed to counteract extinction processes. Hence, each paradigm, by means of highlighting different aspects of spatial community dynamics, predicts changes in local community composition based on the rate of dispersal and on patch and species characteristics.

1.3.2. Observed Spatial Patterns of Microbial Diversity

Examples of cosmopolitanism in the microbial world mostly come from studies on certain protist species. Finlay and Clarke (1999), for instance, found that a small sediment sample from a pond in the UK contained remnants of as much as 78% of all globally identified species within the diverse flagellate genus *Paraphysomonas*. However, there is evidence of ubiquity in the bacterial realms of the microbial world, too. From studies of marine bacterioplankton communities, it seems, for example, that the alphaproteobacterial *Pelagiobacter* cluster could be found in nearly every pelagic marine environment around the globe (Giovannoni *et al.* 1990). Or, the identification of 34 globally occurring clusters that were dominated by or restricted to freshwater bacteria and lead to the coining of the term ‘typical freshwater bacterioplankton’ is another example of bacterial cosmopolitanism (Zwart *et al.* 2002).

Yet, a growing body of evidence, however, shows that microbial organisms vary in abundance, diversity and distribution and over various taxonomic and spatial scales, i.e. microbial assemblages do differ in composition and diversity in different locations (Martiny *et al.* 2006).

Observations of non-cosmopolitan distribution of microbes are found in the study of Cho and Tiedje (2000), who were among the first to provide an example of a ‘distance-decay relationship’, a relationship generally observed for macroorganisms (Nekola & White 1999). They showed that genetic distance between fluorescent *Pseudomonas* strains was related to geographic

distance. Similarly, Papke et al. (2003) could clearly detect certain cyanobacterial *Synechococcus* morphotypes in hot springs in North America, yet they could not observe them in other hot springs around the globe, suggesting a restricted dispersal range because of physical isolation and lack of viability during transport. Whitaker et al. (2003), who studied hyperthermophilic *Sulfolobus* populations, found further evidence of microbial endemism. Finally, Oda et al. (2003) investigated the biogeography of the purple non-sulphur bacterium *Rhodospseudomonas palustris* and found a gradual decrease in genotype similarity as a function of spatial distance.

The relationship between the number of species (taxa) and the size of the area from which samples have been taken (the taxa-area relationship [TAR]) is further evidence for patterns of biogeography. The TAR is one of the most widely studied patterns in ecology and it appears to be one of the few general ecological phenomena (Lawton 1999). Recently, microbiologists have indeed reported patterns of TARs comparable to such of macroorganisms. Horner-Devine and colleagues (2004b), for instance, describe a TAR for bacteria over a scale of centimetres to hundreds of metres in contiguous salt marsh sediments. Bell et al. (2005a) detected strong taxa-area relationships for insular bacterial assemblies in water-filled tree holes.

A pattern that has become obvious is that the answer to the question of whether or not microbial organisms are ubiquitously distributed around the globe or show patterns comparable to such of macroorganisms may depend on the phylogenetic resolution applied (e.g. Cho & Tiedje 2000; Acinas *et al.* 2004). High-throughput sequencing technologies, allowing greater sampling acuity and depth compared to traditional culture-independent methodologies regarding the number of sequences, may provide the necessary tools to answer both the questions about the extent of microbial diversity and its spatial distribution. In the end, with increasing sampling effort it might well be that 'everything is everywhere'.

1.4 Mechanisms Influencing Freshwater Bacterioplankton Diversity

With respect to aquatic bacterioplankton communities, a series of factors and mechanisms, both local and regional in nature, have been identified to exert influence on local community composition and richness.

1.4.1 Local Interactions

Local deterministic or system-intrinsic processes are local interactions within species, between species and/or between species and the local environment. Several studies have identified numerous local factors that poten-

tially influence bacterioplankton community composition (BCC), such as pH (e.g. Lindström *et al.* 2005; Yannarell & Triplett 2005), ultraviolet radiation (e.g. Warnecke *et al.* 2005), quality and quantity of dissolved organic matter (DOM) (e.g. Crump *et al.* 2003; Eiler *et al.* 2003), inorganic nutrients (e.g. Fisher *et al.* 2000), primary productivity (e.g. Horner-Devine *et al.* 2003) and food web interactions (e.g. Thingstad 2000; Jürgens & Matz 2002; Simek *et al.* 2005; Kent *et al.* 2006). Grazing and DOM are probably the factors investigated the most amongst the environmental factors listed above. See Hahn (2006) or (paper I) for an overview of local factors and interactions influencing BCC.

1.4.2 Regional Processes

Dispersal (i.e. the locomotion of an individual from one location [emigration] to another [immigration]), historical legacies (e.g. dispersal limitation, past environmental conditions), speciation, extinction and geographical and climatic conditions have been circumscribed as regional or system-extrinsic processes. As to regional processes, a distinction has to be made between direct and indirect effects. As an example, a region can exert indirect influence on a local bacterioplankton community via affecting the lake's local environmental conditions, such as pH, salinity and DOM quantity and quality – in fact all local factors that have been shown to have a profound effect on BCC. An additional indirect effect could be the geographic position of a lake within the surrounding landscape (e.g. Yannarell & Triplett 2005; Nelson *et al.* 2009). Direct regional effects, with regard to a lake and its residing bacterioplankton community, could be, for instance, the effect of import and establishment of bacterial cells from the regional metacommunity (e.g. Lindström *et al.* 2006) (see The Metacommunity Framework). Yet, regional processes have, in comparison to local interactions and factors, less frequently been studied, possibly because they are more difficult to incorporate and measure, since they act on a much larger scale, both spatially and temporally.

1.4.3 Local versus Regional Drivers of Bacterioplankton Diversity

Studies integrating both local and regional aspects of bacterioplankton community assembly plus investigating the relative importance of local versus regional dynamics for local BCC are yet few in numbers. In any case, Lindström *et al.* (2006), Crump *et al.* (2007) and Nelson and colleagues (2009) found signatures of mass effects in freshwater systems of short water residence time (WRT). Other findings, however, indicate that BCC in lakes is primarily driven by species-sorting processes (Van der Gucht *et al.* 2007;

Jones & McMahon 2009). Langenheder and Ragnarsson (2007) then again identified both species-sorting and regional processes at work in affecting bacterioplankton communities in rock pools.

It has to be noted that studies on the biogeography of bacterioplankton have almost exclusively concentrated on the comparison of community composition amongst sites, i.e. β -diversity, rather than on α -diversity that is the diversity within sites.

2. The Present Thesis

2.1 Aims

The primary goal of my doctoral thesis was to gain a more in-depth understanding of the mechanisms governing freshwater bacterioplankton diversity. The key objective was to identify ecological linkages between lake bacterioplankton and bacterial communities in connected streams and the surrounding terrestrial landscape. Overall, it addresses one fundamental question:

- To what extent is local freshwater bacterioplankton diversity structured by local environmental interactions and/or regional processes?

Paper I is a classical literature review, compiling findings of patterns in the spatial distribution of freshwater bacterioplankton diversity. Of additional interest with regard to the biogeography of bacterioplankton in inland waters was the proposition of a conceptual model, invoking metacommunity theory, to obtain mechanistic insight, to generalise patterns, and to make predictions. Paper II synthesises results from a broad array of empirical approaches to metacommunities, examining whether and to what extent the studies followed the assumptions made by metacommunity theory or whether and to what extent theory failed to capture the realities of natural systems. Papers III, IV, and V depict observational field studies, investigating the mechanisms underlying BCC (papers III and IV) and bacterioplankton richness (BR) (paper V). Paper III aimed at analysing the relative importance of mass effects rather than that of species-sorting processes in structuring freshwater bacterioplankton assemblages. Paper IV explored the potential importance of neutral processes for bacterioplankton community assembly. Finally, paper V investigated local and regional mechanisms and their respective relevance for driving local freshwater bacterioplankton richness (α -diversity).

2.2 Material and Methods

2.2.1 Study Sites

The three field surveys (papers III, IV and V) were carried out in various regions in Sweden. Paper III is based on sampling eight lakes and their respective inlets in Uppland. Paper IV draws its results and conclusions from assessing 13 lakes in Uppland, nine lakes in Jämtland, 35 rock pools in the Ängskär nature reserve along the Baltic Sea coast and 40 lakes and 18 streams in the region of Småland. Finally, paper V is built on a survey of 14 lakes in Jämtland.

2.2.2 Abiotic and Biotic Characterisation

Water chemistry analyses included measurements of total phosphorus (Tot-P), total nitrogen (Tot-N), total organic carbon (TOC), Chlorophyll-a (Chl-a) and absorbance at the three wavelengths of 250, 365 and 436 nanometres³. Bacterial abundance was enumerated by epifluorescence microscopy of acridine-orange-stained cells (Hobbie *et al.* 1977) and bacterial production was measured via incorporation of tritiated thymidine into deoxyribonucleic acid (DNA) (Bell 1993).

2.2.3 Molecular Methodologies

In order to explore BCC (papers III and IV) and BR (paper V), two molecular, culture-independent methodologies were applied: terminal-restriction fragment length polymorphism (t-RFLP) (Liu *et al.* 1997; Marsh 1999; Osborn *et al.* 2000) and 454 pyrosequencing (Ronaghi *et al.* 1998; Rothberg & Leamon 2008), respectively.

Terminal-Restriction Fragment Length Polymorphism (t-RFLP)

t-RFLP is a polymerase chain reaction (PCR)-based community-profiling or -fingerprinting methodology adopted to obtain a qualitative and (semi-) quantitative representation of different phylotypes in a sample. It allows for a rapid, inexpensive and reproducible assessment and, thus, for a comparative analysis of microbial communities. It is, however, important to note that t-RFLP, as other community-fingerprinting methodologies, provides only an overview of the most dominant and abundant phylotypes within a community.

At first, the 16S rRNA gene (rDNA), extracted from the natural communities studied, was PCR-amplified, using a fluorescently labelled 5' primer.

³ Absorbance at 436nm was used as a measure of water colour, while the ratio between absorbance at 250nm and absorbance at 365nm can be used as a measure of the quality of DOM.

Amplicons were subsequently digested (that is they were cut into fragments that differ in sequence length depending on sequence variation) by means of restriction enzymes. Finally, fluorescently labelled terminal-restriction fragments were separated and detected using a DNA sequencer, thereby generating a molecular “fingerprint” of the specific communities on focus. These community fingerprints allow for the detection of differences among and within communities over time.

In paper III, both 16S rRNA and 16S rDNA were co-extracted from lake and stream bacterioplankton communities in an endeavour to target both the total (16S rDNA) community and its metabolically active (16S rRNA) fraction. t-RFLP analysis was, hence, preceded by a reverse transcription step in which 16S rRNA was synthesised into complementary DNA.

454 Pyrosequencing

454 pyrosequencing is a high-throughput sequencing technology, which has been introduced into microbiology within the last five years. It is a culture-independent technology that can generate, at present, up to one million sequences per run, which can be up to 450 base pairs in length. As such, high-throughput sequencing technologies allow sequencing – and thus detection – of a number of microbial taxa that could not be reached with traditional molecular sequencing methodologies. The data generated by 454 pyrosequencing allow obtaining improved estimates of microbial richness and a more accurate mapping of community assembly.

Once DNA was extracted from natural bacterioplankton lake communities, the bacterial hypervariable regions V3 and V4 of the 16S rRNA gene were PCR-amplified, using individually bar-coded 3' primers. Amplicons were, in a final step, 454-pyrosequenced at the Norwegian High-Throughput Sequencing Centre (NSC; University of Oslo, Oslo, Norway; <http://www.sequencing.uio.no>), using a 454 GS FLX system (454 Life Science, Branford, CT, USA) and Titanium chemistry.

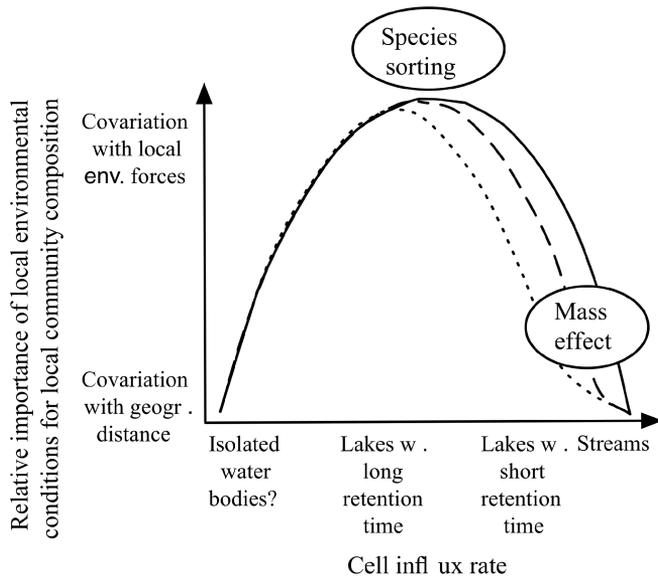
2.3 Results

2.3.1 Biogeography of Bacterioplankton in Inland Waters (Paper I)

The mechanisms by which microbial communities assemble are still poorly understood, yet it has become apparent in recent years that microbial assemblages do differ in diversity in different locations (Martiny *et al.* 2006). Variation among bacterioplankton communities has been reported to be temporal and spatial that is both among and within habitats. Moreover, it has become clear from this review that microbial diversity and its distribution

over space are governed by a complex interplay of both local and regional drivers. Thus, the question is not whether local or regional processes are of importance in structuring bacterioplankton diversity, but rather how relevant local interactions are in comparison to regional processes and vice versa. But for all that, studies analysing the importance of regional spatial effects in relation to local drivers for bacterioplankton community assembly are still rare.

In an effort to compile findings of the spatial scaling in bacterioplankton diversity, we developed a theoretical model, generalising observed patterns in the spatial distribution of bacterioplankton taxa with regard to WRT, thereby invoking metacommunity theory (Figure 2). The model allows for a relationship between the relative importance of local environmental drivers for local BCC and the systems' exchange rate of bacterial cells. We, thus, assumed that BCC in rivers and lakes of short WRT is to the largest extent influenced by mass effects and less by local environmental drivers. In contrast, the rate of bacterial cell import from the catchment to lakes characterised by longer WRTs does yet allow global dispersal, however, dispersal rates will not be high enough to overrule the influence of the local indigenous habitat. In these latter systems, species-sorting processes best describe dynamics in local bacterioplankton assemblies.



Low plasticity and/or high growth rate	—————
Medium plasticity and/or growth rate	- - - - -
High plasticity and/or low growth rate

Figure 2. Conceptual figure, describing the possible relative importance of local environmental properties for local bacterioplankton community composition (BCC) in inland waters, in dependence of cell influx rate to the system. Different scenarios are expected depending on functional plasticity of taxa within the metacommunity and/or cell growth rates. The figure also shows under which circumstances species sorting or mass effects is most likely to determine BCC. The scenario in the lower left corner corresponds to dispersal limitation. It still remains to be shown if inland water bodies isolated to the extent of dispersal limitation do exist.

2.3.2 Empirical Approaches to Metacommunities – a Review and Comparison to Theory (Paper II)

To comprehensively evaluate metacommunity empirics and theory we examined (i) whether and to what extent experiments in the laboratory and observations in the field do follow the assumptions made by metacommunity theory and (ii) whether and to what extent metacommunity theory with its four paradigms succeeds in capturing the realities of natural systems.

In general, empirical studies did relate rather imperfectly to the four paradigms, experimental, however, more so than observational approaches. Thus, the majority of experimental studies was not exclusively designed to test a certain paradigm. Yet, if experimental findings could be related to metacommunity theory, differentiation in particular between the species-sorting and the mass-effects paradigms was hampered. In contrast, a much higher proportion of observational studies was explicitly outlined to test one or several paradigms. Yet again, findings from observational studies could, in most cases, not unambiguously be assigned to a specific paradigm.

Moreover, in spite of being at the very heart of metacommunity theory, empirical metacommunity approaches often lacked the inclusion of concise measurements of actual dispersal rates.

Our results, furthermore, show that theory mostly addresses permanent habitat patches with discrete boundaries and so do most empirical studies, focussing on insular metacommunity types (e.g. lakes, ponds, islands, moss patches). Yet, metacommunities observed in nature often are not permanent in character nor do they have distinct boundaries.

2.3.3 Species Sorting Affects Bacterioplankton Community Composition as Determined by 16S rDNA and 16S rRNA Fingerprints (Paper III)

The primary objective of this study was to analyse the relative importance of mass effects rather than that of species-sorting processes in structuring bacterioplankton assemblages. We hypothesised that (i) bacterioplankton communities in lakes of short WRT were assembled by mass effects due to high cell import rates, (ii) the importance of mass effects depended on season as a

consequence of changes in water flow and, thus, changes in cell import rates, and (iii) the importance of mass effects depended on whether the total community (16S rDNA) or its active fraction (16S rRNA) is targeted. To test for the abovementioned hypotheses, bacterioplankton assemblages of eight lakes differing in WRT and their main inlets were sampled on four occasions that differed in water flow regime.

BCC similarities between lakes and their main inlets on the four dates of sampling ranged from 21% to 78% and from 18% to 83% for 16S rDNA and 16S rRNA, respectively (Figure 3). However, no obvious relationship between WRT and lake:inlet BCC similarities could be observed. Considering all data from all seasons, lake:inlet BCC similarities for both 16S rDNA and 16S rRNA were significantly positively correlated with cell import per cell production rates. Yet, lake:inlet BCC similarities were also significantly negatively correlated with local environmental heterogeneity between lakes and their inlets. What is more, cell import per cell production rates were significantly negatively correlated with environmental heterogeneity. Results from partial Spearman's rank correlation analyses showed that for both types of fingerprints the degree of association between lake:inlet BCC similarity and cell import per cell production rate became insignificant once the effect of environmental heterogeneity was controlled for. Yet on the contrary, the relation between lake:inlet BCC similarity and environmental heterogeneity remained significant, once the effect of cell import per cell production rate was removed.

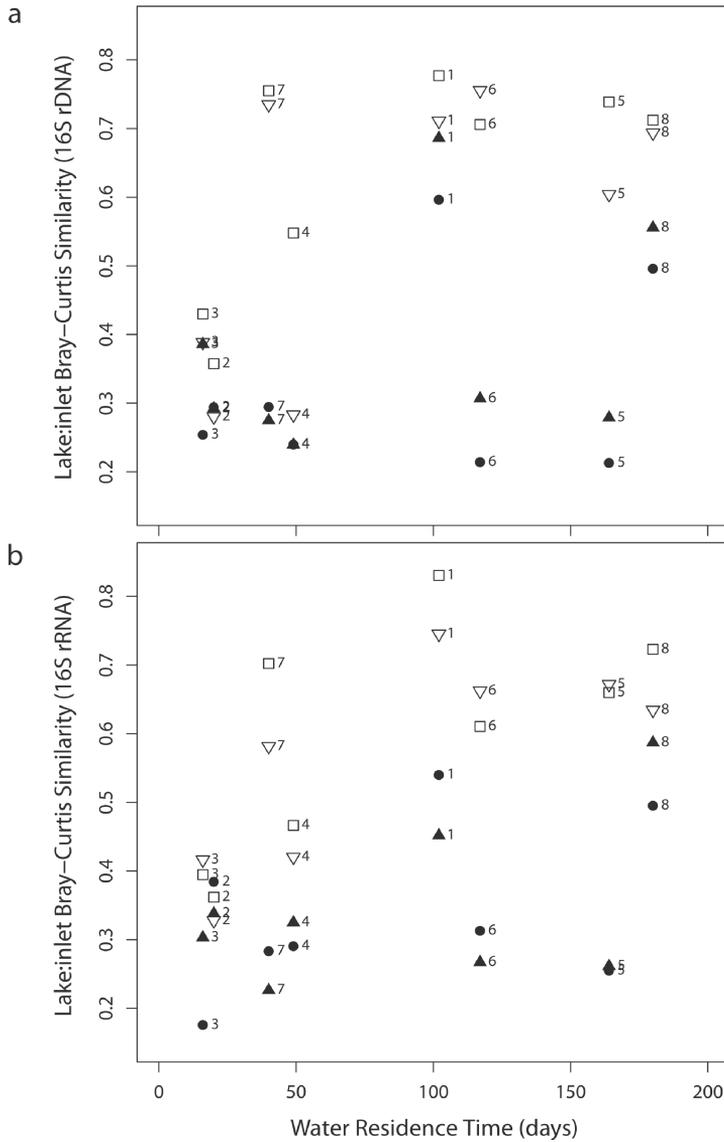


Figure 3. Relationship between lake:inlet Bray-Curtis similarities in 16S rDNA (a) and 16S rRNA (b) t-RFLP fingerprints and water residence time (WRT) for the eight studied lakes over all four seasons (winter [□], spring [▽], summer [●] and autumn [▲]). The eight studied lakes are labelled as follows: 1, Velängen; 2, Stensjön; 3, Norrsjön Almunge; 4, Fibysjön; 5, Lumpen; 6, Ramsjön; 7, Norrsjön Norreda; and 8, Siggeforasjön.

2.3.4 Regional Invariance Among Microbial Communities (Paper IV)

Bacterioplankton communities in each of five different regions were assessed in view of the neutral model. Here, (i) the degree of similarity between bacterioplankton assemblies and (ii) the amount of variation in local bacterioplankton abundances that could be explained by regional relative bacterioplankton abundances were explored. Communities being more similar than could be expected by chance were termed invariant. Also, the higher the proportion of abundances and detection frequencies of local bacterioplankton taxa that could be explained by regional relative abundances, the more invariant the communities were amongst each other. Finally, (iii) we investigated how the extent of invariance changed in dependence of the local environment.

Local bacterioplankton communities were in all five regions more similar to each other than could be expected by chance. In addition, locally abundant aquatic bacteria were, in general, also regionally widespread. As such, the neutral model could explain most of the variation in bacterioplankton detection frequency (85%). Finally, the degree of invariance among bacterioplankton assemblages decreased with increasing environmental variation within the region.

2.3.5 Productivity Influences Freshwater Bacterioplankton Richness in Oligotrophic Lakes (Paper V)

The primary objective of this study was to investigate local and regional mechanisms and their respective relevance for driving local BR (α -diversity). We hypothesised that BR would increase with (i) nutrient loadings, (ii) lake area and catchment area, and as a consequence thereof (iii) with a lake's position within a landscape (measured as lake chain number [LCN], i.e. lakes lower down in the landscape have a higher LCN). To do so, we surveyed fourteen lakes that share origin and climate.

Our results showed that BR did not increase with increasing LCN. In addition, BR did neither increase with increasing lake area nor with increasing catchment area. Yet, BR was significantly correlated to local environmental parameters, such as nutrient content (Figure 4).

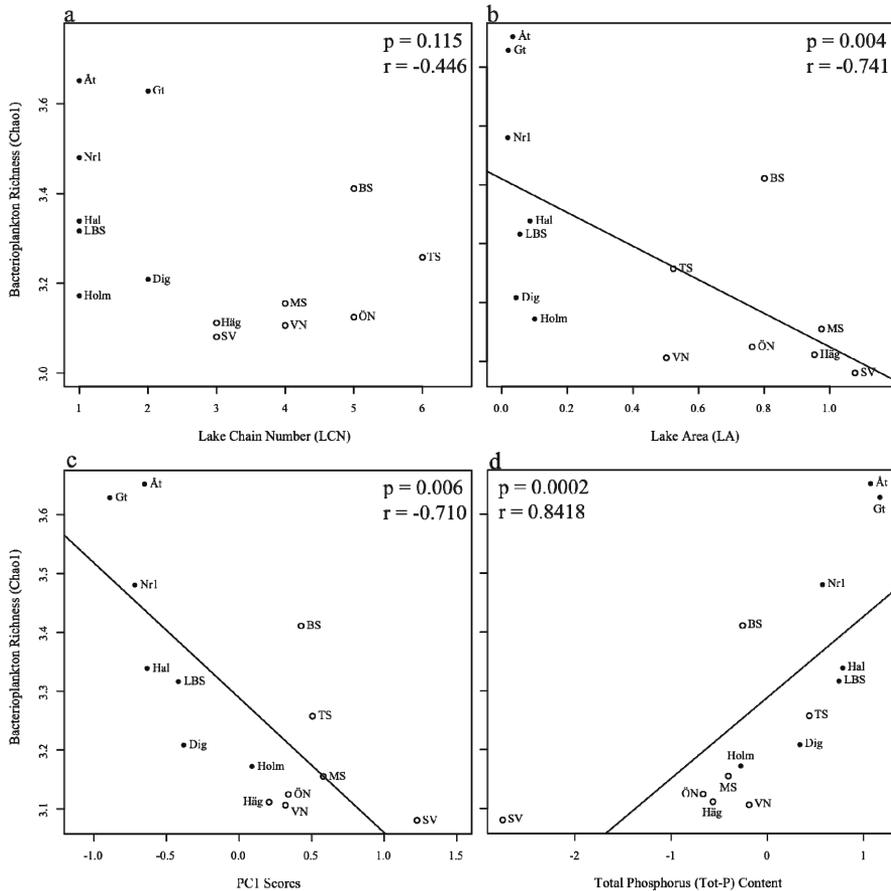


Figure 4. Relationship between bacterioplankton richness (BR) and (a) lake chain number (LCN), (b) lake area, (c) the environment (PC1) and (d) total phosphorus content (Tot-P). Closed circles correspond to the lakes high up in the landscape (LCN of one and two), while open circles refer to lakes lower down in the landscape (LCN of three to six).

Furthermore, BR in lakes higher up in the landscape (lakes of LCN one and two) differed significantly from that in lakes further down (lakes of LCN three to six). Several local environmental parameters showed similar patterns, i.e. higher values were observed for lakes higher up in the landscape.

2.4 Discussion

In the observational study described in paper III, we were interested in whether regional dispersal mechanisms (mass effects) rather than local envi-

ronmental lake properties (species sorting) were of greater importance in structuring bacterioplankton assemblies in eight freshwater lakes. The set of lakes that was selected ranged in WRTs previously proposed by Lindström *et al.* (2006) to be short enough for the communities to experience mass effects. We assumed that an event of mass effects would bring about high lake:inlet BCC similarities because of the high number of imported bacterial cells, which in turn are a result of short WRT and high seasonal water flow. Thus, high lake:inlet BCC similarities should be obtained at short WRTs and lake:inlet BCC similarities should be correlated with rates of cell import per cell production. Considering the third hypothesis, mass effects were expected to be more pronounced when targeting 16S rDNA compared with 16S rRNA. In the latter case, a weaker relationship between cell import and lake:inlet BCC similarities is expected.

Despite sampling lakes of short WRT, both low and high lake:inlet BCC similarities were observed over the entire range of WRT, except for the shortest WRTs when lake:inlet BCC similarities were consistently low. Moreover, variation in lake:inlet BCC similarities was not well explained by variation in cell import per cell production rates. Instead, lake:inlet BCC similarities co-varied better with local environmental heterogeneities between lakes and their inlets, suggesting that mass effects were of rather minor importance when compared with species-sorting dynamics in explaining bacterioplankton community structure in the lakes studied. Yet, it cannot be excluded that individual bacterioplankton populations were subject to mass effects. It further cannot be excluded that mass effects could occur in lakes of even shorter WRTs than were included in this study as well as at extremely high water flow events, such as during snow melt or heavy rain storms; events that were not sampled in this study.

Several previous studies did observe signatures of mass effects exerting influence on a lake's BCC due to high cell import rates (Crump *et al.* 2004; Lindström *et al.* 2006; Crump *et al.* 2007; Nelson *et al.* 2009). Yet, the question has been raised as to at what cell import rates the point differentiating between mass effects and species sorting should come to lie (paper I). Focusing on lakes, Lindström *et al.* (2006) suggested a discrimination point at a WRT of 100 days, whereas results from Nelson *et al.* (2009) suggested one at 68 days. As we did not find any signatures of mass effects on BCC in our studied lakes of short WRT (16–180 days), our data (paper III) do not support conclusions drawn by these researchers. Thus, it remains to be shown whether this point of discrimination in fact could be at much shorter WRTs, such as <2 or <3 days, as has been suggested by Crump *et al.* (2004; 2007) for estuaries and streams, respectively.

This point of discrimination, however, needs not to be the same for all types of systems. For instance, in systems of high growth rates, such as eutrophic lakes, a greater input of cells (i.e. shorter WRT) might be needed to overrule the internal production of cells. Hence, species-sorting dynamics

rather than mass effects might be more pronounced in such systems. Results from a study of 98 relatively eutrophic lakes indicated an overwhelming importance of species sorting for BCC as opposed to mass effects (Van der Gucht *et al.* 2007). They, further, proposed that more oligotrophic lake systems should be rather prone to mass effects dynamics when compared with more eutrophic lakes. Unfortunately, we were not able to test this hypothesis because of the fact that our lakes were all predominantly mesotrophic. Moreover, as Nelson *et al.* (2009) did not observe mass effects structuring BCC in a headwater lake of extremely short WRT (<5 days) either, it is likely that there is no such thing as a universal point of discrimination between mass effects and species sorting. However, it seems that there are not yet enough data available to consistently determine how high rates of cell import to lakes in nature ought to be to trigger mass effects.

In paper III, we further investigated whether there were differences in the mechanisms structuring BCC depending on whether the total community or only its active fraction was analysed. The observed similar results for 16S rDNA and 16S rRNA fingerprints is an interesting finding, considering the emerging pattern that a non-negligible proportion of cells within a microbial community is assumed to be either dormant, damaged or dead (del Giorgio & Gasol 2008) and that growth rates of bacterial cells are related to cellular RNA content (e.g. Bremer & Dennis 1987). Hence, 16S rDNA and 16S rRNA fingerprints are expected to yield different results, which has been shown in studies conducted in marine and/or coastal aquatic ecosystems (e.g. Schäfer *et al.* 2001; Rodriguez-Blanco *et al.* 2009). Whether the observed similarity in 16S rDNA and 16S rRNA from our study might be a pattern typical for freshwater bacterioplankton communities cannot be said because of a shortage in studies in such systems. However, marine environments differ greatly from freshwater ecosystems and are, for example, generally poorer in nutrient content compared with lakes. As it has been shown that the number of substrate respiring, i.e. active, bacterial cells increases with system productivity (del Giorgio & Scarborough 1995), bacterial production (del Giorgio *et al.* 1997) and cell growth (Sherr *et al.* 1999), it is possible that the most active phylotypes were also the most abundant ones in our study, thus resulting in similar 16S rDNA and 16S rRNA fingerprints. A relatively recent study conducted by Jones and Lennon (2010) recorded results that are in line with the abovementioned, namely that the proportion of dormant bacteria was relatively low in productive ecosystems. With regard to local and regional drivers of bacterioplankton community assembly, they suggested that regional environmental cues and dormancy synchronise the composition of active communities across the landscape, while decoupling active microorganisms from the total community at local scales (Jones & Lennon 2010).

In another β -diversity study of bacterioplankton communities from five different regions (paper IV), we showed that locally abundant aquatic bacteria were also regionally widespread, causing communities to be more similar than could be expected by chance and, thus, suggesting neutral dynamics to be acting upon BCC. Positive correlations between occurrence and local abundances are patterns predicted by neutral theory (Hubbell 2001) and are well established for many metazoan metacommunities. However, patterns of such have, until recently, received little interest within microbial ecology. Yet, Sloan et al. (2006) and we (paper IV) show that it could indeed be a common pattern also for microbial metacommunities. There have been several, non-mutually exclusive explanations suggested to explain why frequently occurring taxa are locally abundant; explanations, which can be divided into three loosely defined groups. First, some organisms might be more abundant and widespread than others because the resources they use are more abundant and widespread or they, in general, can grow on a wider spectrum of resources. Second, more abundant and widespread organisms are more likely to be rescued via, for instance, source-sink dynamics. Third, ecological equivalence among organisms in combination with dispersal will result in locally abundant organisms occurring frequently (Hubbell 2001; Economo & Keitt 2008). Sloan et al. (2006) have shown that the latter in particular can be the case for microorganisms.

None of the explanations can however be really discarded here. Besides, they are likely to act in coherence. However, neutral models, being the only models currently making any testable and quantitative predictions regarding detection frequency and mean relative abundance, fit relatively well to the bacterioplankton metacommunity studied here. This finding suggests that many taxa may indeed be ecologically similar to each other. Yet, regional relative abundances explained less variation in local relative abundances when environmental variations within the five regions were high. Thus, spatial variation in some environmental properties seemed to increase the importance of taxa-specific traits and species-sorting processes for bacterial assemblages between sites, suggesting that regional invariance at least partly depends on some resources being abundant and widespread.

Studies of the relative importance of local versus regional drivers of microbial diversity and its spatial scaling have almost exclusively focused on β -diversity (e.g. paper III and IV) that is comparing the composition of communities among sites. In paper V, however, we placed emphasis on bacterioplankton richness, i.e. α -diversity, rather than on community composition. Results from paper V indicate that local environmental lake characteristics are also important in driving differences in BR, more specifically that BR increased with nutrient content. Our results, further, indicate that the trophic status of these lakes accounted for most of the variation detected in BR amongst the fourteen study lakes, suggesting that richness is related to

system productivity. This productivity-diversity relationship, a frequently reported relationship in macroecological studies of plants and animals (Rosenzweig 1995; Mittelbach *et al.* 2001), has recently been observed in the microbial realm, too (Bohannan & Lenski 1997; Fisher *et al.* 2000; Kassen *et al.* 2000; Horner-Devine *et al.* 2003). Horner-Devine and others (2003) saw that different bacterioplankton groups differed in response to changes in productivity, exhibiting both unimodal and U-shaped relationships. The positive relationship between BR and nutrient content found in our study, however, rather relates to a linear relationship that is we observed a higher BR at higher levels of productivity. However, it is crucial to note that the lakes surveyed are all highly oligotrophic in nature and, thus, the observed linear relationship between BR and productivity might fall into the positive linear phase located on the left side of the expected hump-shaped productivity-richness curve. Finally, ecologists have repeatedly observed relationships between productivity and the number of eukaryotic organisms. Our study with others demonstrates that this productivity-diversity relationship might very well be a relationship generally valid across all groups of organisms.

Interestingly, results from paper V show no signs of a positive TAR. This is a finding of particular interest, contradicting theoretical, macroecological predictions and contrasting recent microbial observations that have reported a positive TAR for microbial eukaryotes and bacteria (Green *et al.* 2004; Horner-Devine *et al.* 2004b; Bell *et al.* 2005a; Reche *et al.* 2005). This discrepancy between results from our study and the abovementioned studies is, amongst other reasons, very likely methodological in nature. Both traditional sequencing and community profiling methodologies were employed in the studies mentioned above, however, these techniques markedly undersample communities (Curtis & Sloan 2005; Curtis *et al.* 2006; Woodcock *et al.* 2006) and, thus, most likely provide less reliable richness estimates. By employing a 454-pyrosequencing approach, we achieved a much greater sampling effort, analysing an average of 30 248 sequences per sample and, thus, obtaining a more reliable richness estimate in comparison to the ones obtained from the other studies (Green *et al.* 2004; Horner-Devine *et al.* 2004b; Bell *et al.* 2005a; Reche *et al.* 2005). Another reason for this lack of TAR could be the issue of scale. Drakare and colleagues (2006) found that TARs were significantly affected by the spatial scale and the types of organisms or habitats involved and concluded that mechanisms underlying richness at different scales strongly affect the shape of TARs. Yet, we did not observe a TAR at either scale of lake or catchment area, which might indicate that neither scale is appropriate in describing a microbial habitat. However, since our study excluded most of the methodological limitations of previous studies reporting positive TARs for microorganisms, it re-opens the debate about

whether TARs can indeed be found in the microbial world and, thus, are indeed universally valid, applicable to all organisms from all domains of life.

Finally, implementing metacommunity theory into microbial ecology has allowed taking a step forward in understanding mechanisms of spatial microbial community ecology, as the interdependence between local interactions and regional processes can now be tackled operationally in experiments and observational studies, allowing to pose questions within a theoretical framework. However, our review (paper II) has identified some caveats that occur naturally, when carrying out empirical studies within a framework. We found that most empirical metacommunity approaches only partially tested assumptions of or predictions from the four metacommunity paradigms (e.g. Beisner *et al.* 2006; Crump *et al.* 2007; Langenheder & Ragnarsson 2007; Van der Gucht *et al.* 2007; Nelson *et al.* 2009) (paper III and paper IV). As the various mechanisms proposed by metacommunity theory operate in parallel in nature, it is, indeed, intriguingly difficult for empirical studies to disentangle them. In addition, we have recorded a bias towards the testing of species-sorting dynamics. Hence, more studies have to be carried out incorporating more than one aspect of metacommunity theory. Furthermore, empirical metacommunity approaches often lack the inclusion of concise measures of actual dispersal rates. In aquatic bacterial metacommunities only one route of dispersal was examined at a time, which most often was water-related dispersal via streams and rivers (Crump *et al.* 2004; Lindström & Bergström 2005; Lindström *et al.* 2006) (Crump *et al.* 2007; Nelson *et al.* 2009) (paper III). Bacterial organisms can, though, enter an aquatic system via several different routes, e.g. surface flow and subsurface flow inputs, resuspension from the sediments back into the water column, atmospheric deposition (wet [rain, snow] and dry [dust]), and vector import. Only Jones *et al.* (2008) investigated the relative importance of atmospherically dispersed bacterial cells for local bacterioplankton community assembly. Yet, considering only one respective route of dispersal most probably leads to an underestimation of bacterial cells being imported to an aquatic ecosystem, which can, in turn, result in a concealment of the actual mechanisms influencing community assembly. Therefore, actual measurements of dispersal as well as dispersal properties, frequency and range are essential in order to grasp both ecological and evolutionary processes (Shurin *et al.* 2009).

2.5 Conclusion and Perspectives

Briefly, the following can be concluded from the articles presented in this thesis:

1. Freshwater bacterioplankton communities are assembled by means of local, deterministic and neutral, stochastic processes.
2. Freshwater bacterioplankton richness was influenced by local environmental properties, in particular by system productivity. The finding that landscape position and local environmental conditions co-varied suggests that the landscape dictates environmental properties, which then directly structure local bacterioplankton diversity.
3. The metacommunity framework, emphasising the interplay between local interactions and regional processes as a driver of community dynamics, has emerged as a valuable concept within which to address questions of microbial diversity and its spatial scaling. Our results, however, highlight a gap between theoretical and empirical research on metacommunities, indicating that empirical approaches, in particular experiments, did not relate well enough to the paradigms and that the paradigms are not in all cases suitable abstractions for depicting processes in nature.

Distinctively unravelling local from regional drivers of bacterioplankton diversity remains a challenge that is important to meet to gain a more thorough understanding of the mechanisms that underlie bacterioplankton diversity. Clearly, more studies are needed to investigate regional processes driving local bacterioplankton diversity, despite the fact that regional processes are indeed more difficult to incorporate in comparison to local interactions, since they act on a much larger spatial and temporal scale. Yet, with the prospect of alterations because of changes in our climate (e.g. more and heavier winds and rainfalls) regional dynamics might gain more importance. Furthermore, more studies are needed to explore the relative roles of local and regional drivers of bacterioplankton diversity.

Another challenge that needs to be met is that of clearly delineating microbial diversity and its extent. This is a challenge that may be met with the employment of high-throughput sequencing technologies but also through refining existing theory and developing a body of new microbial theory, theory that is parsimonious, general, consentient, and predictive, i.e. a theory of microbial ecology with a set of underlying rules to describe and predict the behaviour of the microbial world as a system.

In this search for theory we must, however, not forget to turn our attention to some – in my opinion – crucial aspects within the microbial realm. Probably one of the most prominent issues to address is that of spatial scale. For instance, we were not able to observe a positive taxa-area relationship (paper IV) on neither the lake- nor the catchment-area scale, whereas Bell and colleagues, for example, did record such a relationship at a much smaller scale (Bell *et al.* 2005a). Therefrom emerging is the question of what is a spatially

defined microbial habitat, a question to which a satisfactory answer has yet to be found.

Another crucial aspect is that of microbial dispersal, that is the immigration and emigration dynamics into and from microbial assemblages. Concise recordings of dispersal and its properties are important not only in light of metacommunity theory but also with regard to the dispute over the extent of microbial diversity and its spatial scaling. The study of the spatial scaling of microbial diversity will benefit tremendously from concise observations of dispersal in that it will allow insight into the question of whether microbial ubiquity, i.e. unlimited dispersal, or dispersal limitation and, hence, microbial endemism prevails.

Closely linked to the question of how microbial organisms are distributed over space and time is that of the physiological state of microbes that is whether microorganisms are actively metabolising or dormant. Dormancy is one trait that allows organisms to contend with temporal variability of local environmental conditions, which have shown to be of importance in the structuring of microbial assemblages. The question about the physiological state has, further, important implications for the functioning of ecosystems and diversity dynamics of future generations in that dormant or passive microbes can possibly enter a seed bank.

3. Acknowledgements

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4. Summary in Swedish

Faktorer som styr bakteriers biogeografi i sötvatten

Jorden myllrar av mikroorganismer. Mikroorganismer bebor alla tänkbara habitat på jorden. Deras utbredning utgör därmed själva definitionen för var den yttersta gränsen för biologiskt liv går. Förutom att mikroorganismer finns överallt är de också den organismgrupp som finns i störst antal och är sannolikt också den mest artrika gruppen på jorden. Mikroorganismers evolutionära utveckling speglar jordens utveckling, eftersom de har funnits på jorden under mer än fyra femtedelar av jordens historia. Eftersom de alltså har genomgått 3.8 miljarder år av evolution är deras biologiska mångfald mycket stor, sannolikt större än hos alla andra organismer tillsammans. Mikroorganismer spelar också mycket centrala roller i ekosystem där de utför mycket viktiga processer av stor betydelse för nästan alla andra livsformer på jorden. Därmed är det troligt att mikroorganismers mångfald är av betydelse för hur jordens ekosystem fungerar. Av särskild betydelse för mänskligt liv är de tjänster de gör för människors hälsa, och för bioteknologi, jordbruk och livsmedelsindustrin. Mikroorganismers biologiska mångfald kan därför utgöra en reservoar för upptäckter av nya metaboliska processer och gener som kan visa sig användbara för utvecklandet av nya mediciner och bioteknologiska produkter.

Ett av ekologins främsta fokus för närvarande är att skapa förståelse för hur biologisk mångfald formas och upprätthålls. Anledningen till detta är att man kan anta att bevarandet av biologisk mångfald kräver att vi har mekanistisk kunskap för hur den har skapats. Studier av mikroorganismers mångfald har inte en särskilt lång historia, men har de senaste årtionden ådragit sig mycket uppmärksamhet. Av särskilt stort intresse är deras biogeografi, det vill säga utbredningsmönster i naturen, eftersom sådan information kan hjälpa oss att förstå vilka mekanismer som formar biodiversitet i allmänhet. Trots mikroorganismers centrala roller i ekosystem, och deras (sannolikt) mycket höga biologiska mångfald och det stora intresset under senare år, är studier av mikroorganismers diversitet ännu i ett mycket tidigt stadium. Därför vet vi ännu mycket lite om vilka faktorer som styr mikroorganismers biologiska mångfald över tid och rum.

I studier av biologiska samhällens mångfald har man tidigare fokuserat antingen på betydelsen av lokala deterministiska faktorer, som biologiska interaktioner i samhället och/eller den lokala miljön, eller på processer på en regional nivå som till exempel spridning av organismer mellan samhällen. Under de senare decennierna har dock en förståelse för att båda typerna av processer är av betydelse utvecklats inom den så kallade metasamhällesteorin. Denna teori betonar betydelsen av samspelet mellan lokala och regionala processer för biologisk mångfald. Metasamhället är ett antal lokala samhällen som sammanbinds av spridningen av ett antal arter som samtidigt interagerar med varandra och med sin miljö. Metasamhällesteorin är i sin tur uppdelad i fyra olika men något överlappande perspektiv: "species sorting" (ungefär artsortering), "mass effects" (masseffekt), "patch dynamics" (ungefär platsdynamik) och "neutral model" (neutrala modellen). Varje perspektiv beskriver olika mekanismer som styr biologiska samhällens sammansättning, där lokala och regionala processer har olika stor betydelse.

Syftet med den här avhandlingen är att undersöka i vilken utsträckning den biologiska mångfalden i bakterieplanktonsamhällen i sötvatten struktureras av lokala eller regionala faktorer, det vill säga att applicera ett metasamhällesperspektiv på dessa samhällen. Bakterieplanktonsamhällenas sammansättning och artrikedom undersöktes i svenska sjöar samtidigt som lokala miljöförhållanden (som t ex näringsförhållanden) och regionala faktorer (som t ex cell spridningshastighet och plats i landskapet) kartlades. Bakteriesamhällenas sammansättning och artrikedom undersöktes med hjälp av molekylärgenetiska metoder. Både så kallade fingeravtrycksmetoder (tRFLP) och moderna DNA-sekvenseringsmetoder (454 eller pyrosekvensering) användes.

Avhandlingen innehåller också två litteratursammanställningar där den första visade att bakterieplanktons biogeografi verkar styras av ett komplex samspel mellan lokala och regionala faktorer. I en av fältstudierna i denna avhandling fann vi, dock, att det snarare var de lokala miljöfaktorerna (artsortering) än en massiv spridning av mikroorganismer (masseffekt) som var av betydelse för samhällenas sammansättning. Vidare fann vi att dessa miljöfaktorer var lika viktiga för den mest aktiva delen av samhället som för samhället som helhet. I en annan fältstudie fann vi, dock, en potentiell betydelse av neutrala processer, det vill säga att samhällena skulle formas av stokastiska händelser. Betydelsen av neutrala processer verkade dock minska med ökande heterogenitet i miljön, vilket tyder på att artsortering är viktigare om miljöerna i ett metsamhälle är olika varandra. I en tredje fältstudie undersökte vi bakteriers artrikedom och fann att den var som högst vid högre tillgång på näring. Detta tyder på att bakteriesamhällen är mer artrika i produktiva miljöer. Däremot verkade sjöns placering i landskapet ha liten direkt betydelse för artrikedomen. Den andra litteratursammanställningen samman-

fattar resultat från empiriska metasamhällesstudier av makro- och mikroorganismerna. Resultaten visar att det finns ett glapp mellan empiri och teori, det vill säga att det har visat sig vara svårt att testa teorierna i praktiken. Resultaten visar också att en ganska stor andel av de studier som har utförts har gjorts på mikroorganismerna. Trots att ganska få studier har utförts totalt kan man alltså säga att studier av mikroorganismerna har gett ett relativt stort bidrag till vår förståelse av metasamhällen i allmänhet.

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